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ABSTRACTS

List of Abstracts from the 15th International Symposium on Olfaction and Taste

Givaudan Lecture

From Peppers to Peppermints: Natural Products as Probes of the Pain Pathway

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Natural products have an illustrious history when it comes to deciphering basic cellular and molecular mechanisms that contribute to the detection or modulation of noxious (pain-producing) signals. We, too, have exploited the power of natural products and folk medicine to identify cellular signaling molecules that enable primary afferent sensory neurons to detect chemical and physical stimuli that elicit discomfort or pain. These include members of the excitatory TRP ion channel family that respond to plant-derived irritants such as capsaicin, menthol, and mustard oil. Using genetic, electrophysiological, and behavioral methods, we have tested roles for these channels in a variety of somatosensory modalities, including thermosensation and chemosensation, both in regard to acute nociception and pain hypersensitivity. We have also used natural products (including novel spider toxins) to elucidate mechanisms of TRP channel activation and modulation, with the goal of understanding how these channels integrate information from physical and chemical stimuli to regulate sensory neuron excitability. Recent findings from these studies will be presented and discussed.

Olfaction: From Receptors to Modulation of the CNS

Elucidating the Molecular Mechanism of 11-*cis*-Vaccenyl Acetate Recognition in *Drosophila*

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The *Drosophila melanogaster* odorant-binding protein (OBP) LUSH mediates olfactory responses to two different ligands: one, ethanol, is a nonspecific environmental cue; the other, 11-*cis*-vaccenyl acetate (cVA), is a species-specific aggregation and mating pheromone. Flies lacking *lush* are defective in their avoidance of high ethanol concentrations (Kim et al., *Genetics* **150**:711-721, 1998), and also are not attracted to cVA (Xu *et al. Neuron* **45**:193-200, 2000). These defects can be rescued by either expression of LUSH or addition of the protein exogenously. These studies suggest that LUSH may have a more complex role in olfaction than simply acting as a passive odorant carrier. We have solved the X-ray crystal structure of LUSH bound to cVA to 1.4 resolution. From this structure we have identified a key interaction between cVA and F121 in LUSH that we hypothesize induces an "active" conformation of the protein. Substitution of this residue with alanine diminishes both pheromone-induced and spontaneous depolarizations of cVA-sensitive olfactory receptor neurons (ORN) *in vivo*. Furthermore, mutation of a neighboring residue, D118, to alanine results in a protein that induces ORN depolarizations in the absence of cVA. The X-ray crystal structure of D118A-LUSH without cVA indicates that this substitution allows the protein to adopt the same conformation as the WT protein with cVA, thus providing a mechanistic explanation for the activating effect. These results provide strong support for the hypothesis that LUSH is the primary ligand for the olfactory receptor complex in cVA sensitive T1 olfactory sensilla. This data also explain how this single OBP can mediate recognition of both attractive and repulsive stimuli.

Olfaction: From Receptors to Modulation of the CNS

Odor Coding by a Mammalian Receptor Repertoire

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Deciphering olfactory encoding requires a thorough description of the ligands that activate each odorant receptor. In mammalian systems, however, ligands are known for only a handful of over 1000 odorant receptors, greatly limiting our understanding of olfaction. We performed high-throughput screening of ligands for mammalian odorant receptors using a large repertoire of mouse and human odorant receptors expressed in heterologous cells. We identified excitatory ligands for 52 mouse and 10 human odorant receptors, greatly expanding our knowledge of receptor-ligand interactions. We used the resulting interaction profiles to develop a predictive model relating physicochemical odorant properties, receptor sequences, and their interactions. Our model can predict a tested receptor's response to a novel odorant ($d' = 0.63$, $p < 0.001$) and a novel receptor's response to a tested odorant ($d' = 0.22$, $p < 0.001$). This provides a large dataset of mammalian receptor-ligand interactions to constrain the search for rules underlying olfactory transduction and a framework for identifying active ligands for untested receptors in the mouse and human odorant receptor repertoire.

Olfaction: From Receptors to Modulation of the CNS

Plasma Membrane Calcium ATPase 2 Knock Out Shows Slower Calcium Clearance from Olfactory Sensory Neurons and Deficits in Olfactory Driven Behavior

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Odorants initiate signal transduction in mammalian olfactory sensory neurons (OSNs) that leads to increased intracellular Ca^{2+} from the opening of cyclic nucleotide gated channels. The Ca^{2+} is then cleared preparing the cell for the next stimulation. Several candidates for Ca^{2+} clearance include the Na^{+}/Ca^{2+} exchanger (NCX), plasma membrane calcium ATPases (PMCAs), and ER calcium pump (SERCA). We show here the significance of the PMCAs in Ca^{2+} clearance from OSNs after stimulation and olfactory driven behavior. We know from immunofluorescence and RT-PCR that all 4 isoforms of the PMCAs are expressed in mouse OSNs. It matters very much

that the PMCAs are not evenly distributed and that not all are in the cilia, because they differ in their kinetics and affinities. The assortment of PMCAs in a cell shapes the Ca^{2+} signal, which affects signaling and adaptation. Here we show that OSNs stimulated with either 60 mM KCl or IBMX/Forskolin are slower to clear Ca^{2+} from cell bodies and dendritic knobs if they are missing PMCA isoform 2 (PMCA2 KO mice were a gift from Dr.Gary Shull). Both wild type and PMCA2 KO OSNs treated with a PMCA inhibitor carboxyeosin (CE) are significantly slower to clear Ca^{2+}. The rate of Ca^{2+} clearance from the OSN knob is slowed 34% with CE, 34% with CPA to inhibit SERCA, and 35% with low-Na Ringers to inhibit NCX Thus PMCAs play a significant role in OSN Ca^{2+} clearance and that the loss of even one PMCA (PMCA2 which has the highest affinity for Ca/calmodulin) can alter Ca^{2+} signal kinetics. *Does this matter to olfactory driven behavior?* We will demonstrate that the PMCA2 KO mice show behavioral deficits in learning a two bottle conditioned olfactory avoidance and a conditioned fear response when an odor is paired with shock.

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Olfaction: From Receptors to Modulation of the CNS

The *in vitro* and *in situ* Effects of Insulin on Olfaction in Mice

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The role of insulin pathways in olfaction are of significant interest with the rising incidence of *Diabetes mellitus* and associated metabolic and neuronal co-morbidities. It is difficult to understand the full scope of insulin function in olfaction because of a large data gap between insulin-evoked biochemical and behavioral effects. To address this, experiments were conducted to ascertain the function of insulin *in vitro*, followed by biochemical and behavioral analysis of insulin effects *in situ*. The insulin receptor (IR) kinase is expressed at high levels in the olfactory bulb (OB), where it is found to suppress the current of a dominant *Shaker* channel, Kv1.3, via tyrosine phosphorylation of critical N- and C-terminal residues. We report that the adaptor protein post-synaptic density 95 (PSD-95) disrupts insulin-evoked Kv1.3 current suppression, demonstrating a role for adaptor proteins as indirect ion channel modulators. Kv1.3 co-immunoprecipitated and co-localized with PSD-95 and IR in the OB, demonstrating a scaffolding interaction. We optimized 5 day intranasal hormone delivery in awake mice via intracellular clefts in the cribriform plate. Intranasal insulin delivery evoked robust phosphorylation of Kv1.3 in the OB, as well as increased channel protein-protein interactions with IR and PSD-95. Intranasal insulin delivery increased short- and long-term object memory recognition in the mice, evoked anxiolytic behavior, increased odor discrimination via odor habituation paradigms, but did not significantly modify odorant threshold. Thus, insulin-evoked ion channel modulation and alteration of protein-protein interactions appear to affect olfactory-related behaviors, suggesting a mechanism for a metabolic hormone regulating glucose utilization to influence olfaction.

Olfaction: From Receptors to Modulation of the CNS

Gaba-Mediated Regulation of the Activity-Dependent Olfactory Bulb Dopaminergic Phenotype

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The majority of olfactory bulb (OB) periglomerular interneurons are GABAergic, and distinct subsets of these interneurons co-express other neuroactive molecules such as dopamine (DA). Terminal differentiation of the OB DA phenotype requires activity-dependent expression of tyrosine hydroxylase (TH). To establish the molecular mechanisms necessary for activity-dependent TH expression, neonatal forebrain slice cultures were prepared from transgenic mice expressing GFP under the control of the 9kb TH upstream gene regulatory region. These studies revealed that the induction of TH/GFP expression under depolarizing conditions (25mM KCl) is completely inhibited by nifedipine, an L-type Ca+2 channel blocker, and partially inhibited by w-agatoxin, a P/Q Ca+2 channel blocker. This combined action of both L and P/Q-type Ca+2 channels is similar to the established mechanism for synaptic release of GABA from periglomerular interneurons. These findings suggest the novel concept that the initiation and maintenance of the OB DA phenotype is coupled to its co-expressed GABAergic phenotype. Our studies also revealed that exogenous application of GABA further increased TH/GFP expression levels in depolarized slice cultures. This GABA-mediated increase of TH/GFP expression was blocked by inhibitors of either GABA-A or GABA-B receptors as well as inhibitors of metabotropic and ionotropic glutamate receptors. Although previous studies have shown that GABA is sufficient to both depolarize OB interneuron progenitors and activate L-type Ca+2 channels, GABA, by itself, was not sufficient to induce TH/GFP expression in our studies. Instead, the data indicate that induction of TH/GFP expression specifically required glutamate-mediated depolarization and activation of L-type Ca+2 channels.

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Olfaction: From Receptors to Modulation of the CNS

Olfactory Deafferentation of Adult Mice Transsynaptically Alters AMPA Receptor Expression in Cells of the Main Olfactory Bulb External Plexiform Layer

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Altered expression of AMPA receptors and their subunits have been linked to stimulation-dependent changes in synaptic efficacy within the brain. We are studying AMPA receptor expression within the main olfactory bulb (OB). We previously showed that neonatal naris occlusion reduced the number of interneuron cell bodies that were immunoreactive (IR) for the GluR1 AMPA receptor subunit in the external plexiform layer (EPL) of the adult mouse OB. Our more recent studies showed that the number of GluR1-IR interneurons was reduced following olfactory deafferentation of adult mice expressing the GABAergic interneuron marker glutamic acid decarboxylase 65 (GAD65). The number of double GluR1-IR- and GAD65GFP-positive interneurons that were immunoreactive for parvalbumin (PV), another inhibitory interneuron marker, was also reduced. Moreover, comparison of the number of GluR1-IR interneurons that expressed GAD65GFP and/or PV with the total number of GluR1-IR cells suggested that GluR1 expression was reduced in the relatively abundant tufted cells and sparse interneurons of the EPL that were positive for GluR1 but negative for GAD65 and PV. The reduced GluR1-IR of the interneurons and tufted cells was not likely due to cell death, because sensory deafferentation resulted in little neuronal degeneration and it did not significantly reduce GluR1-IR cell density or total cell numbers. These results suggest that olfactory input might transsynaptically regulate GluR1 expression by tufted cells with subsequent transsynaptic regulation of GluR1 expression by interneurons within the OB EPL. Additional studies of AMPA receptor plasticity are currently underway.

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Stem Cells in Sensory Epithelium Development and Regeneration

Development and Regeneration in the Mammalian Inner Ear: Cell Cycle Control and Differentiation of Sensory Progenitors

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Sensory hair cell loss is the leading cause of deafness in humans. The mammalian cochlea cannot regenerate its complement of sensory hair cells, and thus at present, the only treatment for deafness due to sensory hair cell loss is the use of prosthetics such as hearing aids and cochlear implants. In contrast, in non-mammalian vertebrates such as birds, hair cell regeneration occurs following the death of hair cells and leads to the restoration of hearing. Regeneration in birds is successful because supporting cells that surround the hair cells begin to divide when hair cells are lost and are able to subsequently differentiate into new hair cells. Although these cells exist in mammals, they do not normally divide or transdifferentiate when hair cells are lost, and so regeneration does not occur. To understand the failure of mammalian cochlear hair cell regeneration, we have been studying the molecular mechanisms that underlie cell division control and hair cell differentiation, both during embryogenesis and in the postnatal mouse. In this presentation, I will discuss the molecular basis for the timing of cell cycle exit in the embryo, and how this is coordinated with differentiation to produce the correct number of hair cell and supporting cell precursors to build a functional organ of Corti. I will also discuss the role of the Cip/Kip cell cycle inhibitors and Notch signaling in the control of stability of the differentiated state of early postnatal supporting cells. Finally, I will present data indicating that some early postnatal mammalian supporting cells retain a latent capacity to divide and transdifferentiate into sensory hair cells. Together, these observations make supporting cells important therapeutic targets for continued efforts to induce hair cell regeneration.

Stem Cells in Sensory Epithelium Development and Regeneration

Fate Mapping Mammalian Taste Bud Progenitors: New Insights, Challenges and Beyond

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Despite some neuronal characteristics, taste receptor cells arise from the local epithelium unlike other sensory receptors, which derive from neurogenic ectoderm. Like other epithelial appendages, taste organs form as epithelial placodes, followed by intervention of mesenchymal core to form taste papillae. Taste buds differentiate in the papillary epithelium around birth. However, evidence for a lineage relationship between the embryonic placodes and functional taste buds is primarily indirect. Likewise, while mesenchyme plays a role in the morphogenesis of most epithelial appendages, its function in mammalian taste bud and papilla development is unclear. To understand the developmental relationship of taste buds and papillae, and the interplay between papillary epithelium and mesenchyme, we used a fate mapping approach to indelibly label either embryonic taste placodes, or the cranial neural crest-derived mesenchyme and followed the postnatal fates of these cell populations. With the inducible ShhcreERT2 mouse line crossed to R26R reporter line, we demonstrate embryonic Shh-expressing taste placodes are taste bud progenitors, which give rise to the differentiated taste cells. In contrast, with Wnt1-Cre mediated recombination, we show that cranial neural crest-derived mesenchyme contributes only to the mesenchymal core of taste papillae and not to taste buds or papillary epithelium. Recently, we have shown that these taste bud progenitors are specified by Wnt/ß-catenin, a key pathway in the induction of other epithelial appendages. We are now exploring the role of WNT signaling with respect to its function within the taste bud progenitor population and its impact on papillary morphogenesis.

Stem Cells in Sensory Epithelium Development and Regeneration

Wnt5a in Tongue and Taste Papilla Development

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Wnt10b has a critical role in formation of fungiform papillae, via canonical *Wnt* signaling. In a gene array experiment we found that *Wnt5a*, which can signal in either the canonical or non-canonical pathway, was increased 8 fold in anterior tongue of rat embryo organ cultures, compared to posterior tongue. In Western blots, Wnt5a was expressed in tongues from embryonic (E) day 13 through 16. Immunoreactions in E15 tongue demonstrated Wnt5a in epithelium and mesenchyme. To learn if *Wnt5a* has a role in development of papillae, we compared tongues of E15.5 - 17.5, *Wnt5a* -/- mutant mice with those of wild type littermates. Length of oral tongue in *Wnt5a* null mouse was reduced to 60% of wild type length. Portions of the tongue that were anterior, or posterior, to the anterior-most border of the intermolar eminence were reduced by similar proportions. However, pharyngeal tongue in null mice was reduced to only 25% of wild-type. Whereas length of oral tongue was severely compromised in null mice, width was similar to that in wild type. Thus overall, area of the anterior tongue was substantially reduced and shape was radically altered. In the face of a much truncated anterior tongue area, numbers of fungiform papillae were not different on mutant tongues relative to wild type. This separates genetic programs for papilla number from those for tongue shape and size. The single circumvallate papilla on posterior tongue also was sustained, but with topographical shrinkage and shape alteration in *Wnt5a* mutant tongues. Results demonstrate a role for *Wnt5a* in tongue and circumvallate papilla size and shape, in distinction to roles for *Wnt10b* in establishing fungiform papilla number.

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Stem Cells in Sensory Epithelium Development and Regeneration

Opposing Actions of Cell-Intrinsic Factors and Secreted Signals Regulate Neurogenesis in Olfactory Epithelium

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In mouse olfactory epithelium (OE), growth and differentiation factor 11 (GDF11), an activin-like TGF-β expressed by olfactory receptor neurons (ORNs) and late-stage neuronal progenitors, acts to inhibit both proliferation and neuronal differentiation of neuronal progenitor cells. *Foxg1*, which encodes a forkhead-box transcription factor known to be required for OE development, is co-expressed with *Gdf11* in much of developing OE; and FoxG1 is known to interact with Smad transcription complexes to inhibit expression of TGF-β target genes. Together, these observations raise the possibility that

Foxg1 regulates OE development by inhibiting *Gdf11*'s negative action on neurogenesis. We characterized neurogenesis in detail in *Foxg1*$^{-/-}$ OE, and found a severe loss of cells in the OE neuronal lineage, including *Sox2*-expressing neural stem cells, apparent as early as the olfactory pit stage. By birth, neurogenesis has terminated and only remnants of OE can be found in *Foxg1*$^{-/-}$ embryos. Remarkably, the depletion of neuronal cells in the OE resulting from loss of *Foxg1*, as well as nasal cavity morphogenesis, are substantially rescued when embryos are also made null for *Gdf11* (*Foxg1*$^{-/-}$;*Gdf11*$^{-/-}$ mice). Importantly, rescue is dependent on *Gdf11* gene dosage, with an intermediate level of rescue evident in *Foxg1*$^{-/-}$;*Gdf11*$^{+/-}$ compound mutants. Rescue is accompanied by modifications in expression of both *p21Cip1* and *follistatin* (Fst), providing mechanisms by which GDF11 signaling and FoxG1-regulated transcription can interact in vivo. Thus, our data indicate that the *Gdf11*-mediated antineurogenic signal in OE is negatively regulated by *Foxg1*, and these two genes together are largely responsible for controlling the expansion and progression of OE neurogenesis during development.

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Stem Cells in Sensory Epithelium Development and Regeneration

Onset of Odorant Receptor Expression

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Olfactory sensory neurons (OSNs) express 1 of ~1,000 odorant receptors (ORs). OSNs projecting axons to the same glomeruli express the same OR protein, although they are distributed within restricted regions of the olfactory epithelium (OE). As the OSN axons navigate from the OE to the olfactory bulb (OB), they reorganize and project to specific glomeruli based on OR expression, among other cues. This expression pattern is not achieved simply via retrograde signals from the OB after synapse formation because: 1) ORs are expressed during embryonic development prior to synapse formation; and 2) ORs are also expressed in mice lacking OBs. ORs are expressed prior to synapse formation, but it remains to be established when ORs are first expressed during development. The aims of this work were 3-fold: 1) study onset of OR expression for a subset of ORs; 2) determine if there is a preferential zonal or chromosomal OR expression choice during embryogenesis; and 3) perform a quantitative analysis of specific OR expressing OSNs. We found that the onset of OR gene expression is asynchronous. For example MOR244-1 is first expressed at very early stages of olfactory development, while MOR245-3 only appears late in embryogenesis. Interestingly, OR onset does not seem to be stochastic during development; i.e. ORs on some chromosomes appeared overrepresented early in development. Moreover, expression of ORs from the same region/zone have differential onsets of expression, and the profile of numbers of cells expressing a given OR is not uniform, but varies by OR; some ORs had profiles that increased with age while some had a more transient expression. Our results provide compelling evidence that OR choice could be an important determinant of glomerular targeting during embryogenesis.

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Stem Cells in Sensory Epithelium Development and Regeneration

Transcriptional Control of Epidermal Morphogenesis

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The epidermis is the primary barrier that protects the body from dehydration, mechanical trauma, and microbial insults. This barrier function is established during embryogenesis through a tightly controlled stratification program. One gene that is critical for controlling epidermal morphogenesis is p63, a transcription factor that can be expressed as isoforms that contain (TA) or lack (ΔN) a transactivation domain. Of these, ΔNp63 isoforms are the predominantly expressed p63 isoforms in late embryonic and postnatal epidermis. To determine the role of ΔNp63 proteins, we generated an epidermal-specific inducible ΔNp63 knockdown mouse model. We found that downregulating ΔNp63 expression in postnatal epidermis caused severe epidermal defects, including aberrant keratinocyte differentiation and impaired basement membrane formation, culminating in the development of severe skin erosions. Interestingly, these lesions were indistinguishable from lesions that develop in patients with AEC, an ectodermal dysplasia caused by mutations in ΔNp63α. Follow-up studies demonstrated that, during epidermal morphogenesis, ΔNp63α initially induces expression of a keratinocyte-produced extracellular matrix protein, Fras1, which is required for maintaining the integrity of the epidermal-dermal interface at the basement membrane. Subsequently, ΔNp63α initiates epidermal terminal differentiation by inducing IKKα, a regulator of epidermal, skeletal, and craniofacial morphogenesis. Together, our data provide novel insights into the role of ΔNp63α in epidermal morphogenesis and homeostasis, and may contribute to our understanding of the pathogenic mechanisms underlying disorders caused by p63 mutations.

Umami Symposium I: Umami Reception in the Oral Cavity: Receptors and Transduction

Receptors and Transduction of Umami Taste Stimuli

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L-glutamate and 5′- ribonucleotides such as GMP and IMP elicit the "umami" taste, also known as the fifth taste. This talk will review recent advancements in our understanding of umami taste receptors and their downstream signaling effectors in taste receptor cells. Several G protein-coupled receptors that bind umami stimuli have been identified in taste buds, including the heterodimer T1R1 + T1R3, and the truncated glutamate receptors taste-mGluR4 and taste-mGluR1. Further, ionotropic glutamate receptors are expressed in taste cells, and may play a role in glutamate transduction or signaling between taste cells and/or nerve fibers. Knockout of T1R1 or T1R3 reduces, but does not eliminate responses to umami stimuli, suggesting that multiple receptors contribute to umami taste. The signaling effectors downstream of umami receptors involve Gβγ activation of PLCβ2 to elicit Ca^{2+} release from intracellular stores and activation of a cation channel, TRPM5. In fungiform and palatal taste buds, T1R1 + T1R3 is co-expressed with GαGustducin, but the Gα proteins involved in circumvallate taste buds have not been identified. Previous physiological studies in our lab and other labs have shown that L-glutamate elicits multiple types of responses in rat taste cells isolated from both fungiform and circumvallate papillae, including both depolarization and hyperpolarizaton. In most cases, however, L-glutamate elicits an increase in intracellular Ca^{2+}, likely via release from intracellular stores. We are currently using transgenic mice expressing GFP in specific subsets of taste cells to correlate responses to umami stimuli with specific taste cell types.

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Umami Symposium I: Umami Reception in the Oral Cavity: Receptors and Transduction

Processing Umami and Other Tastes in Mammalian Taste Buds

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Umami taste is initiated by interactions between L-glutamate (and related compounds) and G protein-coupled receptors GPCRs. Taste GPCRs and their downstream effectors for umami, sweet, and bitter compounds are expressed by one class of cells in taste buds termed Receptor, or Type II cells. When they are stimulated, Receptor cells release a taste neurotransmitter, ATP, via a novel synaptic mechanism involving secretion through pannexin 1 gap junction hemichannels. Another taste cell type is also involved in sensing chemical stimuli. This is the Presynaptic, or Type III cell. Presynaptic taste cells possess synapses (hence their name) but do not express taste GPCRs. Presynaptic cells respond directly to salty and sour (acid) tastants, presumably by transduction mechanisms involving ion channels. Presynaptic cells are also stimulated by ATP secreted from Receptor cells. When activated by sour stimuli or by ATP from Receptor cells, Presynaptic cells release the taste transmitters serotonin (5-HT) and norepinephrine (NE). These findings suggest that there is an intriguing interplay between at least two taste cell types and multiple taste neurotransmitters when taste buds are stimulated by umami and other tastes.

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Umami Symposium I: Umami Reception in the Oral Cavity: Receptors and Transduction

Multiple Receptor Systems for Umami Taste in Mice

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Recent molecular studies proposed that T1R1/T1R3 heterodimer, mGluR1 and mGluR4 might function as umami taste receptors. However, the roles in umami taste of each of these receptors have not been made clear. In this study, we analyzed response characteristics of individual glutamate sensitive fungiform taste cells and chorda tympani fibers in mice, then, investigated contribution of each of these receptors to umami responses. Recordings from mouse single fibers and taste cells revealed that both glutamate sensitive fibers and taste cells were classified into sweet-best (S-type) and mono sodium (or potassium) glutamate (MSG or MPG)-best (M-type). Each type was further classified into 2 subgroups: one type showing synergistic effect between MSG and IMP (S1, M1) and the other type showing no synergism (S2, M2). In T1R3- or TRPM5-KO mice, S1-type was absent, but S2, M1 and M2 types still remained, supporting the existence of multiple receptors, transduction pathways and fiber types for umami taste. Glutamate responses of M-type taste cells and fibers were reduced by addition of metabotripic glutamate receptor antagonists, AIDA and CPPG, suggesting that mGluR1 and mGluR4 may function as umami taste receptors in M-type cells. These results suggest that umami taste is mediated by multiple receptor systems in the taste bud of mice.

Umami Symposium I: Umami Reception in the Oral Cavity: Receptors and Transduction

Genetic Tracing of the Gustatory Neural Pathways Originating from T1R3-Expressing Sweet/Umami Taste Receptor Cells

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Neural pathways conveying taste information from the tongue to the brain underlie the gustatory information coding and processing. It is an enigma how the gustatory information is conveyed from the sweet/umami and bitter taste receptor cells (TRCs) devoid of conventional synapses. To visualize the gustatory neural pathways of sweet/umami sensation, we here established transgenic mouse lines in which a trans-neuronal tracer, wheat germ agglutinin(WGA), was faithfully and robustly expressed in sweet/umami TRCs under the control of mouse T1R3 gene promoter/enhancer. WGA protein was transferred to a subset of neurons in the geniculate and nodose/petrosal ganglia. Furthermore, WGA protein was observed in a subpopulation of neurons in the rostro-central region of the nucleus of solitary tract. This indicates that WGA has undergone trans-neuronal transfer into the central nervous system. However, no WGA immunoreactivity was detectable in the taste bud cells with synapses that co-express aromatic l-amino acid decarboxylase and putative sour taste receptor PKD2L1. Also, no such immunoreactivity was found in the brain regions more centrally located along the gustatory neural pathways including the parabrachial nucleus, thalamus, amygdala, and gustatory cortex. These results imply that the gustatory neurons innervate the sweet/umami TRCs, and that there is a sweet/umami information pathway directly transmitting to the gustatory neurons from TRCs. This study uncovered a precise map of sweet/umami information pathways from TRCs to the nucleus of solitary tract in the brainstem.

Umami Symposium I: Umami Reception in the Oral Cavity: Receptors and Transduction

Behavioral Studies of Umami: Tales Told by Mice and Rats

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Psychophysical research with rats and mice has been instrumental in understanding umami taste transduction and perception. Although early studies suggested that an NMDA-like receptor detected substances that elicit an umami taste, studies using a variety of methods with both rats and mice indicate that the mechanisms for detecting umami stimuli are much more complex. When the G-protein-coupled receptor T1R1+T1R3 was discovered, it was believed to be the principle umami receptor and a more broadly-tuned L-amino acid receptor. Since then, however, results from a number of behavioral studies, like molecular and physiological studies, suggest that other receptors may contribute to umami taste. For example, deleting the T1R3 receptor in knockout mice (KO) elevates detection thresholds for

monosodium glutamate (MSG) and L-alanine only slightly. In conditioned taste aversion studies, T1R3 KO mice show bidirectional generalization of the aversion between MSG and L-alanine, indicating that these substances elicit similar tastes and yet these KO mice can rather easily discriminate between the tastes of the two amino acids. Alternatively, behavioral evidence supporting another putative umami receptor, taste-mGluR4, has been growing. For example, in rats the mGluR4 antagonist CPPG decreases an aversion to MSG while increasing the generalization of the aversion to L-arginine. Thus behavioral data from rats and mice acquired with a variety of complementary methods suggest other putative umami receptors, including the taste-mGluR4, taste-mGluR1, and possibly NMDA-like receptors. This talk will present an overview of relevant behavioral studies with rats and mice and some preliminary data showing that multiple receptors in the oral cavity contribute to umami taste.

Supported by NIH DC007617 and NSF IOB-0450350.

The Alarm Pheromone: 70 Years after von Frisch

The Alarm Reaction in Fishes

Kjell B. Døving

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In 1938 Karl von Frisch, working on the sense of hearing in European minnows, discovered a "schreck reaktion" (alarm reaction) when the fish were exposed to water from injured conspecifics. Von Frisch worked out the essentials of this behavior; he and his collaborators found that the substances evoking the alarm reaction were stored in specialized 'club cells' of their skin, and that the olfactory system detected these substances and mediated the behavior. The alarm reaction was once thought to be restricted to the carps and their relatives, but it is seems to be present in all teleosts investigated, although the displayed behavior varies between species. Crucian carps show a darting with vigorous swimming and go into hiding in the bottom mud to avoid potential danger. The sensitivity to skin extracts increases with age, approaching ng/L at a length of 70 mm. The fish olfactory epithelium contains three types of olfactory sensory neurones: crypt cells, microvillous cells, and ciliated cells, which are thought to mediate behaviors related to reproduction, food search, and alarm respectively. In crucian carps, information from the ciliated sensory neurones is transmitted into the medial part of the medial olfactory tract. The alarm reaction is lost if and only if this part of the olfactory tract is cut. Recordings of the spike activity from neurons in the 'alarm region' of the olfactory bulb of crucian carp, demonstrate that there are unique neurons responding only to skin extract of conspecifics and not of other carp species. This finding indicates that the alarm substances have a species-specific composition and a corresponding specific odorant receptor. In spite of several attempt to isolate and identify the alarm substances, they remain mysteriously hidden in the realm of chemical richness.

Amino Acids as Chemical Stimuli for Fish

Tasting and Smelling Amino Acids in the Water: 40 Years after Bardach

John Caprio

LSU, Baton Rouge, USA

Advances in our understanding of the chemoreception of amino acids by fish since the classical work of J.E. Bardach and colleagues will be presented. During the latter 1960s, his laboratory at the University of Michigan performed key experiments on the sense of taste in fish and heavily influenced the development of the field of fish chemoreception.

Ligand-Binding Properties of Taste and Smell Receptors

Special NIDCD Workshop: Ligand-Binding Properties of Taste and Smell Receptors

Barry Davis

Taste and Smell Program, NIDCD, Bethesda, USA

The response to the program announcement, "The Structural Analyses of Ligand-Binding Properties of Taste and Smell Receptors" (http://grants.nih.gov/grants/guide/pa-files/PA-07-126.html) has been very good, given the size of the research community that works on the problem. However, the success rate of these applications is considerably lower than the overall success rates of other types of taste and smell applications. The purpose of the workshop is to identify the scientific and peer-review issues that contribute to this lower success rate. What are the barriers to progress in this area of research and how can the NIDCD develop research resources and research strategies to facilitate forward movement? Panel Members: Barry Knox (Syracuse), Marianna Max (New York), John Ngai (Berkeley), Steve Munger (Baltimore) and Charles Leutje (Miami). Moderator: Barry Davis. The Workshop is open to the AChemS/ISOT attendees. No special registration is required for this workshop. Seating will be limited.

Evolution of Pheromonal Communication in Insects

A Comparative Genomics Screen for Novel Olfactory Molecules

Richard Benton[1] and Leslie B. Vosshall[2]

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The olfactory systems of vertebrates and invertebrates display remarkable similarities in their neuroanatomical organisation and neurophysiological sensory coding properties. However, while all known vertebrate olfactory and pheromone receptors belong to the G protein-coupled receptor superfamily, insects have evolved a novel family of polytopic transmembrane proteins to mediate odour detection. To identify molecules that act with these receptors in *Drosophila*, we have performed a bioinformatics screen, based upon the assumptions that such molecules will display the same insect-specific conservation and restricted tissue expression as insect odorant receptors. This screen yielded all previously identified classes of olfactory molecules as well as a large number of novel genes that we find expressed in diverse populations of chemosensory neurons. We will describe our current analysis of some of these molecules, which reveals insights into the molecular mechanisms of odour detection by odorant receptors and new olfactory sensory pathways.

Funding: University of Lausanne, European Research Council, Helen Hay Whitney Foundation (R.B.); NIH, McKnight Endowment Fund for Neuroscience (L.B.V.).

Evolution of Pheromonal Communication in Insects

The Olfactory Pathway of Adult and Larval *Drosophila*: Conservation or Adaptation to Stage-Specific Needs?

Reinhard F. Stocker

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Tracing of olfactory projections based on odorant receptor expression has led to a quite complete receptor-to-glomerulus map in adult *Drosophila*. While most of the glomeruli may be involved in processing of food odors, others appear to be more specialized, e.g. responding to CO2 or pheromonal cues. Recent studies have shed new light on signal processing in the antennal

lobe and in higher centers. Newly detected cholinergic excitatory local interneurons appear to provide substrates for the broad odor tuning properties of projection neurons. In the mushroom bodies, projection neurons establish an intricate divergence-convergence network with their target cells, allowing complex modes of signal transfer. In the lateral horn, projection neurons innervating 'normal' glomeruli and those innervating candidate pheromone glomeruli appear to segregate. Hence, food and pheromone information may be handled by separate channels, consistent with discrete behavioral meanings of the two kinds of signals. The olfactory pathway of the larva shares the general layout of its adult counterpart, with a number of simplifications: The presence of only 21 glomeruli suggests a reduction of primary olfactory dimensions compared to adults. The existence of a pheromone-sensing subsystem is unlikely. Larval glomeruli are targets of single, unique sensory neurons rather than being sites of convergence as in the adult. Projection neuron outputs are restricted to single, restricted areas in the mushroom body. Their target cells either innervate one or several of these areas creating substrates for elementary odor coding or coincidence detection. In conclusion, olfactory discrimination capacities of the larva are very likely reduced, consistent with the requirements of a substrate feeder.

Evolution of Pheromonal Communication in Insects

Evolution of Pheromonal Communication in Insects: Sexual Dimorphism of Antennal Lobes

Nicholas J. Strausfeld, Carolina Reisenman

ARL Division of Neurobiology, University of Arizona, Tucson, USA

The origin and evolution of the insect olfactory system is still enigmatic. Whereas modern neopteran insects are equipped with glomerular antennal lobes that supply targets in the protocerebrum, such as the mushroom body calyces and the lateral horn, observations on basal monocondylic archaeognathans show them to reveal olfactory pathways that are more similar to those of malacostracan crustaceans than to dicondylic insects. This suggests that the transition from the marine to terrestrial habitat may have principally forced the evolution of novel olfactory sensilla while retaining the basic groundplan of the antennular lobe and at least one of its protocerebral projections. Thus, the very first detritovorous insects were equipped with glomerular antennal lobes. However, extant neopteran insects show obvious sex-specific arrangements in their antennal lobes, the lepidopteran macroglomerular complex being the archetypal manifestation of such sexual dimorphism. When and how often did such dimorphism evolve and what were the evolutionary constraints underlying this innovation? In this talk we will offer examples of dimorphisms across the Lepidoptera, even amongst species where there is no obvious dimorphism of the antennae. We will also review sex-specific antennal lobes in the Coleoptera, Diptera, and other neopteran groups. Mapping such traits onto a neopteran phylogeny suggests that dimorphic antennal lobes may have evolved convergently in several lineages and that their evolution provides a fascinating counterpart to the evolution of sexual dimorphism of the optic lobes.

Evolution of Pheromonal Communication in Insects

Shaping the Male Courtship Posture by a Gustatory Pheromone in *Drosophila*

Daisuke Yamamoto[1], Masayuki Koganezawa[1] and Takashi Matsuo[2]

[1]Tohoku Univ Grad Sch Life Sciences, Sendai, Japan and [2]Tokyo Metropol Univ, Hachioji, Japan

Mating behavior of *Drosophila melanogaster* males is composed of several distinct behavioral elements, i.e., orientation toward a female, tapping the female abdomen, unilateral wing extension and vibration to generate courtship songs, licking the female genitalia, attempted copulation and copulation. This series of mating behavior is highly stereotyped and does not, in principle, require prior training. The most prominent feature among these is unilateral wing vibration, which is only observable in males when they are engaged in courtship activities. We found that bilateral wing extension by courting males toward a female dramatically increases when a class of sensory neurons expressing *Gustatory receptor(Gr)32a* is prevented from functioning by targeted expression of Tetanus toxin (TNT) in these cells. Amputation of the tarsus of a foreleg results in an increase in the occurrence of bilateral wing extension, suggesting that *Gr32a*-expressing neurons in the foreleg tarsi convey a signal that directs the male fly to use a single wing and not two wings at a time during courtship. We also found that an odorant binding protein is required for normal unilateral wing extension to occur during courtship.The hypothesis of the pheromonal regulation of unilateral wing extension during male courtship is substantiated by the fact that electrical activities are induced in sensory hairs that house *Gr32a*-expressing neurons upon stimulation with extracts of fly cuticle. The central projections of tarsal *Gr32a*-expressing neurons terminate close proximity to the dendritic branches of sexually dimorphic *fruitless*-expressing interneurons (mAL) in the suboesophageal ganglion. It awaits rigorous demonstration as to whether *Gr32a*-expressing neurons have functional connections with mAL neurons.

Evolution of Pheromonal Communication in Insects

Desat1 and the Evolution of Pheromonal Communication in Drosophila

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In most animals, sensory communication is necessary to recognize individuals of the same species. In particular, chemical signals (pheromones) are ubiquitously used for mate discrimination. To continue its existence, each species must maintain a tight genetic link between the emission and the reception of its species-specific signals. If the emission and reception systems diverge, a new species with individuals using new sensory signals may arise. We discovered that the desat1 gene can change both the production and the perception of sex pheromones that are exchanged during the courtship ritual by Drosophila melanogaster flies. This is the first exemple of a single gene acting on both sides (emission / reception) of inter-individual sensory communication. If the function of desat1 on the pheromone production (it codes a desaturase introducing double bonds on hydrocarbon chains) is clear, its involvment on pheromone perception remains unclear. Using genetic and molecular approaches, we discovered that these phenotypes are not causally connected but rather depend on a separate or pleiotropic control. The desat1 promoter is made of regulatory sequences each of which can drive desat1 expression in tissues involved either in pheromone production or in pheromone perception. These sequences are used in promoter-specific driver lines to target separate pheromonal tissues and to measure the functionnal consequences of this manipulation. We hypothezise that the functionnal pleiotropy of desat1 is related to its complex regulation and/or to multiple transcripts all of which code a similar enzyme. The ultimate goal of this study is to understand how pheromonal communication can drive the evolution and formation of new species.

Evolution of Pheromonal Communication in Insects

Molecular Evolution of Moth Sex Pheromone Desaturases

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Moth sex pheromone communication has evolved to make use of complex blends of relatively simple long-chain fatty acid precursors. Species

specificity is derived from the unique stereochemistry of double bonds introduced into exact locations along the hydrocarbon backbone of fatty acids, which are reduced and then undergo a variety of chain-shortening and functionalization reactions to form the pheromone blend. Key enzymes that have evolved to function in this system are the acyl-CoA desaturases, which catalyze the introduction of the double bonds. An overview of the evolution of these enzymes is given here.

Impact of Oronasal Inflammation on Taste and Smell

Impact of Oronasal Inflammation on Taste and Smell

Nancy E. Rawson[1,2] and Liquan Huang[2]

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Chronic inflammatory disorders of the nasal and oral cavities can result from a variety of external and internal causes, such as infections, chemical exposures, traumatic injuries or surgery, cancer, medications or radiation therapy. The impact of these conditions on chemosensation has been characterized to varying degrees, and these chemosensory losses can be severe and long-lasting. In spite of the adverse impact on the patients, our understanding of the specific mechanisms underlying inflammation-associated chemosensory loss are limited, and therapeutic options are few and often are ineffective or only transiently effective. The lack of consistent diagnostic tools and criteria for defining these disorders has presented a challenge to researchers attempting to understand the chemosensory impact of inflammation. However, new insights into inflammatory pathways and tools to examine their activity in chemosensory tissues provides an opportunity for identifying targets for new therapeutic approaches. This symposium will bring together researchers with diverse perspectives to present the current views, new findings and approaches, and highlight needs for further research in this area.

Impact of Oronasal Inflammation on Taste and Smell

Psychchophysical Effects of Nasal and Oral Inflammation

Antje Welge-Luessen

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Nasal inflammation in all its variations - viral, bacterial or allergic is the probably the most common cause of olfactory disorders in patients. Onset and underlying pathophysiologic mechanisms of the different forms of nasal inflammation differ, however these conditions can not only reduce olfactory function but can also induce permanent anosmia. In contrast to nasal inflammations oral inflammations causing taste disorders are less common even though they routinely develop in patients receiving radiotherapy. This talk will analyze underlying pathophysiologies in both nasal and oral inflammatory conditions, depict the different clinical appearances and discuss the impact on psychophysical smell and taste testing in patients. A more precise analysis and differentiation of the disorders might contribute to counselling and giving correct advice concerning the prognosis of the existing disorder.

Impact of Oronasal Inflammation on Taste and Smell

Analysis of the Olfactory Mucosa in Chronic Rhinosinusitis

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Millions of people suffer from chronic rhinosinusitis (CRS), and the impact of this disease on the olfactory mucosa (OM) is dramatic. Our goal is to comprehensively assess structural changes in the OM of CRS patients with varying severity, in order to better understand the processes underlying OM degradation and repair. Epithelial integrity in OM biopsies was evaluated using histological and immunohistochemical methods to establish cellular (i.e., neurons, supporting cells, basal cells and glands) and inflammatory profiles (i.e., macrophages, neutrophils, eosinophils, T-cells, B-cells and dendritic cells). We have examined over 50 CRS subjects, aged 18 – 63 yrs, and over 20 control subjects, aged 23 – 56 yrs, and have defined specific histopathological remodeling patterns and cellular profiles associated with varying degrees of inflammation. Morphometric analyses suggest three OM remodeling patterns in our CRS patients: goblet cell hyperplasia, damage/erosion, and squamous metaplasia. While OMP immunoreactive (ir) cells were present to varying degrees in all groups, fewer CK18-ir supporting cells but more infiltrating inflammatory cells were present in OE that exhibited damage/erosion or squamous metaplasia remodeling than in OE with goblet cell hyperplasia remodeling; the latter was more similar to OE from controls. Exploratory data mining methods are being used to analyze quantitative measurements to identify patients with similar OM profiles and subsequently to correlate these profiles with clinical characteristics and outcome. While it is not known if the OM remodeling patterns we have noted correspond to specific stages or distinct pathways of the disease, these analyses will provide direction for further mechanistic research in CRS.

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Impact of Oronasal Inflammation on Taste and Smell

Transgenic Mouse Model for the Study of Chronic Rhinosinusitis-Associated Olfactory Dysfunction

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Inflammatory sinonasal disease is a common cause of human olfactory loss. Although obstruction of airflow is often a contributing factor, inflammation also directly affects olfactory epithelium structure and function. Currently, the mechanisms underlying sinusitis-associated olfactory loss are poorly understood and treatment options are limited. One impediment to the study of sinusitis-associated olfactory loss has been the lack of an animal model. To address this need, we have developed a transgenic mouse model of inducible olfactory inflammation wherein there is temporally-controlled expression of tumor necrosis factor alpha (TNF) by sustentacular cells. Analysis of these mice reveals a progressive inflammatory infiltrate into the olfactory epithelium. After 4-5 weeks of inflammation, there is a marked loss of olfactory sensory neurons. Electroolfactogram responses are diminished by 50% at 2 wks of inflammation, preceding the loss of neurons from the epithelium, and nearly absent after 6wks of inflammation. After discontinuation of TNF expression, rapid resolution of inflammation and reconstitution of the epithelium occurs, with normal EOG responses within 2 wks. In sum, the inducible olfactory inflammation mouse demonstrates physiologic and histologic features of chronic sinusitis-associated olfactory dysfunction. Initial characterization suggests that TNF-induced inflammation leads to olfactory loss through early direct effects on sensory neurons, and later by destruction of normal olfactory epithelial architecture. Despite widespread damage after 6 wks of inflammation, there is complete recovery once the TNF expression is stopped. This model holds promise for improving current knowledge regarding inflammation-associated olfactory loss, and for developing novel treatment strategies.

Impact of Oronasal Inflammation on Taste and Smell

Inflammation and Taste Disorders: Mechanisms in Taste Buds

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Taste disorders, including taste distortion and taste loss, are frequently associated with inflammatory conditions, such as infections and autoimmune diseases. How inflammation affects taste sensation remains largely unknown. We recently demonstrated that taste bud cells express receptors for interferons (IFNs), a group of inflammatory cytokines that are highly induced during viral and bacterial infections and overproduced in autoimmune diseases. IFN-γ receptor IFNGR1 is preferentially expressed in type III and a subset of type II taste bud cells. Inflammatory stimuli such as lipopolysaccharide (LPS) and double-stranded RNA (dsRNA) polyinosinic:polycytidylic acid (poly(I:C)), mimicking bacterial or viral infection, activate the IFN pathways and up-regulate the expression of IFN-inducible genes in taste buds. Systemic administration of IFNs augments apoptosis of taste bud cells in mice. In addition, administration of LPS and poly(I:C) rapidly dampens the expression of *c-fos* in taste bud-containing epithelium, but not in nontaste lingual epithelium, indicating that inflammation may also affect taste signaling. Using quantitative real-time RT-PCR analysis and immunofluorescent staining, we found that taste bud cells express several Toll-like receptors (TLRs) including TLR3 and TLR4, the primary receptors for dsRNA and LPS, respectively. By recognizing pathogen-derived molecules, TLRs play essential roles in pathogen detection and activation of innate immune reactions. The preferential expression of TLRs in taste buds suggests that inflammatory stimuli can directly trigger inflammatory response in taste bud cells. Together, these findings suggest that inflammation may alter taste function through both TLR and inflammatory cytokine receptor signaling pathways.

Supported by NIH/NIDCD grants.

Impact of Oronasal Inflammation on Taste and Smell

Nasal Neurogenic Inflammation

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"Neurogenic inflammation" is the immediate mucosal or skin reaction to injury mediated by the release of neurotransmitters from Type C neurons into local tissue. Scratching the skin activates the highly branched nociceptive neurons to release calcitonin gene related peptide (CGRP) that causes vasodilation. In mucosal tissues, capsaicin, acid pH and other chemicals activate specific receptors on nociceptive nerves to elicit a wide array of visceral sensations and local mucosal responses. Unilateral hypertonic saline provocations were performed in human nasal mucosa to study these responses. Sensations of pain, nasal congestion and rhinorrhea ("drip") increased with tonicity. Doses sufficient to cause pain ratings of ~4 on a 10 cm scale caused unilateral reactions that did not recruit parasympathetic reflexes. The weight of recovered secretions, total protein, mucin, lysozyme, urea and substance P content increased in dose dependent fashion. However, there were no changes in albumin concentrations indicating that vascular permeability was not activated. Neurokinin 1 receptor mRNA was localized to submucosal glands by in situ RT-PCR using human nasal mucosal tissue sections. This indicated that the human axon response was limited to glandular and potentially epithelial exocytosis with no alteration in vascular permeability. Acute sinusitis and allergic rhinitis subjects had significantly elevated pain, fullness, and drip sensations, and greater protein secretion suggesting neurotrophic factors released during inflammation increased axon response effects. In contrast, Chronic Fatigue Syndrome subjects had heightened sensations without any dose response for glandular secretions. Dysfunctional axon responses may contribute to the syndrome of non-allergic rhinitis in Chronic Fatigue Syndrome.

Umami Symposium II: Post-Ingestional Effects of Umami: Visceral Detection of Glutamate

Glutamate Signaling Systems During Feeding and Digestion Processes

Kunio Torii

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Every animal needs to ingest sufficient quantities of nutrients for maintenance of homeostasis and normal growth as well as reproduction. Appetite and preference for foods containing various nutrients are changed depending upon physiological needs that are varied reflecting life style, aging, metabolic disorders etc. Whenever animals are faced with a particular nutrient deficit, their physiological state is deteriorated, and this systemic change is recognized by the brain to trigger a food-search behavior to alleviate the deficit. This food-related behavior may involve the senses of vision, smell, texture and an especially important cue, taste. In addition memory may be important due to similar nutritional deficits from previous experiences. Animals should have to inject either energy sources for activity or dietary protein for maintenance of body structure, by means of balance for consumption and dietary intake as required. The recognition of dietary protein intake by the brain is composed of cephalic and visceral nutritional information to notify into the brain. Glutamate residue is dominant in the dietary protein composition among 20 kinds of amino acid indicating that umami taste evoked by glutamate is major cue to dietary protein intake. The brain takes in nutritional information from the tongue to the alimentary organs to control appetite and metabolic regulation due to maintenance of homeostasis for 20 kinds of amino acids. So this symposium is conducted to discuss about physiological and nutritional aspects of umami materials, especially glutamate during and post-prandial state of meal, biochemically and physiologically.

Umami Symposium II: Post-Ingestional Effects of Umami: Visceral Detection of Glutamate

The Roles of T1r Taste Receptors and Gustducin in Enteroendocrine Cell Responses to Sugars, Fatty Acids and Amino Acids

Robert F. Margolskee, Bedrich Mosinger and Zaza Kokrashvili

Department of Neuroscience, Mount Sinai School of Medicine, New York, USA

Many taste signalling elements are expressed in enteroendocrine cells where they underlie multiple chemosensory functions of the gut. We have found that the L type enteroendocrine cells in human duodenum that express GLP-1 (glucagon like peptide-1) also express T1r taste receptors, gustducin, and many other taste transduction elements. In mice we have observed that gustducin is co-expressed in intestinal enteroendocrine L cells with GLP-1, GLP-2 and PYY (peptide YY). Knockout mice lacking T1r3, Trpm5 or gustducin are severely deficient in their gustatory responses to sugars, sweeteners, MSG and other umami stimuli. We have found that gustducin knockout mice also have post-ingestive deficiencies in enteroendocrine cell secretion of GLP-1 and GIP in response to sugars and fatty acids introduced directly into the gut by gavage or duodenal lavage. Gustducin knockout mice also have disrupted regulation of their plasma levels of insulin and glucose. We have recently begun to examine effects of MSG and amino acids on hormone release from enteroendocrine cells in wildtype and knockout mice. GLP-1 release from NCI-H716 cells, an L cell line that expresses gustducin

and taste receptors, was promoted by sugars, and sweeteners, and blocked by the sweet antagonist lactisole or siRNA for gustducin. Dietary sugar and sweeteners also appear to use taste receptors on enteroendocrine cells in a signaling pathway that leads to increased enterocyte expression of sodium-dependent glucose transporter-1 (SGLT1), the rate-limiting step for dietary carbohydrate absorption. T1r3 and gustducin null mice fail to upregulate SGLT1. Modulating hormone secretion from enteroendocrine gut 'taste cells' may provide novel treatments for obesity, diabetes and malabsorption.

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Umami Symposium II: Post-Ingestional Effects of Umami: Visceral Detection of Glutamate

Vagal Nerve Response to Amino Acids in the Gut

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The alimentary canal includes the mouth, stomach, and intestines, and is connected to the brain by 1000's of chemosensory neurons. In contrast to the understanding of the lingual taste system the chemosensory functions of other regions of the alimentary canal are mostly obscure. Sensory pathways for coding stimuli, such as sweet, bitter, umami, etc., might function in the gut-brain system to assess the quality and initiate the appropriate digestion of ingested nutrients. We directed assessed the flow of nutritional signaling to the brain by recording the electrical responses of gut chemosensory nerve fibers in the rat. Because chemosensory signaling represents complex coding patterns we analyzed the response profile of many individually recorded fibers simultaneously, an approach that is distinctly different from traditional nerve recording methodology. Currently, we are working to determine the sensory coding of amino acid signaling in the upper intestine and liver. We tested the effects of glutamate, histidine, isoleucine, leucine, lysine, valine, and saline control on vagal afferent activity. None of these amino acids had immediate effects on vagal signaling when infused into the hepatic portal vein. Intra-portal infusion of glutamate produced an excitatory effect on vagal activity that appeared as a slow increase beginning at 5 min after infusion. Glutamate infusion into the lumen of the duodenum also produced an increase in vagal activity from 1 to 5 minutes after infusion. Peptone, a hydrolyzed protein, did not produce an effect on intestinal vagal afferent activity. Overall, this research will provide insight into the role that ingested amino acids, acting on gut-brain signaling pathways, play in the control of nutrition, gut function, and feeding behavior.

Umami Symposium II: Post-Ingestional Effects of Umami: Visceral Detection of Glutamate

Effects of Free Dietary Glutamate on Gastric Secretion in Dogs

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Amino acid glutamate abundant in many foodstuffs is the most effective stimulator of the abdominal afferents from the vagus. However, the physiological role of dietary free glutamate in reflex and endocrine control of gastric secretion is still poorly understood. Experiments were performed with 6 male mongrel dogs with surgically prepared small gastric pouches according to Pavlov (innervated) or Heidenhain (vagally decentralized). In overnight fasted animals secretion in the gastric pouch was induced by infusion into the main stomach of liquid amino acid diet lacking free glutamate. Supplementation of 10-100 mmol/L free glutamate to the diet caused a powerful increase of the output of gastric acid, pepsinogen and fluid. This effect of glutamate on the gastric secretion was potentially attenuated by the denervation of the gastric vagus (Heidenhein model). With Heidenhein model, the pentagastrin-induced secretion was also enhanced by 100 mmol/L glutamate applied through a fistula into the main stomach. Application of glutamate solution alone up to 100 mmol/L or saline into the main stomach did not affect gastric secretions in any of present experimental models. We conclude that free glutamate at doses not exceeding its common concentrations within dietary foods substantially enhances gastric secretion induced by direct application of amino acids into the stomach. This effect of free glutamate on gastric secretion is achieved predominantly by potentiation of vago-vagal reflexes, presumably gastric glutamate-sensing by the vagus. We think that free glutamate fortification to daily meal or enteral liquid diet might improve the quality of life in the hospitalized patients with gastrointestinal troubles, especially in the elderly people with gastric dyspepsia.

Umami Symposium II: Post-Ingestional Effects of Umami: Visceral Detection of Glutamate

Brain Functional Changes in Rats Administrated with the Most Preferable Concentration of Monosodium L-glutamate in the Stomach

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Postingestive consequences (nutrition, satisfaction, food memory, etc.), as well as oro-nasal sensory stimuli (taste, smell, and texture), are the key factors for determining preference and appetite for foods and fluids. Recent studies have demonstrated expression of taste receptors and its transduction elements in the gastrointestinal (GI) epithelium, suggesting an existence of chemical sensing systems in the oral and GI tract. Especially, the gastric vagal afferents respond specifically to intragastric administration with monododium L-glutamate (MSG) among 20 amino acids through production of mucosal bioactive substances such as nitric oxide and serotonin. However, there has been little direct evidence concerning contribution of the brain on perception of food-derived chemosensory signals in the GI tract. We have demonstrated the spatio-temporal activation of forebrain regions, including the cortex, hypothalamus, basal ganglia, and limbic system following intragastric delivery of taste substances (MSG, glucose, and NaCl at 60 mM) by using functional magnetic resonance imaging (fMRI) in α-chloralose anesthetized, 12-15 h fasted rats. Some areas were commonly activated and some distinctly activated by these 3 taste substances. Subdiaphragmatic total vagotomy (TVX) substantially eliminated brain activation induced by MSG and NaCl but not by glucose. Blood glucose levels increased significantly at 20-40 min after administration with glucose. These results clearly suggest that post-oral taste substances can activate higher brain centers via neural (vagal) or humoral signaling pathway.

Umami Symposium II: Post-Ingestional Effects of Umami: Visceral Detection of Glutamate

Clinical Trial of Glutamate for the Improvement of Nutrition and Health in Elderly

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Glutamate (Glu) has been known to enhance palatability of food and thus appetite. Recently improvement in gastrointestinal functions by Glu has also

been found. Aged hospitalized persons often have poor appetite. Through our previous study we found that the meals of such persons contained only half the free-Glu of ordinary meals. At first we have done the pilot study to observe the effect of Glu supplementation on QOL in hospitalized aged persons (9 women and 2 men with mean ages of 88.3 and 74.5years, respectively). Meals supplemented with Glu (0.5% w/w) were given for 2 months. Since by the first study we could confirm the usefulness of MSG on the health of the elderly, we have done the second study (case-control study) with bigger number (15 each for the control and experimental group, respectively) and longer period (3 months). Food intakes were measured daily and energy and nutrient intakes were calculated. Anthropometric and blood biochemical parameters were measured before the beginning of the study and at 30 and 60 days. Behaviors were recorded by video as well as through observation by the staff and care-givers. Clear improvements were observed in cognitive score, eating behavior, emotional expression and verbal communication. In conclusion, for hospitalized aged persons, supplementation of MSG (0.5% w/w) for 2 to 3 months was effective in improving QOL.

Do Environmental Agents Enter the Brain Via the Olfactory Mucosa to Induce Neurodegenerative Diseases?

Introduction: Do Environmental Agents Enter the Brain Via the Olfactory Mucosa to Induce Neurodegenerative Diseases?

Richard L. Doty

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Environmental agents, including viruses, prions, metals, and neurotoxins, have been implicated in the etiology of some neurodegenerative diseases, most notably Alzheimer's (AD) and Parkinson's (PD). The presence of smell loss and the pathological involvement of the olfactory pathways in their formative stages, along with evidence that xenobiotics can readily enter the brain via the olfactory mucosa, have led to the notion that some such diseases are caused or catalyzed by agents that enter the brain via this route. Evidence for and against this 'olfactory vector hypothesis' is the topic of this symposium. Dr. Doty will present the history of this concept, including early studies showing that polio virus can enter the brain via the nose. Dr. Genter will describe xenobiotics capable of entering the brain via the olfactory receptor cells and will present data regarding ion transporters known to move divalent metals and other agents from the nasal cavity into the brain via the olfactory receptor cells. Dr. Prediger will discuss his recent animal research concerning the induction of symptoms of PD in rodents by introducing the pro-neurotoxin MTPT into their olfactory mucosa. Dr. Hawkes will review the neuropathological studies of Braak and associates which imply that PD first appears within the olfactory bulbs and the dorsal motor nucleus of the vagus, describing his 'dual hit hypothesis' of PD. Dr. Zanusso will describe the potential implications of his finding, published in a *New England Journal of Medicine* article in 2004, that the pathologic prion protein $P^{rP^{Sc}}$ is consistently found in the olfactory bulb and tracts of patients with Creutzfeldt-Jakob disease.

Do Environmental Agents Enter the Brain Via the Olfactory Mucosa to Induce Neurodegenerative Diseases?

Uptake of Materials from the Nasal Cavity and into the Blood and Brain: Are We Finally Understanding these Processes at the Molecular Level?

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It has been known for quite some time that materials that enter the nasal cavity gain access to the blood stream and the central nervous system. These materials range from viruses to pharmaceuticals to toxicants such as metals. The mechanism(s) by which this transfer occurs, however, has remained obscure. Recently, divalent metal transporter-1 (DMT1, also known as solute carrier SLC11A2) has been shown to be important in the uptake of manganese (Mn) from the nasal cavity into both the bloodstream and the brain in rats. Similarly, the organic anion transporter OAT6 (SLC22A20) is reported to transport both endogenous molecules and toxicants. Both DMT1 and OAT6 are localized to non-neuronal cell types in the olfactory epithelium. Our labs have shown that the metal transporters ZIP8 and ZIP14 (SLC39A8 and SLC39A14) immunolocalize to olfactory sensory cells and olfactory nerve bundles in the olfactory mucosa. ZIP8 and ZIP14 have been shown to transport a variety of transition metals, including Mn, iron, and cadmium. In addition, we have found abundant immunoreactivity with both ZIP8 and ZIP14 antibodies in the ciliated cells of the nasal respiratory epithelium. Our observations, together with those of other investigators, are beginning to shed light on the molecular mechanisms by which materials are transported into the bloodstream and brain from the nasal cavity.

Do Environmental Agents Enter the Brain Via the Olfactory Mucosa to Induce Neurodegenerative Diseases?

The Risk is in the Air: Intranasal Administration of MPTP to Rodents Reproducing Clinical Features of Parkinson's Disease

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Many studies have shown that deficits in olfactory and cognitive functions precede the classical motor symptoms seen in Parkinson's disease (PD) and that olfactory testing may contribute to the early diagnosis of this disorder. Although the primary cause of PD is still unknown, epidemiological studies have revealed that its incidence is increased in consequence of exposure to certain environmental toxins. In the present study, we demonstrated that rats treated with intranasal (i.n.) infusion of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) at low concentrations (0.1 mg/nostril) suffered progressive impairments in olfactory, cognitive and motor functions. Moreover, i.n. administration of MPTP reduced the expression of the enzyme tyrosine hydroxylase in the olfactory bulb and substantia nigra of rats, resulting in a significant reduction of dopamine concentration in the olfactory bulb, prefrontal cortex and striatum, but not in the hippocampus. These results reinforce the notion that the olfactory system represents a particularly sensitive route for the transport of neurotoxins into the central nervous system that may be related to the etiology of PD. In

addition, the time course of the olfactory, cognitive and motor impairments verified in rats treated intranasally with MPTP, which appears to be correlated with different stages of the human PD, suggest that the MPTP intranasal model in rats may provide new insights into the underlying mechanisms of PD pathogenesis.

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Do Environmental Agents Enter the Brain Via the Olfactory Mucosa to Induce Neurodegenerative Diseases?

Detection of the Pathological Prion Protein in the Olfactory Epithelium of Subjects with Sporadic Creutzfeldt-Jakob Disease

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The human transmissible spongiform encephalopathies (TSE), or prion diseases, include a number of sporadic, inherited and infectious neurodegenerative disorders characterized by a conformational modification of the host cellular prion protein (PrP^{C}) into an insoluble and protease-resistant isoform, termed PrP^{Sc}. Prion diseases are neuropathologically characterized by neuronal loss, spongiform degeneration, gliosis, and abnormal PrP^{Sc} deposition in central nerve cell processes and synaptic regions. Sporadic Creutzfeldt-Jakob disease (sCJD) accounts for about 85% of all human TSE. Recent studies from our group have demonstrated that the peripheral and central olfactory pathways are involved in the pathology of sporadic Creutzfeldt-Jakob disease (sCJD). Strikingly, deposition of PrP^{Sc} has been detected in post-mortem olfactory neuroepithelium, but not in contiguous respiratory mucosa in subjects with definite sCJD (Zanusso et al., NEJM 2003). In a subsequent study we performed olfactory biopsy in living patients with probable sCJD providing evidence that PrPSc deposition in the olfactory mucosa occurs relatively early during the disease course (Tabaton et al., Ann Neurol in press). However, it is still unclear whether the detection of PrP^{Sc} in the olfactory neuroepithelium and bulb might represent a primary site of PrP^{Sc} formation following somatic mutations in adult-born neurons or the result of the centrifugal spread of PrP^{Sc} from the brain. Here we present the most recent results obtained in sCJD patients.

Do Environmental Agents Enter the Brain Via the Olfactory Mucosa to Induce Neurodegenerative Diseases?

Parkinson's Disease: The Dual Hit Theory Revisited

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Accumulating evidence suggests that sporadic Parkinson's disease (sPD) has a long prodromal period during which several non-motor features develop, in particular, impairment of olfaction, vagal dysfunction, and sleep disorder. Early sites of Lewy pathology are the olfactory bulb and enteric plexuses of the foregut. We propose that a neurotropic pathogen, probably viral, enters the brain via two routes: a) nasal, with anterograde progression into the temporal lobe b) gastric, secondary to swallowing of nasal secretions in saliva. These secretions might contain a neurotropic pathogen that, after penetration of the epithelial lining, could enter axons of the Meissner's plexus and via transsynaptic transmission reach the preganglionic parasympathetic motor neurons of the vagus nerve. This would allow retrograde transport into the medulla and from here into the pons and midbrain until the substantia nigra is reached and typical aspects of disease commence. Evidence for this theory from the perspective of olfactory and autonomic dysfunction is reviewed and the possible routes of pathogenic invasion are considered. It is concluded that the most parsimonious explanation for the initial events of sPD is pathogenic access to the brain through the foregut and nose – hence the term 'dual-hit'.

Impact of Bitter Taste on Human Nutrition and Health

Impact of Bitter Taste on Human Nutrition and Health

Wolfgang Meyerhof

German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany

Many bitter substances are toxic although a direct relationship between bitterness and toxicity has not been established. Most bitter compounds are of plant origin with ~10% of the plant species contain toxic glycosides or alkaloids. Other bitter compounds contained in our food are being generated during food processing by heating or fermentation. Still others are produced during food aging. Thus, many bitter substances appear to be present in the daily diet of humans. Generally, bitter taste is innate and elicits aversive reactions preventing the ingestion of bitter and potentially toxic food. However, some bitterness is tolerated or even desired in certain food items and beverages. Moreover, our tolerance towards bitterness increases from childhood to seniority. Thus, we may hypothesize that bitterness influences the choice of food and subsequently, diets, nutritional status and eventually health. So far, no clear link between bitter taste perception and food selection has been established. Yet, the recent cloning and analyses of T2R/TAS2R bitter taste receptor genes enabled researchers to approach this problem now. The symposium combines biochemistry, genetics, food chemistry, psychophysics, and nutritional science to explore intake behavior. New findings based on the identification and functional characterization of bitter taste receptors and their variants, of the polymorphic nature of TAS2R genes and the distribution TAS2R alleles in the human populations, as well as their phylogenetic analyses will be presented. Moreover, advanced human psychophysical studies, innovative methods for detecting the taste-giving bitter substances in food and beverages, and novel studies of intake behavior in relation to tasting abilities advanced our knowledge of the impact of taste on nutrition.

Impact of Bitter Taste on Human Nutrition and Health

Functionally Distinct TAS2R Bitter Taste Receptor Variants

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The perception of bitter compounds in mammals is mediated by about 30 bitter taste receptors (TAS2Rs). The fact that thousands of structurally diverse bitter compounds are detected by a relative low number of TAS2R genes has raised the question of how broadly tuned bitter taste receptors are. Utilizing functional assays we and others have deorphaned a considerable number of mostly human TAS2Rs. From those experiments one can conclude that the average TAS2R recognizes multiple bitter compounds and that, in turn, single bitter substances activate more than one TAS2R. Mutagenesis studies on selected TAS2Rs are currently performed in our

laboratory to investigate how the observed broad tuning is achieved while maintaining the specificity necessary for selective interactions with chemical found in the environment. Additionally, the successful deorphanization of so many bitter taste receptor genes has created the opportunity to screen the highly polymorphic TAS2R gene family for functional differences arising from non-synonymous single-nucleotide polymorphisms. Although such studies are still in their early phase, the discovery of several functional polymorphisms in hTAS2R genes indicates that bitter taste might be highly individualized among humans. Consequently, the bitter taste receptor repertoire of an individual might profoundly influence food selection and ultimately health. Variability of bitter taste receptor genes is not only restricted to polymorphisms in their coding regions, also the cellular expression pattern of individual TAS2R genes is not uniform thereby creating an inhomogeneous population of bitter taste receptor cells. This may allow, at least on a cellular level, the discrimination between bitter substances.

Impact of Bitter Taste on Human Nutrition and Health

Genetic Dissection of Human Taste Perception

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Inherited variation plays a major role in perception of bitter and other tastants in humans. The paradigm for this variation has been large differences in the perceived bitterness of thiol-containing compounds exemplified by phenylthiocarbamide and propylthiouracil (PTC and PROP). These compounds are sensed through the bitter receptor encoded by the TAS2R38 gene, which exists in two major forms, designated the major taster and major non-taster alleles, in populations worldwide. Other bitter substances have also been shown to produce dichotomous responses meditated by inherited variants of bitter receptors, such as aloin/aristolochic acid and the TAS2R43 receptor. We have extended these genetic methods to other taste modalities, and recently applied them to variation in sweet taste perception. A naturally occurring form of the TAS1R2 gene, differing from the most common form at 3 amino acid positions, confers a reduced sensitivity to sweeteners in *in vitro* assays. Genetic association studies with candidate genes reveal additional genetic contributions to variation in sweet perception, and suggest that genetic variation in non-protein coding regions make major contributions to this phenotypic variation in the population.

Impact of Bitter Taste on Human Nutrition and Health

The Genetics of Bitter Taste and its Impact on Nutrient Evaluation

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Our individualized perceptions of food vary as a function of several genetic factors such as the set of alleles we possess that code for our taste receptors and their transduction components. The structure that any of our taste receptors take (based on our alleles) impacts their responses to sets of ligands. Presumably the stimuli that served as a pressure for selection of taste receptor alleles came from foods and potential ingesta. Accordingly, we established a direct link among the compounds that stimulate a specific bitter receptor variant, the foods that contain these taste compounds, and the variation in the perception of these foods among those who express these receptor forms. Specifically, we examined the glucosinolate thyroid toxins in cruciferous vegetables and the bitterness perception of them among people who vary in their TAS2R38 bitter taste receptor genotype. We found that subjects who possess less responsive forms of TAS2R38 perceive less bitterness from specifically those vegetables that contain glucosinolates compared to subjects with more responsive forms of this gene. These two sets of subjects do not differ in their perception of other families of bitter vegetables. Whether differences in bitter taste perception impact the variety and amounts of food ingested is yet to be determined. Reflexive responses to these tastes may be overridden by learning from life experiences and food familiarity. There is some evidence that preschoolers who perceive less bitterness from propylthiouracil will eat more vegetables in an experimental setting than do the subset of children who perceive more bitterness from it. Whether adults will similarly follow basic sensory reflexes in the face of conflicting social influences will be determined by future studies.

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Impact of Bitter Taste on Human Nutrition and Health

Oral Astringency and Bitter Taste of Food – Discovery of Chemical Stimuli, Sensory Activity, and Beyond

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Consumer studies showed that bitter or astringent tasting foods often tend to be rejected, but in certain foods and beverages such as coffee some bitterness is tolerated or even expected. Although it is believed that the food taste stimuli are known, recent studies showed that many of the key players still remain orphan. Using the sensory activity as the guide for determining the structure of a compound, natural product chemistry combined with analytical human psychophysics enabled the discovery of various previously unknown key food tastants as shown by three examples: (i) In contradiction to the literature, the bitter taste of coffee was found to be not caused by caffeine, but by novel *O*-caffeoyl-quinides and phenylindanes generated upon bean roasting. (ii) The stimuli inducing the bitter taste of a matured cheese were mapped and identified as peptides with previously not reported sequences, amongst which the peptide YPFPGPIHNS exhibited the lowest thresholds of 0.05 mmol/L. (iii) In contradiction to the literature, not flavan-3-ols, but a series of novel *N*-phenylpropenoyl-L-amino acids (PPAAs) were identified as the key contributors to the astringency of cocoa. As these tastants might exhibit additional physiological activities after having activated our gustatory sensing systems, we investigated potential activities in the stomach. For the first time, coffee bitter compounds were found to stimulate gastric acid secretion in stomach cells and the astringent PPAAs from cocoa were identified as inhibitors of *H. pylori* adhesion on human stomach epithelium, thus demonstrating the multifunctionality of some taste actives.

Impact of Bitter Taste on Human Nutrition and Health

Genetic Variation in Taste Sensitivity to Prop and its Relationship to Taste Perception, Food Preferences and Diet Choice - Connecting the Dots

Beverly J. Tepper

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The ability to taste bitter thiourea compounds and related chemicals is a well-known human trait. The majority of individuals perceive these compounds, typified by the bitterness of 6-n-propylthiouracil (PROP) and phenylthiocarbamide (PTC), as moderately-to-extremely bitter. Approximately 30% of the population is taste blind to these substances. It has been hypothesized that PROP/PTC tasters are more sensitive to other bitter tastes, sweetness, spiciness of chili peppers, astringency of alcohol and the texture of fats.

Tasters may also show lower preferences for foods with these taste qualities than non-tasters who show the opposite set of responses (lower taste sensitivities and higher preferences for these sensory qualities). This pathway is illustrated in the following model: PROP Sensitivity → Food Perception → Preference → Selection Robust associations between PROP status and taste perceptions have been well documented. However, subsequent links to food preferences and diet selection have been more difficult to demonstrate. This is not surprising given the complexity of human eating behavior that is influenced by numerous factors including other genetic predispositions, food attitudes, personality traits and environmental variables. A variety of experimental approaches have provided insights into these relationships including short-term feeding studies, multivariate modeling, and cross-cultural studies, to name a few. Our laboratory has been using PROP screening to investigate individual differences in the selection of bitter foods, especially bitter tasting vegetables that may have long-term implications for diet and health. This presentation will review emerging findings in this field and explore some novel approaches to address this issue.

Olfaction in Birds: A Dedication to the Pioneering Spirit of Professor Bernice Wenzel

Olfaction in Birds: A Dedication to the Pioneering Spirit of Professor Bernice Wenzel and Betsy Bang

Gabrielle A. Nevitt[1] and Julie C. Hagelin[2]

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The notion that birds are anosmic is a deeply rooted opinion among scientists and laymen alike. However, growing evidence suggests that the sense of smell is of fundamental importance among birds. Interest in avian olfaction has emerged from studies conducted in unrelated laboratories, and on a variety of species, from domesticated chickens and homing pigeons to seabirds such as auklets, petrels and albatrosses. New research efforts have done much to show that olfaction is critical to a variety of behaviors, and new and exciting insights into the use of olfaction in social behavior and individual recognition have recently been proposed. In the early 1960s and 1970s, two pioneering women paved the way for current studies on avian olfaction. Betsy Bang was the first to produce a comprehensive anatomical atlas describing olfactory structures in birds, while Bernice Wenzel was one of the first researchers to document physiologically that birds possessed a functional sense of smell and that olfaction was used in a variety of behaviors. The purpose of this symposium is to honor these two ''Pioneers of Avian Olfaction'' by presenting a series of presentations on new research exploring the sensory biology and ecology of olfaction in birds. The work highlighted draws from a wide phylogenetic base, and explores this fascinating sense from several different disciplines, including neurobiology, foraging ecology, and social behavior.

Olfaction in Birds: A Dedication to the Pioneering Spirit of Professor Bernice Wenzel

Avian Chemoreception: An Electrophysiological Approach

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Studies in this laboratory have provided the first detailed physiological evidence for olfactory and trigeminal chemoreception in an avian species. Investigations in the chicken (*Gallus domesticus*) indicate that the activity of avian olfactory bulb neurones closely resembles that of other vertebrates, exhibiting variable spontaneous temporal firing patterns with mean firing rates between those reported for mammals and reptiles. Application of odours directly to the olfactory epithelium showed that like mammals, avian olfactory bulb neurones respond in the form of inhibition and excitation with accompanying changes in temporal firing pattern. When exposed to a range of concentrations of a single odour, responsive neurones exhibited an ability to discriminate small step-changes in concentration producing clear stimulus response relationships. Although it is well recognized that trigeminal innervation of the nasal, oral and ocular epithelium has a chemoreceptive role, few studies have described the characteristics of individual trigeminal receptors responding to noxious chemical stimulation. Avian trigeminal chemoreception was investigated by examining the responses of single mucosal units. Slowly and rapidly adapting nasal mechanoreceptors were identified, some of which exhibited chemical sensitivity when exposed to irritant gases. These results demonstrate that polymodal nociceptors are present in avian nasal mucosa and represent the first attempt to quantify the responses of single trigeminal receptors to a range of concentrations of noxious airborne chemicals. These findings demonstrate how an electrophysiological approach can improve our understanding of the sensory physiology underlying avian chemoreception.

Olfaction in Birds: A Dedication to the Pioneering Spirit of Professor Bernice Wenzel

Olfactory Navigation in Homing Pigeons: The Last Challenge

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One of the most debated issues in animal navigation concerns the nature of stimuli used by homing pigeons to determine their position with respect to their home loft after displacement to unfamiliar locations. Over thirty years ago Floriano Papi and his collaborators released a group of anosmic pigeons and observed that they were unable to orient and find their way home. This striking result led them to propose the olfactory navigation hypothesis, according to which pigeons are able to associate the odours carried by the winds with the direction from which they blow at the home area; once displaced they recognise the prevalent odours and determine the direction of displacement. The last challenge to the olfactory navigation hypothesis came from the discovery of putative magnetoreceptors innervated by the ophthalmic branch of the trigeminal nerve and located in the upper beak. These findings raised the question whether the navigational impairment observed after manipulations of the olfactory system was due to possible accidental damage to the trigeminally innervated magnetoreceptors. We compared the navigational performance of pigeons subjected to section of the ophthalmic branch of the trigeminal nerve with that of birds with section of the olfactory nerve, either inexperienced or subjected to training flights after the surgery. Our results suggested that trigeminally mediated magnetic information is neither sufficient nor necessary for pigeon navigation and is not involved in the development of the navigational map. By contrast, inexperienced pigeons subjected to olfactory nerve section showed impaired navigational abilities. Moreover, when the surgery was performed in young pigeons the development of the navigational map was compromised even after an intensive flight training program.

Olfaction in Birds: A Dedication to the Pioneering Spirit of Professor Bernice Wenzel

Olfaction in Petrels: From Homing to Self-Odour Avoidance

Francesco Bonadonna

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In the sixties, Betsy Bang for the first time showed the complexity of the olfactory apparatus of procellariiform birds (petrels and albatrosses) that

suggested an important role of olfaction in their ecology. Shortly after, another brilliant researcher Pr. B. Wenzel made petrels known for their well developed olfactory neuroanatomy as well as for their sensitivity to food-related scents. Later, it was also shown that olfaction is critical for ''homing to the nest'' processes. We have expanded on these findings and demonstrated in several petrel species the role of a nest burrow olfactory signature in homing. The nature of this olfactory signature depends on the mate's odour. In fact, Antarctic prions (*Pachyptila desolata*) and blue petrels (*Halobaena caerulea*) in our Y-maze experiments demonstrated that individuals discriminate between own and mate odours. However, while they are attracted by the mate odour, they prefer the odour of a conspecific bird to their own. While traditionally examined from the perspective of homing mechanisms, these results have drawn attention to the possible use of chemical signals in birds' social behaviours such as individual recognition and/or mate choice. Indeed, petrel life-history traits suggest that an olfactory mediated mate choice may have evolved in this group to ensure genetic compatibility between mates. We have recently shown that a single bird's semio-chemical profile is more similar to itself, year after year, than to another bird. As a result, a novel function of olfaction emerges in petrels: the perception of a chemical signal that may broadcast individual's identity as well as quality leading to an optimal mate choice.

Olfaction in Birds: A Dedication to the Pioneering Spirit of Professor Bernice Wenzel

Exploring the Mechanisms of Individual Odor Recognition in Burrow-Nesting Procellariiforms: A Potential Role for the MHC

Terence W. O'Dwyer

Adult procellariiforms use olfaction for foraging, homing and mate recognition. Species within this order also form life-long pairs, lay a single egg per breeding attempt, and nest on remote islands. Burrow-nesting species rear their chick in dark, underground burrows which adults are able to relocate using scent cues. While it is recognized that the chicks of burrow-nesting procellariiforms are sensitive to prey-related odors before leaving their nest, the development of individual odor recognition is less well understood. This talk will review ongoing research investigating these questions in two species of burrow-nesting procellariiform, the Leach's storm petrel, *Oceanodroma leucorhoa,* and the Gould's petrel, *Pterodroma leucoptera*. We recently demonstrated that Leach's storm-petrel chicks can recognize personal odors. In two choice tests, 4-6 week old chicks consistently chose their own nest material over nest material of a conspecific, They also preferred their own nest material to similar organic material collected from the petrel colony. These chicks do not leave their burrow prior to fledging; thus, they do not need to recognize nest-specific odors for homing. However, because nest material is scented by the chick and its parents, we reasoned that, chicks may be learning to recognize kin-related odors which may later play a role in the context of mate choice. The source of individual-specific odors in petrels is not clear but in other vertebrates, such as mice, lizards and humans, personal odors are associated with the major-histocompatibility complex (Mhc). We are now focusing research on whether this diverse multi-gene region is also involved in individual odor recognition and mate choice in petrels using both the Leach's storm-petrel and the Gould's petrel as model systems.

Sniffing Underwater: Olfactory Capabilities of Aquatic Mammals

Underwater Sniffing in Semi-Aquatic Mammals

Kenneth C. Catania

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It has been widely held that mammals cannot use olfaction underwater because it is impossible to inspire air and hence transport odorants to the olfactory epithelium. This conclusion has been used to explain the reduction or absence of olfactory systems in some semi-aquatic and fully aquatic mammals. I will present findings of a novel mechanism for underwater olfaction in semi-aquatic star-nosed moles (*Condyluracristata*) and water shrews (*Sorexpalustris*). While foraging underwater, each species exhaled air bubbles onto objects of interest and then re-inspired the bubbles. Underwater sniffing during aquatic foraging occurred at a rate similar to that observed in terrestrial small-mammals (8-10hz). Both species were able to follow scent trails (fish or earthworm) underwater. One species of terrestrial shrew (*B. brevicauda*) was trained to retrieve food from underwater, but did not exhibit underwater sniffing. The discovery of this behavior in two semi-aquatic species suggests it may be common to mammals that forage underwater. In addition to describing these results, I will discuss the more general sensory specializations and nervous systems of each species.

Chemical Senses and Other Aging Sensory and Motor Systems

A Systems Approach to Studying the Chemosenses and Aging: Moving from Populations to Mechanisms

Wen G. Chen

National Institute on Aging, Bethesda, USA

Following the initial report on the associations between the decline in odor detection and cognitive impairment or dementia, increasing evidence is accumulating to support links between certain aspects of sensory/motor dysfunction and cognitive impairment or dementia. For instance, visual spatial memory changes have been reported in many Alzheimer's disease (AD) patients. In addition, recent data suggest that changes in the motor system including reduced strength and walking speed can antedate the onset of AD by years, raising the possibility that age-related mobility changes may be associated with the development of AD. Although the roles of the auditory, somatosensory, and taste systems in neurodegenerative diseases have yet to be established, some emerging evidence has raised interesting possibilities that changes in certain neural processes such as neurotrophin regulation or inflammation may serve as a common mechanism underlying age-related sensory changes and neurodegenerative diseases. This presentation will lead off the symposium on Chemical Senses and Other Aging Systems with an overview that focuses on the recent advances and new challenges in studying age-related sensory and motor changes. The goal of this symposium is to stimulate research interests in areas cross-cutting multiple systems and disciplines and to encourage discussions and explorations into common mechanisms that underlie age-related changes in chemical senses and other age-related sensory and motor systems that are relevant to neurodegenerative processes including dementia. We hope that these efforts will help us to develop new tools and strategies to prevent or intervene with the progression of these devastating aging problems at a much earlier stage.

Chemical Senses and Other Aging Sensory and Motor Systems

Olfactory Dysfunction in Presymptomatic Alzheimer's Disease

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Alzheimer's disease (AD) impairs olfaction, but it is uncertain how early this occurs in the disease process and whether the effect can be accounted for by

other behavioral or genetic markers of the disease. We administered the Brief Smell Identification Test (BSIT) to 471 older people without dementia or cognitive impairment who then completed annual clinical evaluations and brain autopsy at death. BSIT score was associated with more rapid decline in episodic memory (estimate = 0.014, SE = 0.004, p <0.001, from mixed-effects model) and with increased risk of developing incident mild cognitive impairment (MCI) [hazard ratio = 0.874, 95% CI: 0.812, 0.941, from proportional hazards model], even after controlling for age, sex, education, baseline level of episodic memory, and possession of an apolipoprotein E ε4 allele. In 34 people who died without evidence of cognitive impairment, lower BSIT score was associated with higher level of AD pathology (composite measure of plaques and tangles from 5 brain regions), even after controlling for level of episodic memory function when olfaction was assessed and the ε4 allele (and for age at death, sex, education, and time from olfactory testing to death; estimate = -0.061, SE = 0.027, p = 0.034, from linear regression model). These analyses suggest that among older people without any clinical manifestations of AD (or MCI), olfactory dysfunction is related to both the level of AD pathology in the brain and the risk of subsequently developing prodromal symptoms of AD (i.e., episodic memory decline, MCI) and that these associations persist after accounting for the effects of other recognized behavioral and genetic markers of the disease.

Chemical Senses and Other Aging Sensory and Motor Systems

Effects of Aging on the Human Taste System

Susan S. Schiffman

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Losses in taste perception (e.g. increased thresholds) as well as distortions of gustatory function (i.e. dysgeusia) occur with greater frequency in older individuals, and these changes are exacerbated by certain medical conditions, pharmacologic and surgical interventions, radiation, and exposure to toxic chemicals. Taste disorders have been reported by patients with a broad range of medical conditions including cancer as well as diseases of the nervous, endocrine, and respiratory systems. Medications are very significant factor in taste disorders in the elderly (several studies suggest that up to 33% of elderly individuals experience medication-related alterations in taste), and the contribution of drugs to taste loss is just now beginning to be understood. Older persons with taste disorders often have higher plasma concentrations of a parent drug and lower urinary concentrations of its metabolites than expected of young persons with normal metabolism; this pattern can be due to aging itself, inherited factors, and drug-drug (as well as food-drug) interactions. Drug metabolism occurs through specialized enzymatic systems that convert drugs, the majority of which are lipophilic, into polar (hydrophilic) metabolites that are more water soluble than the parent drug and thus more readily excreted. Cytochrome P450 enzymes, a superfamily of microsomal enzymes, are involved in the metabolism of the majority of prescription drugs (especially family members CYP3A4 and CYP2D6). When drugs that are substrates of CYP3A4 or CYP2D6 (i.e. are metabolized by them) are co-administered with inhibitors of CYP3A4 or CYP2D6, these drug-drug interactions exaggerate pharmacological effects as well as increase the incidence of taste disorders (presumably due to prolonged exposure to higher plasma concentrations).

Chemical Senses and Other Aging Sensory and Motor Systems

A Rodent Model of Age-Related Odor Memory Impairment

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Research in humans suggests that memory for olfactory stimuli may be particularly affected by age-related brain changes. Other studies suggest that odor memory problems may be an early indication of cognitive impairment and Alzheimer's disease. Studies involving aged rats have offered insight into how age-related brain changes may result in impaired cognition. However, very little research has examined odor memory in aged rats. Therefore, it is unclear whether aged rats are a good model for understanding how age-related brain changes might result in odor memory impairments in older humans. In a series of studies, young (6 month old) and old (24 month old) rats were tested on a variety of tasks to measure olfactory learning and memory. The first task examined age-related differences in discrimination and reversal learning for olfactory and visual stimuli. The second task examined the ability of young and old rats to learn an associative contextual learning task involving olfactory and visual cues. The third task examined age-related changes in conditioned flavor preference. The results demonstrate that old rats are able to perform olfactory discrimination tasks as well as young rats. However, old rats show significant age-related impairment on reversal learning, contextual learning, and conditioned preference tasks involving olfactory stimuli. The results suggest that aging may have a similar deleterious effect on odor memory in rats and in humans. The findings may have important implications for the selection of memory paradigms for future research studies on aging. In addition, the use of an animal model to investigate the effects of aging on odor memory will allow researchers the ability investigate how age-related neuroanatomical and neurochemical changes may result in impaired odor memory.

Chemical Senses and Other Aging Sensory and Motor Systems

Visual Motion Processing for Spatial Orientation: Neuronal Mechanisms and Behavior from Monkeys to Man

Charles J. Duffy

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Spatial orientation relies on the integration of multi-sensory cues about self-movement through the environment. Neurons in monkey extrastriate cortical area MST respond to visual and vestibular self-movement cues. We have found that their responses provide a reliable estimate of the direction of self-movement under a variety of environmental and behavioral conditions. In addition, these cells integrate self-movement signals over time to create selective responses to particular paths of self-movement and places in the self-movement environment. Human observers use visual cues about self-movement to update knowledge of their location in the environment, a sense that is greatly impaired in the spatial disorientation of Alzheimer's disease (AD). We have found that AD patients show specific impairments in processing visual cues about self-movement. Psychophysical and neurophysiological measures of these impairments appear to be closely related to their wayfinding deficits. Together, these studies link visual processing with spatial orientation and the capacity to generate and utilize an internal

representation of the environment. This line of research illustrates the utility of a systematic analysis of cortical function as it relates to behavioral impairments in neurological disease.

Chemical Senses and Other Aging Sensory and Motor Systems

Behavioral and Cellular Level Changes in the Aging Somatosensory System

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Impairment of somatosensory function manifested by increased thresholds for tactile, thermal and noxious stimuli is commonly associated with aging. Expression analysis of sensory neurons and nerves of aged mice showed reduced levels of the voltage-dependent sodium channel Nav1.8 and the capsaicin and heat receptor TRPV1. Analysis of growth factors known to modulate channel expression showed NGF and artemin mRNA increased in DRG of aged mice, whereas the artemin receptor GFRa3 decreased. Nearly all GFRa3-positive neurons express TRPV1, a channel required for transmission of thermal hyperalgesia associated with tissue inflammation. Given the decrease in GFRa3 and TRPV1 in aged neurons, we tested thermal sensitivity associated with inflammation in aged mice. Young mice injected with the inflammatory agent complete Freund's adjuvant (CFA), showed transient thermal sensitivity whereas aged mice did not. CFA injection also increased artemin expression in skin of both young and old mice but decreased expression of GFRa3 more so in DRG of aged animals, suggesting artemin signaling was diminished in aging ganglia. The lack of thermal sensitivity to CFA challenge and greater decrease in GFRa3 expression suggests the response properties of neurons that express TRPV1 and GFRa3 are diminished with age. To test this, calcium imaging of isolated primary neurons was used to test the *in vitro* effects of artemin on TRPV1 activation. Artemin potentiated TRPV1 activity in young and old neurons but recovery after activation was faster in young neurons. These findings suggest reduced thermal sensitivity in aged mice is related to decreases in TRPV1 and GFRa3 expression in primary afferents.

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Chemical Senses and Other Aging Sensory and Motor Systems

Possible Neural Bases of Age-Related Hearing Loss: Ear and Brain Mechanisms

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Hearing loss results from damage to the ear (peripheral auditory system) or the brain (central auditory system). Here, some basic structures and functions of the ear and central auditory nervous system will be highlighted as relevant to effects of aging on complex sound processing and auditory perception. The auditory system is altered in two basic ways in cases of age-related hearing loss (presbycusis): 1) Damage to the inner ear, or cochlea, can disrupt the sensory transduction mechanism or the number and nature of input channels that the brainstem auditory system receives from the ear, causing plastic changes in the central auditory system. 2) In some scenarios, age-related damage to the brain can occur somewhat independent of the ear. Implications of age-related deficits of the auditory system for complex sound perception and in relation to age-linked medical conditions will be provided, including implications for age changes in speech processing and language comprehension. Where appropriate and relevant, similarities and differences between age changes in the end-organ and brain pathways of the olfactory and gustatory systems will be compared to those that take place in the auditory system.

Work supported by NIH Grant P01 AG09524 from the National Institute on Aging.

Interspecies Differences in Pontine Taste Processing: Implications for Tasting and Feeding

Interspecies Differences in Pontine Taste Processing: Implications for Tasting and Feeding

Dana M. Small[1,2] and Thomas R. Scott[3]

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In rodents the pons is an important gustatory relay [1] upon which much centrifugal modulation occurs to guide complex feeding behaviors [2]. From the pons two separate projections arise, one which synapses in the parvicellular medial tip of the ventroposteromedial nucleus of the thalamus, which in turn sends axons to taste cortex. This pathway is thought to be largely sensory in nature. A second pathway projects widely to the ventral forebrain areas including hypothalamus, amygdala and is thought to be largely affective in nature. Recent support for this dissociation comes from Hajnal and Norgren who found that lesions of the pons but not the thalamus disrupted dopamine overflow in the accumbens during sucrose licking [3]. Remarkably, the pontine taste relay does not appear to exist in human [4] and nonhuman [5] primates, or if it does exist, its role is much reduced. The aim of this symposium is to outline the role of the pons in sensory and affective processing of taste in rodents and to speculate upon the implications of interspecies differences for tasting and feeding in primates. The specific aim of the introductory comments will be to provide an overview of evidence for these interspecies differences to set the stage for the remaining presentations. [1] AC Spector, (1995); T Yamamoto, T Shimura, N Sakai et al., (1994); PM Di Lorenzo, S Monroe, (1995); PM Di Lorenzo, S Monroe, (1997). [2] RF Lundy, R Norgren, (2001); R F Lundy, R Norgren, (2004). [3] A Hajnal, R Norgren, (2005). [4] JC Topolovec, JS Gati, RS Menon et al., (2004). [5] RM Beckstead, JR Morse, R Norgren, (1980); R. Norgren,(1990), TC Pritchard, R. B. Hamilton, R Norgren, (2000).

Interspecies Differences In Pontine Taste Processing: Implications for Tasting and Feeding

Functional Organization of the Rodent Parabrachial Nucleus

Takashi Yamamoto

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Previous studies in non-primates suggest that the parabrachial nucleus (PBN) is not merely a relay station but also plays an important role in integrating various inputs together with plastic changes of neuronal responses after learning and experience. To further explore the functional features of different subnuclei of the PBN during ingestive behavior, we employed different techniques including electrophysiological unit recording, immunohistochemistry for FOS and phosphorylation of extracellular signal-regulated kinases (pERK) and gene expression analysis using the DNA microarray technologies in rats and mice under different experimental paradigms. FOS and/or pERK neurons were detected in the external lateral subnucleus (els), external medial subnucleus, dorsal lateral subnucleus (dls), central lateral subnucleus, and the central medial subnucleus (cms) depending on the type of taste and visceral stimulation. The expression patterns were different under nutritionally sufficient and deficient conditions, perceptually novel

and familiar conditions and learned and unlearned conditions. As for the possible functions, the rostral part of the els is correlated with general visceral inputs; the caudal part of the els, aversive behavior; the dls, ingestive behavior; the cms, the taste of NaCl. Several genes have been found expressed diffusely in the PBN, others were localized in specific subnuclei. Future directions include correlating the gene expressions with possible functional significance. The limbic and reward systems receive ingestion-related information via the cortical areas in primates, while in rodents the information is sent to these systems mostly via the PBN. The PBN of non-primates is the integrated center with its functions partly corresponding to those of the primate cortex.

Interspecies Differences in Pontine Taste Processing: Implications for Tasting and Feeding

Information Processing in the Parabrachial Nucleus of the Pons: Temporal Relationships of Input and Output

Patricia M. Di Lorenzo

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In rodents the parabrachial nucleus of the pons (PbN) receives information about taste directly from the nucleus of the solitary tract (NTS, the first central gustatory relay). From the PbN, gustatory-related output diverges along two pathways that are functionally distinct. What transformations of incoming information occur in the PbN and how the output is parsed remain unanswered but important questions. Data from simultaneous recordings from NTS and PbN cells suggest that PbN cells receive input both from NTS cells that share the same best stimulus as well as those that do not; however input from an NTS cell with the same best stimulus is more effective in driving the target PbN cell. Consistent with these data are results of cross-adaptation studies suggesting that PbN cells are potentially responsive to all tastants. In general, the time course of the population response in the PbN follows that of the NTS for the first 3 sec of response but is independent of NTS input thereafter. Further, this "coupling" appears to be cyclic across the response interval and differs in the length of the period according to the stimulus. Across neuron patterns (ANPs) of response also show a different time course inthe PbN compared with the NTS: In the NTS ANPs become more similar over the course of the stimulus presentation while in the PbN these patterns remain well differentiated. Finally, like the NTS, taste-responsive cells in the PbN utilize spike timing to conveyinformation about the identity of taste stimuli. Collectively, these data show that the PbN is tightly driven by NTS input early in the response, but is relatively independent of NTS activity thereafter. Processing in the later parts of the response may reflect fine tuning of the signal and/or reciprocal communication with other structures.

Interspecies Differences in Pontine Taste Processing: Implications for Tasting and Feeding

Parabrachial Coding of Sapid Sucrose: Relevance to Reward and Obesity

Andras Hajnal

Departments of Neural & Behavioral Sciences, and Surgery, PennState University, College of Medicine, Hershey, USA

Cumulative evidence in rats suggests that the pontine parabrachial nucleus (PBN) is necessary for assigning hedonic value to taste stimuli. This process is amendable by experience and physiological state, and is involved in palatability-driven overconsumption. In a series of behavioral, neurochemical and electrophysiological studies, our laboratory has investigated the parabrachial coding of sapid sucrose in normal and obese rats. First, using chronic microdialysis, we demonstrated that sucrose intake causes increased dopamine release in the nucleus accumbens, an effect that is dependent on oral stimulation and concentration. We subsequently determined that this response was independent of the thalamocortical gustatory system, but was substantially blunted by lesions to the PBN. Recent acute and chronic extracellular recording studies demonstrated that processing of sucrose-evoked activity in the PBN is altered in rats that develop obesity due to chronic overeating. Specifically, compared to lean controls, we found an overall reduced response to sucrose, and a rightward shift in sucrose concentration-response functions in the obese rats. Our current experiments have revealed that Roux-en-Y gastric bypass not only reduces exaggerated behavioral (preference and lick rate) responses by obese rats to sapid sucrose, but also reverses altered neuronal taste coding in the PBN. Collectively, these observations support the notion that the PBN plays a central role in the sensory-motivation integration of food intake by enabling taste stimuli to engage the reward system and that this function is critical to dietary obesity and weight control.

This research is supported by NIH DK065709, DC00240, and PA-TSF grants.

Interspecies Differences in Pontine Taste Processing: Implications for Tasting and Feeding

Sensory and Homeostatic Functions of the Rodent Parabrachial Nucleus

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In rodents, axonal fibers originating in the gustatory part of the nucleus of the solitary tract (NST) ascend to the parabrachial nucleus (PBN), establishing this pontine structure as part of the central taste pathways. The PBN is also known to express receptors for several peripheral factors known to influence feeding and metabolism, including the adipose tissue-derived hormone leptin. We will review current evidence indicating that pontine processing can influence food intake via the activation of such receptors, with special emphasis on their relative anatomical position within the PBN. It is presently unknown, however, whether these peripheral factors exert control over food intake by directly acting on neurons of the gustatory aspect of PBN or, alternatively, via taste-independent pathways. We will explain how this question is currently being addressed by combining behavioral, pharmacological and neuroanatomical techniques. Of particular interest is whether PBN neurons specifically contacted by axonal fibers arising in the gustatory aspect of NTS express receptors for hormones that influence feeding. We will also discuss the relevance of these studies in light of the presumed absence of taste representations in the primate PBN.

Membrane Targeting of Chemoreceptors

Symposium Overview: Membrane Targeting of Chemoreceptors

Leslie B. Vosshall

The Rockefeller University, New York, USA

Chemosensory receptor proteins that recognize odorants, tastants, and pheromones are membrane proteins synthesized inside sensory receptor cells, but that have to be efficiently trafficked to sensory portions of cells to be fully functional. This symposium will explore the function of accessory factors and co-receptors in facilitating proper expression of chemosensory receptors. Danny Dhanasekaran of Temple University will describe a novel yeast expression system that has been optimized for functional expression of vertebrate odors receptors. Bettina Malnic of the University of Sao Paulo and Hiroaki Matsunami of the Duke University Medical Center will discuss accessory

factors, GEF and TRP, respectively, that assist the trafficking of odorant receptors in heterologous cells. Randy Hall from Emory University will discuss the role of receptor heterodimerization in facilitating membrane trafficking. Finally, Lily Jan of the University of California, San Francisco, will bring the broader perspective of trafficking of potassium channels to this symposium.

Membrane Targeting of Chemoreceptors

Heterologous Expression of Olfactory Receptors for Targeted Chemosensing

Danny N. Dhanasekaran[1], Venkat Radhika[1], Tassula Proikas-Cezanne[2], Jihee Ha[1] and Muralidharan Jayaraman[1]

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With the broad objective of developing a heterologous expression system for mammalian olfactory signaling pathway, we have engineered yeast cells in which the mammalian olfactory signaling pathway is genetically integrated. Our results demonstrate that the prototypic "olfactory yeast" strain *Wif-1* can sense and report the presence of defined chemical agents through the engineered mammalian olfactory system. In this heterologous *S. cerevisiae*-based expression system, the primary components of mammalian olfactory signaling pathway have been engineered and the signaling by rat olfactory receptor is coupled to the expression of green fluorescent protein. By shuttling a library of olfactory receptor ligand-binding pockets into the pre-engineered signaling units of *Wif-1* yeasts, we further demonstrate the ability of these olfactory yeast cells to detect 2, 4-dinitrotoluene. Using this approach, our results have identified the novel rat olfactory receptor Olfr226, as a 2, 4-dinitrotoluene-responsive receptor. Genetic integration of highly discriminatory olfactory system into biologically stable and biochip-adaptable yeast cells, as presented here, can provide an ideal targeted chemosensing platform for detecting diverse chemical molecules. In addition to their potential use in de-orphanizing the superfamily of olfactory receptors, the engineered olfactoryyeast cells should be amenable for high-throughput screening to identify receptor-specific molecular targets

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Membrane Targeting of Chemoreceptors

Functional Expression of Chemoreceptors with the Help of a GEF

Bettina Malnic

University of São Paulo, São Paulo, Brazil

We have previously shown that Ric-8B, a putative guanine nucleotide exchange factor (GEF) interacts with Gαolf and can amplify odorant receptor signaling through Gαolf in heterologous cells. We now further analyzed the mechanisms of Ric-8B function. We found that Ric-8B also interacts with another heterotrimeric G protein subunit: the Gγ13 subunit, which is involved in taste signal transduction, but is also abundantly expressed in the mature olfactory sensory neurons. We show that the Ric-8B protein is localized to the cilia of the olfactory sensory neurons, the site of odorant signal transduction. We also found that Gβ1 is the Gβ subunit which is predominantly expressed in the olfactory neurons, and that Gβ1 co-localizes with Gαolf, Gγ13 and Ric-8B in the cilia of olfactory sensory neurons. We provide evidence that Ric-8B acts as a GEF to regulate Gαolf function, and also show that Ric-8B enhances targeting of the G protein subunits to the plasma membrane in HEK293T cells. These results suggest two possible roles for Ric-8B in the olfactory sensory neurons *in vivo*: Ric-8B may work as an assembly factor that assists in association and targeting of the Golf protein complex to the plasma membrane; and/or Ric-8B may act as a GEF to amplify odorant signal transduction.

Supported by FAPESP and CNPq.

Membrane Targeting of Chemoreceptors

Functional Expression of Mammalian Odorant Receptors by RTP Family Members

Hiroaki Matsunami

Duke University Medical Center, Durham, USA

Although mammalian odorant receptors (ORs) were identified over 15 years ago, we still do not understand how odorant molecules interact with ORs at a molecular level. Previous studies of mammalian ORs have tested small numbers of ORs against large numbers of odorants. Some fundamental properties of the olfactory system, however, require investigation of a wide panel of diverse ORs with a large number of chemically diverse odorants. Previously, we identified OR accessory proteins, RTP1 and RTP2. They are expressed specifically in olfactory neurons, are associated with OR proteins and facilitate the OR trafficking to the plasma membrane when coexpressed in mammalian cell lines. Using this approach, we have performed high-throughput screening using a large repertoire of mouse and human ORs. We used the activation profiles to develop a predictive model relating physicochemical odorant properties, receptor sequences, and their interactions, enabling us to predict a tested receptor's response to a novel odorant and a novel receptor's response to a tested odorant. This provides a basis for understanding how structurally diverse odorant molecules activate the mammalian OR repertoire. Similarly, two families of vomeronasal receptors, V1Rs and V2Rs, are also notoriously difficult to functionally express in heterologous cells. However, coexpression of the RTP family members with V1Rs or V2Rs does not seem to facilitate trafficking of the receptor proteins. We found that calreticulin-4, a homolog of endoplasmic resident chaperone calreticulin, is specifically expressed by the vomeronasal organ. This suggests that the vomeronasal organ has its unique biosynthetic pathway for membrane proteins.

Membrane Targeting of Chemoreceptors

Potassium Channel Traffic

Lily Jan

University of California, San Francisco, San Francisco, USA

The activity of voltage-gated potassium (Kv) channels and inwardly rectifying potassium (Kir) channels, as in the case of other channels and receptors, depends on both the number and the properties of these membrane proteins, as well as their placement on the neuronal membrane. Channel traffic involves cytoskeleton-associated proteins and could be further regulated by neuronal activity. Two examples from our recent studies will be provided: Kv1 channels of the Shaker family are targeted to the axon of vertebrate and invertebrate neurons to enable action potential propagation into a physiologically optimal number of axonal branches without backfiring. Given that only after full assembly of four alpha subunits and four beta subunits could the Kv1 channel exit the endoplasmic reticulum, we needed to first determine which subunit is responsible for axonal targeting. Once the beta subunit was identified as the culprit, we examined two beta subunit-associated proteins, the microtubule plus end binding protein EB1 and the kinesin KIF3, and found both to be critical for Kv1 channel axonal targeting. G protein-activated inwardly rectifying potassium (GIRK, Kir3) channels open in response to activation of G protein-coupled receptors (GPCR) by inhibitory transmitters and neuromodulators. How might the GIRK channel number be regulated by neuronal activity? Our recent study has revealed a signaling pathway involving phosphorylation-dependent modulation of GIRK channel endocytic trafficking.

Membrane Targeting of Chemoreceptors

Olfactory Receptor Interactions with Other Receptors

Randy A. Hall

Emory University, Atlanta, USA

Studies on olfactory receptor (OR) pharmacology have been hindered by the poor plasma membrane localization of most ORs in heterologous cells. We have found that the OR M71 can interact with specific members of the adrenergic or purinergic receptor families, and that these associations facilitate functional expression of M71 at the plasma membrane of heterologous cells. These receptor-receptor interactions involving M71 are highly specific, as numerous other G protein-coupled receptors that were examined do not detectably interact with M71. Moreover, the apparent G protein coupling specificity of M71 can be switched depending on the interacting partner with which it is co-expressed. In addition to these findings demonstrating the capacity of olfactory receptors to undergo heterodimerization with other receptors, we have also observed homodimerization for several different members of the OR family. These studies shed light on the specificity of OR interactions with other receptors and the mechanisms governing olfactory receptor trafficking.

Chemical Senses and Longevity

Modulation of Drosophila Longevity Through Olfaction

Scott D. Pletcher

Baylor College of Medicine, Houston, USA

In the nervous system, ancient signaling pathways detect, decode, and relay environmental input to coordinate metabolism and growth. Indeed, evidence from work in the nematode worm, *Caenorhabditis elegans*, and from our work in the fruit fly, *Drosophila melanogaster*, strongly suggests that aging is regulated by sensory input and that this regulation is evolutionarily conserved. Ablation of specific sensory neurons in the worm increases lifespan, as do mutations in genes required for sensory signal transduction. In many cases these alterations in lifespan require the transcription factor daf-16/FOXO, suggesting an important role for neuroendocrine pathways. Work in our laboratory has shown that, in *Drosophila*, exposure to food-based odorants reduces lifespan and partially rescues the benefits of dietary restriction. Moreover, odorant receptor Or83b loss-of-function, which leaves flies broadly anosmic, results in significantly increased lifespan. While mutant flies have normal size and metabolic rate, they are resistant to starvation and hyperoxia. Efforts to isolate specific populations of odorant receptor neurons that modulate longevity are ongoing. Targeted rescue of Or83b in certain neuronal subpopulations was sufficient to further modulate the lifespan of Or83b mutant flies and, in rare instances, further increase lifespan. Expression in other populations of neurons, however, had very little effect on fly longevity suggesting that small subsets of sensory neurons can have dramatic effects on lifespan.

Chemical Senses and Longevity

Signaling Proteins that Regulate NaCl Taste Sensitivity Modulate Longevity in *C. Elegans*

Gert Jansen[1], Hannes Lans[1], Nathan Bialas[2], Martijn Dekkers[1] and Michel Leroux[2]

[1]Department of Cell Biology, Erasmus MC, Rotterdam, Netherlands and [2]Department of Molecular Biology & Biochemistry, Simon Fraser University, Burnaby, Canada

The nematode *C. elegans* senses environmental cues using 12 pairs of ciliated neurons, the amphid neurons. Each amphid neuron expresses multiple receptors and G proteins, which probably allows specific responses to many different cues, using only few cells. Previous studies have shown that *C. elegans*' life span is under the control of sensory signals detected by the amphid neurons. We determined the life span of loss-of-function (lf) or overexpression (xs) mutants of many sensory specific proteins. This analysis identified five G proteins that extended life span: the Gα subunits *odr-3(lf)*, *gpa-1(lf)*, *gpa-9(lf)* and *gpa-11(xs)* and the Gγ *gpc-1(lf)*. In addition, we found that mutation of *pcrg-1*, the *C. elegans* orthologue of mammalian Pacrg (which shares a promoter element with Parkin, implicated in Parkinson's disease) results in a reduction in life span. All six proteins are expressed in specific subsets of sensory neurons, and at least three, PCRG-1, ODR-3 and GPC-1 localize specifically to cilia. Genetic epistasis analysis revealed a complex signaling network that regulates longevity. In this network *odr-3(lf)* and *gpa-11(xs)* act synergistically and together extend life span more than two-fold, confirming the importance of sensory signals in regulating life span. Behavioral analyses have shown that *odr-3*, *gpa-1*, *gpa-11, gpc-1* and *pcrg-1* also play a role gustatory plasticity, a behavior in which *C. elegans* avoids otherwise attractive NaCl concentrations after prolonged exposure to NaCl, in the absence of food. This behavior involves desensitization of gustatory neurons and sensitization of nociceptive neurons. We propose that not only the mere detection of environmental cues but also the regulation of the sensitivity of sensory neurons contributes to the regulation of life span in *C. elegans*.

Chemical Senses and Longevity

Foxo Transcription Factors: Central Sensors of Environmental Stimuli that Regulate Longevity

Anne Brunet, Eric L. Greer, Max R. Banko

Stanford University, Stanford, USA

Aging is regulated by modifications in single genes and by simple changes in the environment. The signaling pathway connecting insulin, Akt, and FOXO transcription factors integrate environmental stimuli to regulate lifespan. FOXO transcription factors are directly phosphorylated in response to insulin/growth factor signaling by the protein kinase Akt, thereby causing their sequestration in the cytoplasm. In the absence of insulin/growth factors, FOXO factors translocate to the nucleus where they trigger a range of cellular responses, including resistance to oxidative stress, a phenotype highly coupled with lifespan extension. FOXO factors integrate oxidative stress stimuli via phosphorylation and acetylation of specific residues. Oxidative stress stimuli elicit the physical interaction between FOXO and SIRT1 deacetylase, a member of the Sir2 family, which extend longevity in invertebrates. Our recent results indicate that FOXO transcription factors are also regulated in response to nutrient deprivation by the AMP-dependent protein kinase (AMPK) pathway. The energy-sensing AMPK directly phosphorylates FOXO transcription factors at six regulatory sites. AMPK phosphorylation enhances FOXO transcriptional activity, leading to the expression of specific target genes involved in stress resistance and changes in energy metabolism. The AMPK pathway plays a crucial role in the ability of a dietary restriction regimen to extend lifespan in worms. Understanding the intricate signaling networks that translate environmental conditions into changes in gene expression that extend lifespan will be of critical importance to identify ways to delay the onset of aging and age-dependent diseases.

Dendrodendritic Synapses: 40 Years of Progress

Dendrodendritic Synapses: 40 Years of Progress

Charles Greer

Yale University School of Medicine, New Haven, USA

The dendrodendritic synapses that mediate local circuit processing in the olfactory bulb were first identified in the late 1960's, approximately 40 years ago. The principals involved with the initial discovery included Phillips, Powell, Rall, Reese and Gordon Shepherd. Since then a number of labs have taken on the challenge of understanding the organization, function and plasticity of these novel circuits and how they may contribute to information processing in the olfactory pathway. These results have contributed significantly to our understanding of olfactory coding. Moreover, it has been possible to extrapolate these findings to other regions of the brain where similar synaptic specializations occur. This symposium is dedicated to the recent anniversary of olfactory dendrodendritic circuits and the current state of research.

Dendrodendritic Synapses: 40 Years of Progress

A Smell of Olfactory Bulb Interneurons Diversity

Pierre-Marie Lledo, Mariana Alonso, Matthew Grubb and Antoine Nissant

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SVZ astrocytes (B cells) in the rodent brain function as primary progenitors that generate cells throughout life. From the SVZ, neuroblasts migrate toward the olfactory bulb (OB) where they differentiate into interneurons. It was thought that SVZ stem cells were highly plastic and their differentiation might be directed by local demands for specific neuronal types. Here, we will discuss recent data indicating that adult SVZ primary progenitors are heterogeneous and predetermined to generate specific subtypes of OB interneurons. We will present evidence supporting the notion that OB interneurons are generated by B cells not only in the walls of the lateral ventricle but also in the RMS. Hence, specifically targeting RMS astroctytes with lentiviral vectors encoding GFP, we demonstrated that glia cells in the forebrain are able to differentiate into OB interneurons. Ultrastructural observations unambiguously revealed these stem cells' astrocytic nature, while patch-clamp recordings demonstrate their ability to generate interneurons that are functionally integrated within OB circuitry. Interestingly, exposure to an odor-enriched environment increased candidate stem cell proliferation in the RMS and the SVZ, whereas ablation of the olfactory epithelium increased cell proliferation in the RMS only. New neurons in the adult OB can therefore arise from distinct neurogenic niches that are subject to distinct regulation. We conclude that the postnatal periventricular germinal zone offers a unique system for understanding how the generation and recruitment of multiple neuron types are orchestrated.

Dendrodendritic Synapses: 40 Years of Progress

GABA-A Receptor Heterogeneity Modulates Dendrodendritic Inhibition

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In the olfactory bulb, mitral and tufted cells receive GABAergic inhibition at dendrodendritic synapses with granule cells. Dendrodendritic inhibition mediates contrast enhancement between odor stimuli and is important for synchronizing the output responses of principal neurons connected to functionally related glomeruli. Recent studies have revealed a remarkable variability in the subunit composition of $GABA_A$ receptors in dendrodendritic microcircuits, with differential expression patterns of the $\alpha1$ and $\alpha3$ subunits in different subtypes of mitral and tufted cells. In particular, all mitral cells express the $\alpha1$ subunit, whereas $GABA_A$ $\alpha3$ is restricted to a subgroup of mitral cells, as well as to several subtypes of tufted cells. To assess the functional relevance of this heterogeneity, we investigated a mouse strain carrying a genetic deletion of the $\alpha1$ subunit. Ablation of $GABA_A$ $\alpha1$ was partially compensated in mitral cells by receptors containing the $\alpha3$ subunit, resulting in a substantial decrease in the frequency of sIPSCs, as well as a prolongation of their decay time. Evoked inhibition between granule and mitral cells was slower to rise and decay and had a smaller amplitude in alpha1 mutants. Remarkably, these changes in synaptic inhibition were accompanied by a significant reduction in the frequency of field oscillations. Therefore, the subunit composition of $GABA_A$ receptors has a strong influence over rythmic activities in the olfactory bulb network. Together, these data indicate that dendrodendritic circuits in the external plexiform layer segregate into parallel pathways involving distinct $GABA_A$ receptors which are expressed by different subtypes of mitral and tufted cells.

Dendrodendritic Synapses: 40 Years of Progress

Synaptic Mechanisms Governing Spatio-Temporal Activity Patterns in the Olfactory Bulb

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Activity patterns in second-order olfactory brain structures are determined by the interaction between afferent drive from receptors neurons, input from local synaptic networks and the intrinsic properties of output neurons. Classic *in vivo* intracellular recordings demonstrated that synaptic inhibition plays a central role in governing the firing patterns of mitral cells, the principal type in the olfactory bulb. Presumably most of this inhibition arises from granule cells, the most common interneuron in the bulb. Aside from dendrodendritic inputs from mitral cells, relatively little is known about other inputs to granule cells. Using 2-photon guided minimal stimulation (2PGMS) in acute rat olfactory bulb slices, we found that distal and proximal glutamatergic synapses onto granule cells are functionally distinct and exhibit different forms of short-term plasticity. Proximal excitatory synapses arise primarily from "feedback" connections from piriform cortex and facilitate with repetitive activation. Distal dendrodendritic inputs have slower kinetics than proximal inputs and depress with repetitive stimulation. Stimulation of the lateral olfactory tract and the deep cortical layers in a combined OB/anterior piriform cortex slice preparation evoked responses similar to those obtained with distal and proximal 2PGMS, respectively. Proximal excitatory inputs to granule cells activated both AMPA and NMDA receptors. Short bursts of activity in proximal feedback synapses effectively gated reciprocal dendrodendritic inhibition onto mitral cells by temporally relieving the Mg blockade of NMDA receptors that regulate GABA release at distal dendrodendritic synapses. This finding suggests that gamma-frequency bursts in piriform cortex may dynamically regulate lateral inhibition in the olfactory bulb.

Dendrodendritic Synapses: 40 Years of Progress

Dendrodendritic Synapses and Functional Modules in the Olfactory Bulb

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Odorant receptor maps in the glomerular layer of the mammalian olfactory bulb contain zonal, domain and cluster organization. A recent study

indicates that the zonal and domain organization in the odor maps is functionally related to specific behavioral responses (Kobayakawa et al., 2007). Because an individual mitral cell in the mammalian olfactory bulb projects several long lateral dendrites to a variety of directions, it can potentially form numerous dendrodendritic reciprocal synaptic connections with a large number of granule cells that are distributed across different clusters, domains or zones. We show old and new morphological data of lateral dendrites of individual mitral cells in some vertebrate species, and discuss the relation between the spatial extent of lateral dendrites of individual mitral cells in relation to the clusters, domains and zones of the olfactory bulb. In the latter half of this presentation, we report the behavioral state–dependent change in the dendrodendritic synaptic interactions in the rodent olfactory bulb. A majority of synapses in the external plexiform layer of the olfactory bulb are dendrodendritic reciprocal synapses between mitral cells and granule cells, suggesting that the dendrodendritic synapses has a dominant role in the information processing in the olfactory bulb. We asked whether the dendrodendritic synaptic interactions and the information processing mode in the olfactory bulb vary with brain and behavioral states. We show that state-dependent global changes in cholinergic tone modulate granule-to-mitral dendrodendritic synaptic inhibition and thus the information processing mode in the olfactory bulb.

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Dendrodendritic Synapses: 40 Years of Progress

Metabotropic Glutamate Receptors Amplify Lateral Inhibition in the Main Olfactory Bulb (MOB)

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Metabotropic glutamate receptors (mGluRs) are highly expressed at mitral cell-to-granule cell (GC) dendrodendritic synapses, yet their function is less well understand than ionotropic GluRs. GABAergic interneurons in MOB express particularly high levels of Grp I mGluRs, mGluR1 and mGluR5. Our recent studies in rodent MOB slices demonstrate that both Grp I subtypes play key roles in modulating dendrodendritic inhibition in the external plexiform and glomerular layers. GCs are directly depolarized by Grp I agonists such as DHPG. Intriguingly, superficial and deep GCs differentially express Grp I mGluRs, such that DHPG-evoked depolarization of superficial GCs (i.e., those within the mitral cell layer) is mediated by mGluR1, whereas the depolarization of deep GCs is mediated by mGluR5. mGluR-evoked excitation of GCs in turn robustly increases spontaneous GABAergic currents (IPSCs) in mitral cells. Similar results were obtained for external tufted cells in the glomerular layer, indicating that GABAergic periglomerular cells are also excited by activation of Grp I mGluRs. Thus, pharmacological activation of mGluRs increases GABAergic inhibition at multiple loci in the MOB. However, a key question is if endogenous activation of mGluRs modulates the strength of dendrodendritic synaptic transmission. Additional studies showed that inactivation of Grp I mGluRs: (1) reduced mitral cell-evoked excitation of GCs as measured electrophysiologically in individual cells or at the population level with optical imaging of voltage-sensitive dye signals, and (2) nearly completely eliminated mitral cell feedback inhibition. Together, these findings demonstrate that activation of mGluRs by endogenously released glutamate boosts the strength of feedback and feed forward dendrodendritic inhibition in the MOB.

Dendrodendritic Synapses: 40 Years of Progress

Dendritic Excitability and Dendritic Release

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The messages a neuron delivers to its postsynaptic targets are determined by the coupling between electrical activity and neurotransmitter release. Most neurons signal digitally, meaning that action potentials are required to evoke release of transmitter. In non-spiking neurons, subthreshold changes in membrane potential produce graded changes in transmitter release. Here we report that mitral cells of the accessory olfactory bulb release glutamate from their dendrites in both graded and action potential-dependent fashions. Moreover, pharmacological or endogenous activation group I metabotropic glutamate receptors (mGluRs) enhances subthreshold release several fold while having little effect on action potential-dependent release. These results indicate that neurons can dynamically regulate how their local electrical activity is coupled to transmitter output.

Sweet Taste: Receptors, Transduction, and Hormonal Modulation

Symposium: Sweet Taste: Receptors, Transduction, and Hormonal Modulation

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Considerable progress has been made in elucidating the structure and function of the sweet taste receptor, the heterodimer T1R2 + T1R3. This symposium will highlight recent findings concerning the structural basis for ligand binding at the sweet receptor. In addition to receptor-determined parameters, sweet taste may be especially sensitive to modulation by circulating or locally produced hormones; examples of such modulation will be presented. Beyond the periphery, sweet stimuli are represented by distinct patterns of activation in central neuronal circuits. The functional architecture of the gustatory cortex will be discussed, along with new evidence that the representation of sweet taste can be modified by experience. Finally, we will look at recent findings on the hormonal parameters that influence human sweet taste preferences, particularly in gestational diabetes.

Sweet Taste: Receptors, Transduction, and Hormonal Modulation

Especially Sweet: Receptors and Modulators for Sweetener Detection

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Humans and most other mammals like the taste of sweeteners. This strong preference for sweet-tasting things reflects the important nutritional role of sugars. Since both the gustatory and digestive systems recognize sugars and other sweeteners, it is perhaps unsurprising that these systems utilize common molecular mechanisms to transduce sweet-tasting stimuli. For example,

both sweetener-sensitive taste cells and intestinal enteroendocrine cells require the heteromeric T1R2:T1R3 receptor to detect sweeteners. The conservation of molecules used for the detection of sweeteners and the modulation of sweetener-dependent responses indicates common mechanisms for alimentary chemosensation. Indeed, we have found that peptide hormones involved in the regulation of glucose homeostasis are expressed in taste cells, where they modulate sweet taste sensitivity. Thus, efforts to understand the molecular mechanisms underlying sweet taste function will elucidate multiple physiological processes and could shed light on ways in which sensory function can be modulated in the context of an animal's metabolic state.

Sweet Taste: Receptors, Transduction, and Hormonal Modulation

Modulation and Transmission of Sweet Taste Information for Energy Homeostasis

Yuzo Ninomiya, Ryusuke Yoshida, Yoshihiro Murata, Keiko Yasumatsu, Tadahiro Ohkuri, Shinya Shirosaki, Keisuke Sanematsu, Toshiaki Yasuo, Yuki Nakamura and Noriatsu Shigemura

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It is known that the ability to taste sweetness is important for animals to detect carbohydrate source of calories and has a critical role in the nutritional status of animals. Our previous studies in mice demonstrated that sweet-sensitive cells may act as sensors not only for external calorie sources but also for internal energy store, represented by plasma leptin level. Increase in plasma leptin level leads to reduction of sweet sensitivity of taste cells via activation of leptin receptors, followed by reduction of cell excitability. Modulation of perceived sweetness transmitted to the brain through afferent fibers may help maintaining animal's energy homeostasis. However, little is known about potential links between sweet taste and leptin in humans. Then we first addressed this issue by comparing the recognition thresholds for sweet compounds and plasma leptin level. The results indicated that the sweet thresholds have a diurnal variation that parallels variation for leptin levels, with lowest in the morning and highest in the night. This synchronization of diurnal variation in leptin levels and sweet taste recognition thresholds suggests a mechanistic connection between these two variables in humans. It is also unclear how such modulated information from sweet-sensitive taste cells could be transmitted to axons. Then we measured ATP release from mouse sweet-sensitive cells by using a newly developed loose-patch recording configuration. We found that ATP concentration in recording pipetes being inflowed from taste cells increased with increasing spike-frequency of taste cells in response to saccharin. Frequency-dependent increase in ATP concentration indicates importance of action potentials for release of ATP from sweet-sensitive cells.

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Sweet Taste: Receptors, Transduction, and Hormonal Modulation

Sweet Taste in Human Gestational Diabetes Mellitus

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Gestational diabetes mellitus (GDM) is hyperglycemia first identified during pregnancy. Sweet taste is altered in other forms of diabetes, but data are limited in women with GDM. Our laboratory previously reported that, in mid-to-late pregnancy, women with GDM showed increased preference for sucrose-sweetened milks relative to women with normal glucose tolerance (NGT). The objectives of our current work were to compare the time course of sweet taste changes across gestation in women with GDM and NGT, and to relate these alterations to endocrine and metabolic profiles of pregnancy. Dietary intake and food cravings were also investigated. Women with NGT reported increased intake and craving of sweet foods at mid-pregnancy, confirming previous reports in the literature. In women with GDM, circulating insulin and leptin were correlated with liking of glucose solutions and sucrose-sweetened milk, respectively, at mid-pregnancy but not at other time points. Women with GDM also had higher preference for sucrose-sweetened milks late in pregnancy, and a sub-set of these women reported twice the frequency of sweet cravings at this time, as compared to women with NGT. These novel findings suggest that GDM alters the hedonic value of sweet taste. Some of these changes occur in mid-pregnancy and coincide with the development of insulin and leptin resistance, whereas other changes occur late in pregnancy and may be related to dietary restriction. These data raise questions about the role of sweet taste in food preference and appetite in GDM. Further elucidation of mechanisms related to sweet taste alterations could lead to better medical and dietary management of this disease, currently affecting 2-9% of pregnant women.

Sweet Taste: Receptors, Transduction, and Hormonal Modulation

Topographical Representation and Plasticity of Sweet Modality in the Rat Gustatory Cortex

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Among the five senses, gustation has been largely under-studied. Yet, it is of great interest to understand how the brain processes taste stimuli, which play a key role in feeding and survival. Recently, molecular biology studies have sparked new interest in the taste field through the cloning of taste receptors. However, the neural processing occurring in the brain and especially at the cortical level is still largely unknown and subjected to debate. Using genetic tracing, it has been shown that sweet and bitter taste are processed through segregated neuronal circuitries along the gustatory pathway up to the cortical level. This is in disagreement with the evidence that gustatory cortex (GC) neurons recorded in both anaesthetized and behaving animals responded to multiple taste modalities (including sweet and bitter). To investigate the functional architecture of the GC in regard to taste modalities we used *in vivo* intrinsic optical imaging, a technique that has been successfully applied to explore the organization of other neocortical regions. We will present how the sweet modality is represented in the GC and we will compare to the bitter modality representation. We will show that the two taste modalities are represented by distinctive spatial patterns but with common territories. Interestingly, these representations are plastic. We used a conditioned taste aversion paradigm (CTA), a learning paradigm whereby one learns to avoid a taste stimulus (here a sweet taste) previously associated with visceral malaise. We showed that an internal state of malaise induces topographical plasticity of the sweet taste representation in the GC that is associated to behavioral shift of the stimulus hedonic value. We propose that general changes in internal body may be the source of some food intake disorders.

Dendrodendritic Synapses: Old and New Discoveries

Dendrodendritic Synapses: Past, Present and Future

Gordon M. Shepherd

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Studies of the olfactory bulb leading to the discovery of dendrodendritic synapses began in 1959. They involved a rather extensive multidisciplinary approach that included Golgi cell morphology, single cell physiology (extracellular single unit and intracellular recordings), extracellular field potentials, a microcircuit wiring diagram, membrane biophysics, theory of field potentials, cable theory, dendritic electrotonus theory, computational models of mitral and granule cells, prediction by the models of dendritic synaptic interactions, confirmation with electron microscopy using single sections and serial sections, and collaboration on the reports of feedback and lateral inhibitory interactions in 1966 and 1968. Following the discovery of glomerular odor maps in the in the 1970s, the functional significance of the inhibition in processing the maps has been increasingly documented. For the future, the multidisciplinary approach will continue to be essential, incorporating molecular and genetic methods combined with fine structure, physiology, and functional imaging. Computational modeling needs to be closely applied to interpreting dendrodendritic interactions in distributed glomerular units. Studies in the olfactory bulb need to be combined with those in olfactory cortex in order to understand the tight functional loops between the two in odor processing. Methods applied to the awake behaving animal will give critical new insights. Finally, the roles of dendritic mechanisms in perception, memory, and the pathogenesis of disorders such as Alzheimer's need to pursued aggressively. In summary, dendrites and their synapses should continue to provide ideal models for the study of basic mechanisms of cortical integration and the neural basis of smell.

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AChemS Presidential Symposium: Neural Basis of Sensory Experience

Neural Basis for Olfactory Perception: A Historical Perspective

Alan Gelperin

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Sensory information processing and its relationship to sensory perception are studied most directly in the awake behaving animal, whether mouse or human. I will focus on studies attempting to make the causal link between in vivo neural activity patterns and behavioral decision-making by the animal or human subject contributing the neural data. The area of olfactory information processing and its relation to odorant-based decision making has been stimulated by recent technical advances in making in vivo recordings in the awake behaving animal and genetic advances used to supply animal subjects for detailed psychophysical analysis with well-defined alterations in specific circuit elements in the olfactory information processing pathway. These studies have identified a key role for active sampling (sniffing) in olfaction, the critical role of learning and experience in shaping the olfactory percept, and a surprisingly sparse representation of odor information in the awake mouse brain. Some of these ideas have a provocative historical development. Early studies by Moulton, Freeman, and others used chronic recordings in awake rabbits to characterize responses of receptors and mitral/tufted cells before and after odor learning. Recent work has shown that the olfactory bulb has many of the functions of a thalamic relay in gating information flow from receptors to higher cortical centers. Because of this, the context during stimulus presentation, prior experience with the stimulus, and prior learning that the stimulus predicts associated rewards, all have dramatic influences on neural responses and olfactory perception. A major challenge for modern cellular and computational work in olfaction is to further strengthen the links between neural, behavioral and perceptual events.

AChemS Presidential Symposium: Neural Basis of Sensory Experience

Low-Level Mechanisms for Processing Odor Information in the Behaving Animal

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We typically think of sensory systems as generating faithful representations of external stimuli at initial, low-level stages of the nervous system and then performing increasingly complex transformations of these representations as information propagates to higher levels. Likewise, the modulation of sensory codes during behavior – for example, as a function of behavioral context or attentional state – is typically thought to occur at higher nervous system levels. This talk will discuss recent findings from our laboratory demonstrating that, in the olfactory system, odor representations in the behaving animal can be transformed at low levels – as early as the primary sensory neurons themselves - via a variety of different mechanisms. First, changes in odor sampling behavior (i.e. – 'sniffing') can dramatically and rapidly alter primary odor representations by changing the strength and temporal structure of sensory input to the olfactory bulb, effectively shaping which features of the olfactory landscape are emphasized and likely altering how information is processed by the olfactory bulb network. Second, neural substrates exist for presynaptically modulating the strength of sensory input to the bulb as a function of behavioral state. The systems most likely to be involved in this modulation – cholinergic and serotonergic centrifugal inputs to the bulb – are linked to attention and arousal effects in other brain areas. Together, sniffing behavior and presynaptic inhibition have the potential to mediate – or at least contribute to – sensory processing phenomena such as figure-ground separation, intensity-invariance, and context-dependent and attentional modulation of response properties. Thus, 'high-order' processing can occur even before sensory neurons transmit information to the brain.

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Characterization and Coding of Complex Odor Mixtures in the Moth, *Manduca Sexta*

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The odor environment is complex, consisting of a mosaic of behaviorally-relevant and non-relevant odors by which animals must discriminate. This is particularly true for insects, where complex odor mixtures often dictate behavior. Unfortunately, our understanding of how the olfactory system processes complex mixtures and encodes behaviorally-relevant odors is rather limited. To reach an improved understanding, we investigated mixture processing and behavior using the moth, *Manduca sexta*, and floral odors of two important nectar resources for the moth, the plants *Datura wrightii* and *Agave palmeri*. Using a multidisciplinary approach by coupling volatile characterizations, behavioral wind tunnel experiments, and electrophysiology, we have been able to determine how complex mixtures, at natural concentrations, control flight behavior and are encoded in the antennal lobe (AL). Use of a tandem gas chromatography-multi-channel recording system (GC-MR) allowed determination of the key single odorants within the complex floral mixtures that AL neurons are especially responsive to.

Moreover, this system provided comparisons between neural ensemble responses to single odorants in relation to the mixtures. Results demonstrate that neural ensemble responses to the two floral mixtures containing >100 individual odorants can be reproduced by a much smaller subset of odorants (<5). In turn, behavioral results demonstrate these mixtures containing the smaller subset of odorants are as effective as the natural floral mixtures. Finally, spatiotemporal encoding by the AL neural ensemble provides a means by which the olfactory system can generalize between related mixtures while providing enough contrast for discrimination between floral odors.

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Processing of Odor Representations by Neuronal Circuits in the Olfactory Bulb

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Odor information is first represented in the olfactory bulb (OB) by distributed glomerular activity patterns that contain nested spatial maps of primary and secondary molecular stimulus features. Neuronal circuits in the OB transform these input patterns into spatio-temporal patterns of output activity that are transmitted to higher brain regions by mitral cells. To understand the computations associated with this transformation and the function of chemotopic maps, we measured odor-evoked activity patterns across thousands of individual neurons in the intact brain of zebrafish using electrophysiology, temporally deconvolved 2-photon calcium imaging, and transgenic cell type markers. We found that the OB performs multiple computations including a decorrelation of initially overlapping activity patterns, a multiplexing of complementary information, and gain control. The chemotopic representation of primary molecular features is maintained in OB output activity patterns, while secondary chemotopic maps disappear during the initial phase of an odor response. This reorganization is caused by the local sparsening of MC activity within chemotopic foci and promotes the decorrelation of overlapping input patterns. Computational modelling based on measured connectivity patterns indicates that local sparsening and decorrelation are generic features of circuits with an OB-like architecture and depend on the chemotopy of inputs, even though secondary chemotopy is not maintained in the output. These results indicate that topographic maps configure computational properties of circuits and provide insights into the basic functions of the OB.

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AChemS Presidential Symposium: Neural Basis of Sensory Experience

A Searchlight for Meaning in the Olfactory Bulb

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While on the basis of its primary circuit it has been postulated that the olfactory bulb (OB) is analogous with retina, there is at least one feature that sets these two primary sensory relays apart. The OB receives massive centrifugal innervation from olfactory cortex and neuromodulatory centers such as adrenergic neurons in the locus coeruleus and therefore it is expected to undergo plastic changes during learning. Here we show that the responses of the principal cells of the OB -the mitral cells- in response to odors during a go-no go odor discrimination task change transiently in a manner that increases the ability of the circuit to convey information necessary to discriminate among closely related odors. Unlike olfactory cortex where divergent changes in firing rate occur and are long lasting, only a transient divergence in the firing rate of mitral cells to the rewarded and unrewarded odors was observed. In addition to the emergence of divergent individual cell responses there was also a transient overall recruitment of responding neurons to the rewarded and unrewarded odors. Next, restricted adrenergic antagonism was employed within the OB in combination with multielectrode recording. The divergence in individual cell responses was not dependent upon noradrenaline signaling but was delayed in the presence of adrenergic antagonists. Noradrenergic antagonism did cause a drastic reduction in the normal transient increase of single cell responses to the rewarded and unrewarded odors. Taken together these results redefine the function of the olfactory bulb as a transiently modifiable (active) filter used by higher cortical structures to shape odor representations at the output of the olfactory bulb in contextually relevant and behaviorally meaningful ways.

AChemS Presidential Symposium: Neural Basis of Sensory Experience

Pattern Separation and Completion in Olfactory Cortex: The Balance Between Odor Discrimination and Perceptual Stability

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The need for perceptual discrimination must be balanced with the need for perceptual stability. Without an ability to ignore some differences between input patterns, nearly all experiences would be unique events, with each novel presentation of a similar stimulus devoid of previous associations. Computational modeling and experimental data suggest that some cortical circuits balance discrimination and stability through the network emergent functions of pattern separation and pattern completion. Simply put, pattern separation allows partially overlapping input patterns to be discriminated as distinct (de-correlated), while pattern completion is a memory-based phenomenon that allows input patterns to be compared to existing templates and if sufficiently close to those templates, they are "completed" and processed as a match. In olfaction, the need for pattern separation and completion is particularly relevant, as most natural odors derive from complex mixtures, evoking complex spatiotemporal patterns of receptor and olfactory bulb activity. Given this complexity, it is rare for a given stimulus to always have the exact same components, yet it is possible for a noisy or degraded stimulus to reliably evoke a stable percept. On the other hand, if the component make-up changes sufficiently, discrimination ensues. Here, I will describe studies in our lab on piriform cortical single-unit and ensemble processing of complex mixtures as they are morphed by removing or replacing components. The results suggest that cortical ensembles, but not single-units, perform pattern separation and completion as stimulus mixtures are morphed, and that this ensemble activity predicts behavioral performance. These studies help to close the gap between neurobiology and perception.

AChemS Presidential Symposium: Neural Basis of Sensory Experience

Beyond the Olfactory Bulb. Neural Responses to Odor Cues in awake, Behaving Rats

Geoffrey Schoenbaum

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What happens to information about odor cues beyond the olfactory bulb, once they get out into the rest of the brain? In my talk, I'll tell you a story

about how we have used olfactory cues and odor-guided behavior in rats to gain a better understanding of the function of the orbitofrontal cortex and related structures *in humans*. Along the way, I will show neural recording data from awake, behaving rats suggesting that most of the brain seems to care little about what and odor is and much more about what it *means*. This is perhaps a truism in higher order brain regions; however much to our surprise it also seems to be true, to a lesser extent, for piriform cortex in awake, behaving rats.

AChemS Presidential Symposium: Neural Basis of Sensory Experience

The Transformation of Olfactory Input into a Motor Output

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Olfactory inputs are known to elicit motor behaviors, but the underlying neural substrates remain unidentified. We have investigated olfactory-motor transformations in lampreys by using anatomical, electrophysiological and imaging tools and have found a specific neural pathway producing locomotor movements in response to olfactory inputs. This pathway originates from the medial part of the olfactory bulb (OB) that we now show to receive projections from the main olfactory epithelium (MOE) as well as from the accessory olfactory organ, located in the caudo-ventral portion of the peripheral olfactory organ. Lateral OB regions receive projections exclusively from the MOE. A bundle of axons originating from the medial part of OB projects caudally to the posterior tuberculum (PT) in the ventral diencephalon. PT fibers project caudally to the mesencephalic locomotor region (MLR), which controls locomotion by activating locomotor command cells, the reticulospinal (RS) cells. We have used an *in vitro* preparation that includes the peripheral olfactory organ, the forebrain, brainstem, and rostral spinal cord, and observed large excitation in RS cells following application of odorants or pheromones onto the peripheral olfactory organ. Stimulation of the olfactory nerve elicits excitatory post-synaptic potentials in RS cells and calcium imaging revealed excitation in many of them. Stimulating the medial part of OB produces excitatory responses in RS cells, whereas the lateral part of OB does not. Locomotion is elicited by injecting glutamate into the OB. Inactivating parts of this pathway with local injections of transmitter blockers confirms that olfactory inputs reach RS command cells through the PT, and MLR. This work is the first description of an olfacto-locomotor connection in vertebrates.

Chemical Senses and Mechanisms of Neurodegenerative Diseases

Symposium: Chemical Senses and Mechanisms of Neurodegenerative Diseases

Claire Murphy[1,2]

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Olfactory function is significantly and early impaired in neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. Anatomy-based gene expression analysis, epigenetic regulation, calcium channel-mediated neuronal firing function, and adult neurogenesis are four major cutting-edge research directions in studying the mechanisms underlying neurodegenerative diseases. We have invited leading scientists in these research areas to participate in this symposium and to introduce to the chemical senses community their novel insights into the mechanisms underlying neurodegenerative diseases. The core contributors were selected on the basis of their outstanding research contributions and for their ability to communicate critical new findings about the mechanisms of neurodegenerative disease. Insight into the mechanisms underlying olfactory system impairment in neurodegenerative diseases may facilitate both the identification of individuals likely to develop cognitive impairment or dementia who will benefit most from interventions at the earliest incidence of impairment, and the identification of new treatments for neurodegenerative diseases. Thus, this symposium has the following aims: 1) To bring together scientists at the forefront of research on neurogenesis in aging, neurodegenerative diseases, and the chemical senses, 2) To stimulate investigation into the mechanisms that underlie age-related impairment in the chemical senses and the dramatic changes in olfactory function in neurodegenerative diseases, 3) To facilitate the application of state of the art innovative technology, particularly genetic, molecular and cellular biological techniques, to research on aging and the chemical senses, 4) To identify directions for future research in chemosensory science.

Chemical Senses and Mechanisms of Neurodegenerative Diseases

Olfactory Dysfunction in Aging and Alzheimer's Disease: The Apoe E4 Risk Factor for Alzheimer's Disease Alters fMRI Brain Activation in a Cross-Modal Odor Recognition Memory Task

Claire Murphy[1,2], Lori Haase[1,2] and MiRan Wang[1]

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Olfactory function is impaired in old age, dramatically more so in Alzheimer's disease. Individuals with the Apolipoprotein E e4 allele are at significant risk for developing Alzheimer's disease. The current study investigated the underlying cortical substrate for cross-modal odor recognition memory using fMRI in 18 older adults with, and 21 without, the ApoE e4 allele. Participants were presented with 16 common odors for encoding before entering the fMRI scanner. During functional runs at 3T on a GE magnet, participants were presented with words that either represented an odor that had been presented for encoding (target) or one that had not (foil), and distinguished between targets and foils using a button press. Performance on the memory task was recorded as hits, misses, correct rejections and false positives at the button box and the discriminability index (d') was subsequently computed. Brain activity corresponding to memory performance in the two groups was analyzed with AFNI (Cox, 1996). Older adults with the e4 risk factor showed patterns of brain activity that were markedly different from those generated by the older adults without the e4 allele. The differential patterns of fMRI activity suggest altered brain response that may reflect the cortical substrate for differences in performance in those at genetic risk for Alzheimer's disease. These results will be discussed in the context of psychophysical, neuropsychological and fMRI investigations of olfactory dysfunction in aging and dementia.

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Chemical Senses and Mechanisms of Neurodegenerative Diseases

Neural Representations and Cognitive Aging: Evidence from Studies on Olfactory and Spatial Memory Inrodents

Howard Eichenbaum

Boston University, Boston, USA

This lecture will review of evidence from neuropsychological testing and recordings of neuronal activity patterns in young adult and aged rats performing odor and spatially guided memory tests. Rats are exceptionally good in olfactory and spatial memory, and their memory abilities in both domains exhibit the fundamental features shared with declarative memory in humans. Rats and mice can remember the context in which odors were experienced, sequences of olfactory events in unique episodes, and they can construct networks of odor memories that constitute a basic semantic organization. Rats also remember places, sequences of places that compose routes, and they remember networks of routes that constitute spatial maps. These abilities are commonly dependent on the hippocampus, and the neuronal ensembles in the hippocampus encode each of these types of information. Furthermore, these representations are compromised in aging. For example, using signal detection analyses to characterize odor recognition performance, we found that rats with hippocampal damage have a selective deficit in recollection with spared familiarity for odors. Aged rats that are impaired in odor recollection are also impaired in spatial memory, which is related to abnormalities in neural representations of space. Thus it appears the hippocampal system similarly supports declarative memory in the olfactory and spatial domains, and both domains of declarative memory are similarly impaired in aging associated with hippocampal dysfunction.

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Chemical Senses and Mechanisms of Neurodegenerative Diseases

Molecular Mechanisms Regulating Adult Neural Stem Cells and Neurogenesis in the Olfactory System and the Hippocampus

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New neurons are continuously generated from adult neural stem/progenitor cells (NSCs) residing in the subventricular zone of lateral ventricles and the subgranular zone of the hippocampal dentate gyrus in all mammals examined, including humans. During active adult neurogenesis, NSCs generate functional neurons through orchestrated steps, including cell proliferation, fate specification, neuronal migration, axonal and dendritic growth, and finally synaptic integration into the existing circuitry. As in other somatic stem cell systems, neurogenesis from NSCs in the two neurogenic regions of the adult brain is tightly regulated by the highly specialized microenvironment surrounding the NSCs. These ''neurogenic niches'' not only anatomically house adult NSCs, but also functionally control their development in vivo. Using multiple approaches for birth-dating, genetic marking and manipulation of proliferating NSCs and their progeny in the neurogenic regions of adult mice, we have characterized the sequential events of adult neurogenesis in vivo using immunocytochemistry, confocal and electron microscopy, in vivo multiphoton imaging, and electrophysiology. Our studies have identified signaling molecules within the unique neurogenic niche to either positively or negatively regulate various aspects of adult neurogenesis in an activity-dependent fashion, such as GABA. Furthermore, we have also identified essential intrinsic regulators of adult neurogenesis in vivo, such as Disrupted-in-Schizophrenia 1 (DISC1) and its interacting proteins. We hope a better understanding of cellular and molecular mechanisms regulating adult neural stem cells and neurogenesis may lead to novel strategies for functional neuronal replacement therapy after injury or degenerative neurological diseases.

Chemical Senses and Mechanisms of Neurodegenerative Diseases

Chromatin Remodeling in Hippocampus Dependent Learning and Memory

Li-Huei Tsai

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Histone acetylation plays a role in the regulation of gene transcription via chromatin remodeling. Recently, modulation of histone acetylation has been implicated in synaptic plasticity and memory formation. Histone deacetylases are a large family of enzymes that remove the acetyl group from histone proteins and other substrates. We previously showed that administration of the general inhibitor of histone deacetylases (HDACs) sodium butyrate (SB), improved associative and spatial learning in the inducible CK-p25 transgenic mice after massive neuronal loss and synaptic loss in the hippocampus had occurred. In addition, SB treatment facilitated the recovery of consolidated long-term memory that was otherwise lost in the untreated group. Further work suggests that increased dendritic sprouting and synapse number may contribute to the improved learning and recovery of long-term memory resulted from chromatin remodeling. We have since been interested in deciphering the specific HDAC(s) targeted by the HDAC inhibitors that are beneficial to hippocampus dependent learning and memory. Using gain-of-function and loss-of-function mouse models, we have evidence indicating that certain class I HDACs play a role in this process. Potential targets of these HDACs participating in synaptic plasticity and memory will be discussed.

Chemical Senses and Mechanisms of Neurodegenerative Diseases

Cell Proliferation and Death in Peripheral Olfactory Systems

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The olfactory system undergoes neurogenesis, cell migration, synaptic plasticity, and apoptosis beyond the normal short developmental period. Aging encompasses all of these phenomena, but it is not yet clear how cellular aging occurs, nor how aging affects function. The olfactory system provides a unique opportunity to examine the role of environmental versus biological causes of aging. The main olfactory epithelium (MOE) is exposed to odorants, airborne viruses, and toxins, while a vascular pump limits stimulus access to the vomeronasal epithelium (VNE). The role of environment in aging can be examined, as exposure to damaging agents is vastly different in the two epithelia. Here, we establish basal rates of proliferation and apoptosis in the MOE and VNE over the mouse lifespan. In initial studies, we show that BrdU labeling in basal cells in 1 and 2 month old mice is significantly higher ($p < 0.001$) than that seen in either 6 or 24 month old mice. We next asked whether the ability to respond to acute injury, namely olfactory bulbectomy (OBX), also decreases with age. It is not known if the regenerative capacity of either epithelium continues to exist as the animal progresses through advanced life stages. OBX results in rapid death of mature sensory neurons within five days, followed by the massive proliferation of basal cells

and partial reconstitution of the epithelium within 30 days. Unilateral OBX was performed on 2, 6, and 24 month old mice; animals recovered for 5 days and were evaluated for BrdU incorporation in conjunction with GAP-43 and OMP labeling. BrdU incorporation was significantly increased in the OBX VNE versus non-surgery control in all age groups, suggesting that while proliferation rate is normally low in older animals, this rate increases when challenged with an injury.

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Non-Canonical Transduction Pathways in Olfaction–New Views on Olfactory Signaling

A Novel Family of Sensory Receptors in the Nose

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The mammalian nose is a powerful chemosensor, capable of detecting and distinguishing a myriad of chemicals. Sensory neurons in the olfactory epithelium contain two types of chemosensory G Protein-Coupled Receptors (GPCRs): odorant receptors (ORs), which are encoded by the largest gene family in mammals, and trace amine-associated receptors (TAARs), a smaller family of receptors distantly related to biogenic amine receptors. Do TAARs play a specialized role in olfaction distinct from that of ORs? Genes encoding TAARs are found in diverse vertebrates, from fish to mice to humans. Like OR genes, each TAAR gene defines a unique population of canonical sensory neurons dispersed in a single zone of the olfactory epithelium. Ligands for mouse TAARs include a number of volatile amines, several of which are natural constituents of mouse urine, a rich sources of rodent social cues. One chemical, phenylethylamine, is enriched in the urine of stressed animals, and two others, trimethylamine and isoamylamine, are enriched in male versus female urine. Furthermore, isoamylamine is reported to be a pheromone that induces puberty acceleration in young female mice. These data raise the possibility that some TAARs are pheromone receptors in the nose, a hypothesis consistent with recent data suggesting that the olfactory epithelium contains dedicated pheromone receptors, separate from pheromone receptors in the vomeronasal organ. Future experiments will clarify the roles of TAARs in olfaction.

Non-Canonical Transduction Pathways in Olfaction–New Views on Olfactory Signaling

CO_2 Detection by the GCD-Cell System

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The mammalian olfactory system consists of parallel subsystems, one of which is especially intriguing in terms of its unique signal transduction involving cGMP instead of cAMP in their receptor neurons. They project to the necklace glomeruli – a set of interspersing glomeruli that form a ''necklace'' in the caudal end of the main olfactory bulb (MOB). These specialized receptor neurons thus constitute a so-called ''necklace olfactory system'' or ''GCD-cell system'' due to their unique expression of guanalyl cyclase-D (GC-D). Here I will present evidence that carbonic anhydrase type II (CAII), an enzyme that catabolizes CO2, is selectively expressed in a subset of mouse olfactory neurons that express guanylyl cyclase D (GC-D+ neurons) and project axons to necklace glomeruli in the olfactory bulb. Using calcium imaging and electrophysiology, we find that exposure to CO2 activated these GC-D+ neurons, and exposure of a mouse to CO2 activated bulbar neurons associated with necklace glomeruli. CO2 responses required the enzymatic activity of carbonic anhydrase and the opening of c-GMP-sensitive CNG channels. Behavioral tests reveal CO2 detection thresholds of ~0.066%, and this sensitive CO2 detection required CAII activity. These results demonstrate that mice detect CO2 at near-atmospheric concentrations (0.038%) through the GCD-cell system and use cGMP as the second messenger.

Non-Canonical Transduction Pathways in Olfaction–New Views on Olfactory Signaling

Functional Analysis of the Guanylyl Cyclase Type D Signaling System in the Olfactory Epithelium

Frank Zufall

University of Saarland School of Medicine, Homburg, Germany

GC-D neurons are ciliated olfactory sensory neurons (OSNs) that express none of the typical components of the cAMP signaling pathway of canonical OSNs. Instead they express several molecules required for a cGMP second messenger system, including the receptor guanylyl cyclase GC-D and the cGMP-selective cyclic nucleotide-gated channel CNGA3. The existence of these cells in the olfactory epithelium has been known for over a decade and it has been suggested that GC-D cells might respond to hormones or pheromones. A combined approach employing gene-targeting methodology, high resolution electrophysiological and Ca2+ imaging techniques in intact olfactory epithelium, and in vivo analysis has provided insight into the chemosensory role of this unique olfactory subsystem. The results show that a second cyclic nucleotide-based signaling system, which depends on elevation of cGMP, not cAMP, is used for chemodetection by the main olfactory epithelium.

Non-Canonical Transduction Pathways in Olfaction–New Views on Olfactory Signaling

Trpc2: Expression Outside the Mouse VNO

Peter Mombaerts

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We have re-evaluated the notion that Trpc2 knockout mice are equivalent to mice without a functional vomeronasal organ (VNO). By gene targeting we generated in the Trpc2 locus a mutation that is likely to be a null mutation, and we established a homozygous strain in an inbred 129 background. We performed behavioral studies in wild-type or Trpc2 homozygous mice with an intact VNO in comparison to mice with surgical removal of the VNO (termed VNX). We found that Trpc2 homozygous/VNX mice behave similarly to Trpc2 homozygous mice, but differently from wild-type/VNX mice. These results argue against non-specific, general behavioral problems that may be caused indirectly by the VNX procedure itself. We also generated two additional strains with targeted mutations in the Trpc2 locus that result in expression of axonal markers. These mice with tagged Trpc2 loci reveal expression of Trpc2 in the main olfactory epithelium, and projection of axons of Trpc2-expressing neurons from the main olfactory epithelium to glomeruli in the main olfactory bulb. Together with a report of residual peptide-responsive neurons in the VNO of Trpc2 homozygous mice, our results indicate that the Trpc2 mutant phenotype has a complex etiology, which likely also is caused by cells outside the VNO.

Non-Canonical Transduction Pathways in Olfaction–New Views on Olfactory Signaling

Emerging View of Insect Olfactory Receptor Signaling

Kazushige Touhara

The University of Tokyo, Chiba, Japan

There have been a long argument on mechanisms of insect olfactory transduction and the lack of clear consensus on the role of second

messengers in this process. In general, each insect olfactory sensory neuron expresses one member of the olfactory receptor (OR) gene family along with the highly conserved Or83b co-receptor, and the two ORs form a heteromeric complex to function as a chemosensor. In addition, insect ORs lack homology to G protein-coupled ORs in vertebrates and possess a distinct seven-transmembrane topology with the N-terminus located intracellularly. Here we provide evidence that heteromeric insect ORs comprise a novel class of ligand-activated nonselective cation channels. Heterologous cells expressing silk moth, fruit fly, or mosquito heteromeric OR complexes exhibited extracellular calcium influx and cation-nonselective ion conductance upon odorant stimulation. G protein-mediated signaling was negligible in producing the current elicited by odor activation of insect ORs, although some OR complex exhibited a small, ligand-independent sensitivity to cyclic nucleotides. The fast response kinetics and OR subunit-dependent potassium ion selectivity of the insect OR complex support the hypothesis that the OR+Or83b complex itself confers channel activity. Direct evidence for odorant-gated channels was obtained by outside-out patch-clamp single-channel recording of *Xenopus* oocyte and HEK293T cell membranes expressing insect OR complexes. The olfactory signal transduction mechanism in insects is clearly distinct from that in vertebrates and appears to be a unique strategy that insects have acquired to respond to the olfactory environment.

This work was done in collaboration with Vosshall group in Rockefeller Univ. and supported in part by Japan-US cooperative science program funding.

Non-Canonical Transduction Pathways in Olfaction–New Views on Olfactory Signaling

Drosophila Or83b – Receptor or Ion Channel?

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Odorant signals are detected by binding of odor molecules to odorant receptors. These belong to the G-protein-coupled receptor (GPCR) family. They in turn couple to G-proteins, most of which induce cAMP production. This second messenger activates ion channels to depolarise the olfactory receptor neuron, thus providing a signal for further neuronal processing. Recent findings challenge this concept of olfactory signal transduction in insects, since their ORs, which lack any sequence similarity to other GPCRs, are composed of conventional ORs (e.g., Or22a), dimerised with a ubiquitously expressed chaperone protein, such as Or83b in Drosophila. Or83b has a structure similar to GPCRs, but has an inverted orientation in the plasma membrane. Still, G-proteins are expressed in insect olfactory receptor neurons, and olfactory perception is modified by mutations affecting the cAMP transduction pathway. In our experiments we could demonstrate that application of odorants to mammalian cells coexpressing Or22a and Or83b results in nonselective cation currents activated via both an ionotropic and a metabotropic pathway, and a subsequent increase in the intracellular Ca2+ concentration. Expression of Or83b alone leads to functional ion channels not directly responding to odorants, but directly activated by intracellular cAMP or cGMP. Insect ORs thus form ligand-gated channels as well as complexes of odorant sensing units and cyclic nucleotide-activated nonselective cation channels.

Non-Canonical Transduction Pathways in Olfaction–New Views on Olfactory Signaling

Multiplicity of G Protein Signalling Mechanisms in *Drosophila* Olfactory Transduction

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Mechanisms by which G-protein coupled odorant receptors transduce information in insects still need elucidation .We have directly tested the role of mutants in genes encoding $G_q\alpha$, PhospholipaseCβ and a DAG kinase in *Drosophila* olfactory transduction by measuring odorant responses from mutant antennae. Responses to multiple odorants are significantly reduced as measured by field recordings as well as single unit recordings. Our data support a role for a phospholipid second messenger in *Drosophila* olfactory transduction. Interestingly, in olfactory sensory neurons null for the Gq gene, we consistently observed low levels of a residual response to different odorants, suggesting the existence of a second signaling mechanism. The role of other G-proteins that could contribute to the residual responses and their interaction with Gq is under investigation and will be discussed.

Basic Processes in Human Olfactory Cognition

Basic Processes in Human Olfactory Cognition

Rachel S. Herz

Brown University, Providence, USA

Sensory perception is the first step in higher order neurocognitive processing. After stimulus perception, an odor is analyzed or coded through cognitive and associative neurological networks that determine or assign meaning to the odor. This symposium will highlight basic processes that occur during olfactory coding to explain how we come to experience, understand and respond to odors perceptually and cognitively. Using behavioral and neurological approaches, talk topics will highlight the features that underlie olfactory cognition including: memory, expectation, attention, experience, emotion and imagery.

Basic Processes in Human Olfactory Cognition

The Difference is in the Details – A Re-Examination of Human Olfactory Memory

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How do we remember olfactory information? Is the architecture of human olfactory memory unique as compared to memory for other types of stimuli? Ten years ago, a review article (White, 1998) evaluated these questions, as well as the distinction between long and short-term olfactory memory, with three lines of evidence: capacity differences, coding differences, and neuropsychological evidence, though serial position effects were also considered. Based on the data available at the time, the article preliminarily suggested that olfactory memory was a two-component system that was not qualitatively different from memory systems for other types of stimuli. The decade that has elapsed since then has ushered in considerable changes in theories of

memory structure and provided huge advances in neuroscience capabilities. Not only have a large number of studies exploring various aspects of olfactory memory been published since that time, but a model of olfactory perception that includes an integral unitary memory system also has been presented (Wilson & Stevenson, 2003; 2006). Consequently, the structure of olfactory memory is reevaluated in the light of further information currently available with the same theoretical lines of evidence previously considered. This evaluation finds that the preponderance of evidence suggests that, as in memory for other types of sensory stimuli, the short-term/long-term distinction remains a valuable dissociation for conceptualizing olfactory memory, though perhaps not as architecturally separate systems.

Basic Processes in Human Olfactory Cognition

Smell Your Way Back to Childhood: Autobiographical Odor Memory

Maria Larsson and Johan Willander

Department Psychology, Stockholm University, Stockholm, Sweden

Three studies investigated autobiographical odor memory with regard to: (a) whole life-span age distributions, (b) phenomenological experience, (c) semantic processing, and (d) odor imagery. The first study explored influences of cue type (words, pictures, odors) on the retrieval of autobiographical memories. The results showed that odor-evoked events were older than memories evoked by words and pictures. The bump for olfactory evoked information peaked in the first decade of life (<10 years of age), whereas the bump of the word- and picture-evoked age distributions peaked in the second decade (i.e., 11-20 years of age). Also, odor memories were associated with stronger feelings of being brought back in time. A follow-up study investigated the influence of verbal processing on the retrieval of autobiographical olfactory information. The results revealed that semantic knowledge (i.e., the odor name) affected the age distribution of memories and that odor memories were associated with a higher emotional arousal. The third study addressed the influences of olfactory imagery on the age distribution and phenomenological experiences. The results showed that events evoked by odor imagery were older than memories evoked by words. It is suggested that (a) odor evoked memories are older than memories triggered by verbal and visual cues, (b) odor-evoked memories are more emotional and associated with stronger feelings of being brought back in time, (c) knowledge of an odor's name produces a shift from a more perceptually to a more conceptually driven retrieval, and that (d) imagined odor cues mimic the age distribution of events evoked by real odors. Overall, the results indicate that memories triggered by the olfactory sensory system are different from memories evoked by verbal or visual information.

Basic Processes in Human Olfactory Cognition

The Influence of Experience and Attention on Odor Mixture Quality

John Prescott[1], Elodie Le Berre[2], Thierry Thomas-Danguin[2], Noelle Beno[2], Gérard Coureaud[3] and Patrick Etievant[2]

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Odour/taste mixture interactions are strongly influenced both by prior experience with the mixture components and also by whether attention is directed towards the combination as a synthetic whole or analytically as a grouping of distinct elements. The determinants of the extent to which components in an odor mixture will completely blend are incompletely understood, and it is not clear ifeither experience or attentional processes are important in determining mixture quality. We examined the influence of these factors on the perception of those odour mixtures in which a unique quality distinct from those of the components is perceived. Three groups of subjects were either exposed to the individual odorants of a 3-component (characterised by an odour of *Pineapple*) or a 6-component (*Red Cordial*) odor mixture, or were non-exposed (control). Subsequently, half of each group was assigned to either a synthetic task, in which Ss rated how typical (i.e., representative) the mixtures were of the target odor name (*Pineapple* or *Red cordial*) and each of their components, or an analytical task, consisting of evaluations of these stimuli on several scales labelled with the target odor name and odor descriptors of the components. Only for the 3-component mixture was previous exposure to the mixture components found to decrease the extent to which the mixture was a typical example of its label (*Pineapple*). However, subjects engaged in an analytical task rated both 3- and 6-component mixtures as less typical than did subjects engaged in a synthetic task. This study supports a conclusion that perception of the characteristic quality of odor mixtures can be influenced by both perceptual learning and the engagement of optional cognitive strategies.

Basic Processes in Human Olfactory Cognition

Good Olfactory Imagers: How they Could Favor a Deep Odor Processing

Catherine Rouby, Fanny Bourgeat, Fanny Rinck, Johan Poncelet and Moustafa Bensafi

Université Claude Bernard, Lyon - Laboratoire de Neurosciences Sensorielles, Comportement, Cognition, UMR 5020. Institut Fédératif des Neurosciences de Lyon, IFR19, CNRS, Lyon, France

As odor perception, odor imagery is characterized by a large variability between individuals. Our aim was to assess in two studies whether this inter-individual variability is sustained by behavioral differences in actual odor perception. In study 1, 30 subjects from 19 to 26 years of age smelled 3 odorants (Carvone, Isoamyl acetate and Limonene, 10 repetitions of each) and judged intensity, pleasantness, familiarity and edibility. Odorants were diffused using an air-dilution olfactometer. Both odor stimulations and subjects were split in 2 sub-groups (Stimulations: unpleasant odor trials vs. pleasant odor trials according to subject's individual hedonic ratings; Subjects: ''good'' vs. ''bad'' olfactory imagers, ''GOI'' and ''BOI'', according to their scores on an imagery questionnaire). Statistical analysis showed no difference between groups and between stimuli for intensity; a significant difference was found between good and bad imagers for familiarity and edibility: GOI judged all odors as more familiar and more edible than BOI. This is in accordance with previous studies showing an enhanced odor familiarity in good olfactory imagers. In study 2, we set out to characterize whether these effects rely on a deeper processing of smells in GOI by recording the length of their sniffs during odor perception. Eight BOI and 8 GOI were selected and had to perform the same psychophysical tasks of study 1 using a different set of odorants (Cineole, Isoamyl acetate and Heptanal). Results revealed that GOI sniffed longer all odors, and again judged these same odors more edible and familiar. This supports the hypothesis of a deeper odor processing and a better access to odor semantics in good olfactory imagers.

Basic Processes in Human Olfactory Cognition

Perceptual and Neural Pliability of Odor Objects

Jay A. Gottfried

Northwestern University, Chicago, USA

The idea that ''top-down'' factors such as learning and experience help define how an odor is perceived provides an alternative (though sometimes less acknowledged) perspective on odor processing, complementing ''bottom-up'' olfactory models that have focused on odorant chemistry. Human

psychophysical studies increasingly show that the exact same volatile molecule can evoke different odor percepts, within the same individual, and even in a short span of time, indicating that odor quality perception arises not only from the chemical attributes of the smell, but also from the idiosyncratic experiences of the smeller. This presentation will focus on the role of learning and context in modulating human percepts of odor objects, at both the behavioral and neural levels, with some emphasis on our own research involving olfactory fear conditioning in humans. These findings demonstrate that emotional learning can transform perceptually indiscriminable odors into discriminable percepts, highlighting the great capacity of the human sense of smell to improve with experience. At the end of my talk I will briefly consider some of the unique advantages that higher-order cognitive processes confer upon an organism inhabiting an odorous environment.

Nasal Trigeminal Function: Qualitative, Quantitative and Temporal Effects

Symposium: Nasal Trigeminal Function: Quantitative, Qualitative and Temporal Effects

Dennis Shusterman[1], Thomas Hummel[2], Diana Bautista[1], Wayne Silver[3] and Paul Wise[4]

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Nasal trigeminal somatosensation – and in particular trigeminal chemoreception – gives rise to percepts that are phenomenologically intertwined with olfaction, but can be experimentally differentiated. Historically, trigeminal chemoreception has been ascribed to the function of unspecialized neurons termed "polymodal nociceptors," giving rise to what has been regarded as a poorly differentiated sensory modality. Newer psychophysical, anatomic, molecular biologic, electrophysiological, and functional imaging data, however, suggest a more complex sensory system. Psychophysical studies have shown the potential for qualitative encoding, cognitive modulation, and complex concentration-time interrelationships. Electrophysiological recordings – both peripheral and central – have been found to closely mirror stimulus parameters. Functional CNS imaging techniques have provided insight into the central projections of nasal trigeminal afferents, and confirm functional differentiation between nasal trigeminal chemoreception and olfaction. Finally, molecular neurobiology has made tremendous gains in explaining transduction events. We will review nasal trigeminal response, from applied stimulus and signal transduction, to peripheral and central electrophysiological response, and finally to psychophysics in order to gain insight into this sensory system whose complexity is increasingly being recognized.

Nasal Trigeminal Function: Qualitative, Quantitative and Temporal Effects

Molecular and Cellular Mechanisms of Trigeminal Chemosensation

Diana M. Bautista[1], Sven E. Jordt[2], Jan Siemens[1] and David Julius[1]

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In the nasal cavity, trigeminal somatosensory neurons enable us to detect a wide range of environmental stimuli, including pressure, temperature, and chemical irritants. Natural plant-derived irritants have served as powerful pharmacological tools for identifying receptors underlying chemosensation in our somatosensory system, as illustrated by the use of capsaicin, menthol, and wasabi to identify the heat-sensitive ion channel TRPV1, the cold-sensitive ion channel TRPM8, and the irritant receptor TRPA1, respectively. The role of these channels in trigeminal thermosensation and pain will be discussed.

Nasal Trigeminal Function: Qualitative, Quantitative and Temporal Effects

Anatomic and Electrophysiologic Basis of Peripheral Nasal Trigeminal Chemoreception

Wayne L. Silver

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Anatomy: The trigeminal nerve (TN) provides sensory information from the eyes, nose, and mouth. A subset of TN fibers contains the neuropeptides substance P and CGRP and responds to chemical irritants in the environment. Axons in the ethmoid and nasopalatine branches of the TN innervate the nasal mucosa where they ramify repeatedly. TN endings extend close to the nasal epithelial surface stopping at the line of tight junctions only a few micrometers from the surface. A single ethmoid nerve axon may send branches to the nasal mucosa, olfactory bulb and the spinal trigeminal complex. Traditionally, irritants are thought to stimulate free TN endings in the nasal epithelium. Recently, however, solitary chemoreceptor cells (SCCs) have been found scattered throughout the nasal cavity. The SCCs are contacted by trigeminal nerve fibers and express T2R "bitter-taste" receptors, alpha-gustducin and TRPM5.

Electrophysiology: Peripheral trigeminal electrophysiologic recordings in response to irritants have been obtained from the mucosa (negative mucosal potential, NMP) and the nerve to analyze characteristics of trigeminal stimuli. NMP recordings have shown topographic differences in the responsiveness of the mucosa to chemical irritants. Responses to a wide variety of irritants have been recorded from the ethmoid nerve. The more lipid soluble the compound, the lower the threshold. Nerve recordings have also suggested several mechanisms by which irritants elicit responses. Bitter substances elicit responses from the ethmoid nerve and cause a change in respiration indicating stimulation via SCCs. SCCs themselves respond to chemical stimuli and may be contributing to the detection of nasal irritants.

Nasal Trigeminal Function: Qualitative, Quantitative and Temporal Effects

Central Processing of Trigeminal Activation in Humans

Thomas Hummel[1], Emilia Iannilli[1], Johannes Frasnelli[1], Johannes Gerber[2] and Julie A. Boyle[3]

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While numerous FMRI studies have been performed on the processing of olfactory information the intransal trigeminal system so far has not received much attention. In a pilot study stimulants were presented within a constantly flowing airstream birhinally to activate the olfactory (phenylethyl alcohol, H_2S) or the trigeminal (CO_2) nerves. Both, olfactory and trigeminal stimulation activated the ventral insular cortex. Intranasal trigeminal stimulation additonally led to an activation of the midbrain, superior temporal gyrus, anterior caudate nucleus, and the dorsolateral orbitofrontal cortex. Cerebellar activation was reduced relative to odorous stimuli. For all stimuli, right-sided activity was more pronounced. These results suggested that processing of intranasal activation follows a pattern which is, at least to some degree, similar for both trigeminal and olfactory stimulation. This emphasizes the fact that there is a high degree of interaction between the different aspects of the chemical senses. Such interactions can also be seen in patients with acquired olfactory loss who exhibit reduced trigeminal

sensitivity due to the lack of a central-nervous interaction. Both, the orbitofrontal cortex and the rostral insula appear to be of significance in the amplification of trigeminal input which is missing in patients with olfactory loss. On peripheral levels, however, adaptive mechanisms seem to produce an increase in the trigeminal responsiveness of patients with hyposmia or anosmia.

Nasal Trigeminal Function: Qualitative, Quantitative and Temporal Effects

Dynamics of Nasal Chemesthesis

Paul M. Wise and Charles J. Wysocki

Monell Chemical Senses Center, Philadelphia, USA

Dynamics, or how stimulation occurs over time, influences the somatosensory impact of volatile chemicals. Within an experimental session, sensation waxes with steady presentation over seconds to minutes, may reach a plateau, and then may fade. Long-term occupational exposure can desensitize the trigeminal system. Short- and long-term dynamics might be mediated by different mechanisms. For brief intra-nasal exposures, i.e., up to about 10 seconds, studies have systematically manipulated both time (duration of exposure) and concentration to maintain a fixed perceived intensity or a fixed level of detection. A simple mass integration model described the trade-off between concentration and time quite well: a fixed-ratio increase in duration compensated for a fixed-ratio decrease in concentration. However, for most compounds, more than a two-fold increase in duration was required to compensate for cutting concentration in half. For example, for ethanol, an increase in duration of about six-fold was required. For such compounds that display highly imperfect integration, a fixed number of molecules might have a much greater sensory impact when presented over .2 seconds than over .5 seconds. Nasal chemesthesis may be temporally sluggish compared to olfaction, but fine-grained dynamics still matter. Time-intensity ratings of nasal irritation from dynamic stimuli also support this conclusion. Though integration is generally imperfect, compounds vary widely in how for they fall short of perfect time-concentration trading. Current studies are using a structure-activity approach to determine how molecular parameters correlate with how well a compound integrates over time. Such studies, together with more complex manipulations of dynamics, may provide insights into possible underlying mechanisms.

Nasal Trigeminal Function: Qualitative, Quantitative and Temporal Effects

Qualitative Effects in Nasal Trigeminal Chemoreception

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Nasal trigeminal function (including nasal ''chemesthesis'') is important in sensing the quality of inspired air, including its temperature, humidity, and the presence of airborne chemical irritants. Even the sensation of nasal airflow patency is tied to trigeminal stimulation, a fact that is exploited in some over-the-counter pharmaceutical and consumer products. Nasal trigeminal sensations may be described with as varied a set of terms as ''burning,'' ''stinging,'' ''tingling,'' ''itching,'' and even ''cooling.'' Whereas specialized transduction events are becoming more apparent, the manner by which distinct percepts are conveyed to the central nervous system (e.g., by specialized fiber types vs. a coding system) has yet to be elucidated. Despite these physiologic uncertainties, human psychophysical studies of nasal trigeminal chemoreception have shown the potential for qualitative discrimination, cross-stimulus effects, and stereospecificity. A number of these studies are reviewed in this presentation.

The Neuroecology of Chemical Senses

Symposium on ''The Neuroecology of Chemical Senses''

Charles D. Derby

Georgia State University, Atlanta, USA

The symposium on ''The Neuroecology of Chemical Senses'' will highlight research aimed at understanding the molecular basis and evolutionary ecology of chemical communication systems. It fuses the experimental approaches of two fields: chemical ecology, which uses chemistry to explain biological interactions at the level of organisms, populations, and communities; and neuroethology, which explains the cellular basis of behavior. The symposium will include four presentations by young scientists. Ryan Ferrer of the Department of Biology at Seattle Pacific University, will present on ''Neuroecology, Chemical Defense, and the Keystone Concept'', which discusses the chemical ecology of TTX in a system involving newts, cannibalism, and birds of prey. Michiya Kamio of the Department of Biology at Georgia State University, will present a talk entitled ''Crustacean Pheromones: Signaling Sex and Danger'', describing the search for chemicals involved in two forms of intraspecific communication in crustaceans: sex pheromones and alarm signals. Carolina Reisenman of the Division of Neurobiology at the University of Arizona, will present a paper, co-authored with Jeffrey Riffell, on ''Oviposition choice in *Manduca sexta* moths: From Chemical Signals, To Neurons, To Behavior.'' This focuses on understanding the odors that lead these insects to oviposit and feed on plants. Marcus Stensmyr of the Department of Evolutionary Neuroethology at the Max Planck Institute for Chemical Ecology, will present on ''The Evolutionary Neuroethology of Arthropod Chemical Sensing, '' based on his work on molecular approaches to understanding chemical sensing in a variety of arthropods.

The Neuroecology of Chemical Senses

Chemical Communication in the Mating Behavior of Blue Crabs, and Approach to Identifying Signal Molecules

Michiya Kamio

Department of Biology, Brains & Behavior Program, and Center for Behavioral Neuroscience, Georgia State University, Atlanta, USA

Blue crabs, *Callinectes sapidus*, use pheromones in bidirectional courtship chemical signaling between males and females. Males show a courtship-specific behavior - 'stationary courtship paddling' - to pubertal females that are inaccessible. This behavior is a reliable indicator of detection of the female pheromone in a bioassay guided fractionation, but the bioassay is also time-consuming. To overcome this disadvantage, a complementary approach was also applied to identify candidate pheromones – biomarker targeting. This approach uses NMR, LC-MS and CE-MS metabolomics techniques to identify molecules that are unique to or strong biased toward pubertal female urine compared to urine from other individuals. N-acetylglucosamino-1,5-lactone was identified as specific to premolt females. Bioassays show that it is detected by males but it does not evoke the full courtship response. Further biomarker targeting is in progress to identify other minor metabolites as candidate sex pheromones.

Supported by Japan Society for the Promotion of Science (JSPS) Postdoctoral Fellowship for Research Abroad.

The Neuroecology of Chemical Senses

Specialized Noses in the Arthropod Lineage

Marcus C. Stensmyr and Bill S. Hansson

Max Planck Institute for Chemical Ecology, Jena, Germany

The olfactory system directly interfaces with the external world. Changes in the chemical makeup of the environment should accordingly also affect the olfactory system. Specialization towards a single type of resource is a potent way in which the odor landscape is changed and where we can expect the olfactory system to have been adjusted over evolutionary time. I will outline a number of ongoing projects that concern specialized olfactory systems in insects and crustaceans. The Drosophila lineage holds many interesting examples of species with rather unlikely associations and preferences. E.g. D. sechellia which solely feeds on a single species of fruit, which is highly toxic to all other drosophilids and D. endobranchia which is solely found on (and inside) gecarnoid land crabs. Both species also shows altered olfactory systems vis-à-vis their closest relatives. The land crabs themselves represents another highly advanced form of specialization, where the shift from sea to land has caused all encompassing adaptations of the olfactory system in order to operate in the radically different environment. Ongoing work in the group aims at elucidating the molecular, morphological, physiological and behavioral adaptations in the olfactory system of several species of anomuran land crabs.

This work was supported by the Max Plack Society.

The Neuroecology of Chemical Senses

Oviposition Behavior in the Moth *Manduca Sexta*: From Chemical Signals, to Neurons, to Behavior

Carolina E. Reisenman, Jeffrey A. Riffell and John G. Hildebrand

ARLD Neurobiology, Tucson, USA

Olfactory cues play decisive roles in the lives of most insect species, providing information about biologically relevant resources such as food, mates, and oviposition sites. The giant moth *Manduca sexta* offers the advantages of experimentally accessible central neurons for neurophysiological studies and knowledge of olfactory-guided behaviors. This nocturnal insect feeds on floral nectar from a variety of plants (and thus serves as a pollinator), but females oviposit almost exclusively on plants in the family Solanaceae, which they recognize on the basis of olfactory cues. Hostplant-derived volatiles, however, are not static but change in response to environmental factors. In particular, plants respond to herbivory by systemically releasing blends of volatiles that attract natural enemies of herbivores. Thus, female moths should avoid ovipositing on such ''induced'' plants because they are likely to host competitors of the females' larval offspring and to attract parasitoids that would attack those progeny. To date, the sensory-neural bases for host-site selection by moths – and particularly the neural processing of olfactory information about herbivore-induced volatiles – are largely unexplored. This presentation will describe results from chemical-ecological, neurophysiological, and behavioral experiments aimed at understanding the neural mechanisms that allow a specialist insect such as *M. sexta* to evaluate host-derived volatile mixtures to make appropriate oviposition decisions. Importantly, these investigations are focused on a naturally mutualistic insect-plant system, in which both the plant and the insect benefit, thus allowing inferences about adaptive behavior and function of olfactory systems.

Supported by NIH, NSF and CIS (UofA).

The Neuroecology of Chemical Senses

Neuroecology, Chemical Defense, and the Keystone Concept

Ryan P. Ferrer[1] and Richard K. Zimmer[2]

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The emerging field of neuroecology unifies principles from diverse disciplines, scaling from biophysical properties of nerve and muscle cells to community-wide impacts of trophic interactions. Here, these principles are used as a common fabric woven from threads of chemosensory physiology, behavior, and population and community ecology. Effects of the guanidine alkaloid, tetrodotoxin (TTX), and the free-amino acid, arginine, coalesce neurobiological and ecological perspectives. TTX is one of the most potent natural poisons ever described, and it is introduced into stream communities by one host species. In mountain ranges along the Pacifc coast of North America, this compound functions as a chemical defense in adult newts (*Taricha sp.*). When borrowed by resistant consumer species (snakes, *Thamnophis sp.*), however, it is used in chemical defense against higher order predators. Alternatively, TTX serves as a chemosensory excitant that warns conspecific newt larvae of their cannibalistic elders. Behavioral reactions of adult and larval newts are modified by arginine (a precursor to TTX biosynthesis), in association with alternative prey. Adult newts feed preferentially on worms over conspecific young, and arginine is abundant in fluids emitted from these invertebrate prey. Whereas arginine is a strong adult predatory search attractant, it suppresses cannibal-avoidance reactions to TTX in conspecific larvae. A diverse array of physiological traits, expressed differentially across many species, would promote TTX and arginine in keystone roles, with vast ecological consequences at multiple trophic levels. Such cascading effects are predicted to impact profoundly community-wide attributes, including species compositions and rates of material exchange.

IFF Award Lecture: Molecular Systems of Taste

Bittersweet Genetics

Danielle R. Reed

Monell Chemical Senses Center, Philadelphia, USA

Within groups of humans or mice, there are some individuals that are ''blind'' to certain stimuli but have an otherwise normal sense of taste or smell. Genetic tools have been used to find the biological origins of these differences. For instance, the observation that differences exist in sensitivity to sweetness among strains of inbred mice led to the discovery of one subunit of the sweet receptor, *Tas1r3*. Likewise, human differences in the perception of bitterness led to the discovery of a family of receptors and in particular *TAS2R38*. The first step forward for new projects is to establish heritability using classical methods. Toward that goal, projects are currently ongoing using human twins to determine the genetic contribution to perception of odor and taste. Likewise, in mice, projects are ongoing to establish heritability and to identify genes that contribute to sweet intake and preference, as well as non-classical ''tastes'' like that for calcium. One benefit of the well-defined phenotype-genotype relationships observed for *Tas1r3* (mouse) and *TAS2R38* (human) is that we can now dissect the modifiers of these relationships, like genetic background, developmental effects, and learning and experience. Finally, the alleles identified are naturally-occurring and thus may have been maintained in the population by natural selection. Therefore diversity among indivudals in taste and smell probably helps maintain genetic fitness.

What Can Pathology Tell us About Physiology?

What Can Pathology Tell us About Physiology?

Eugene R. Delay[1] and Thomas Hummel[2]

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The intent of this combined symposium/workshop is to help bridge the gap between the clinical realm and the research laboratory by focusing on the experimental analysis of clinical taste and smell dysfunctions for the basic researcher. The clinical literature has a growing mass of experimental evidence showing how disorders such as epilepsy, Alzheimer's disease, stroke, or surgically-induced injury to peripheral nerve, can have devastating effects on olfactory and gustatory functions. Symposium speakers will present current research on a wide range of pathological conditions, or treatments of those conditions, that compromise chemosensory functions in ways that may provide insights into the fundamental physiological functions of theses systems. For example, a loss of function might be an early symptom with diagnostic value that helps the clinician identify the disease state and might also provide clues that help the basic researcher find deviations at the very core of the chemosensory cell's genetic networks and cell signaling pathways. The workshop will introduce the non-clinician to common diagnostic and experimental tests of olfactory and taste functions through demonstrations and hands-on experiences. These demonstrations will include psychophysical tests (e.g., taste strips, UPSIT, the Sniffin' Sticks, the Sniff Magnitude Tests, and electrogustometry), electrophysiological measures (e.g., event-related potentials to odorous stimuli, recording of electro-olfactograms), and functional MRIs in response to olfactory or gustatory activation.

What Can Pathology Tell us About Physiology?

What Can the Olfactory Mucosa Tell us About Pathology?

Alan Mackay-Sim

Griffith University, Brisbane, Australia

An impetus for human stem cell research is to provide cellular models of disease. We generated stem cell lines from 42 persons with Parkinson's disease or schizophrenia or no neurological condition. Duplicate cell lines were initially grown as ''neurospheres'' in serum-free medium containing EGF and FGF2, after which they were passaged into a serum-containing medium and expanded in number as adherent cultures. mRNA was prepared and hybridised against the Illumina human Ref8 Beadchip array. For the majority of cell lines the fluorescence values from the biological replicate arrays were very similar (r>0.99) demonstrating a high level of repeatability. Overall, these cells expressed 10,127 of the 22,184 genes represented on the chip. 2,549 genes were differentially expressed in cells from patients with Parkinson's disease (n=19), compared to cells from controls (n=14). Similarly, cells from patients with schizophrenia (n=9) differentially expressed 1600 genes. There was little overlap in the genes differentially expressed in Parkinson's disease, compared to schizophrenia. Independent qPCR confirmed the findings of the microarrays for 7/8 genes and quantitative ELISA has confirmed the differential expression of one protein identified as a down-regulated gene in Parkinson's disease. Several cell signalling pathways and cell function networks had multiple genes with altered expression in each of the disease states. Functional studies have demonstrated altered cell behaviour consistent with one of these multiply altered cell signalling pathways. We conclude that neural stem cells from the human olfactory mucosa can provide insights into human neurological diseases and disorders. The challenge is to determine if these are cause or consequence of an underlying cellular pathology that is central to the disease

What Can Pathology Tell us About Physiology?

Olfactory Agnosia as a Model for Smell Impairment in Early Alzheimer's Disease

Jay A. Gottfried, Wen Li and James D. Howard

Northwestern Univeristy, Chicago, USA

The sense of smell is commonly disrupted in Alzheimer's disease. Indeed, smelling impairments often coincide with, or precede, the onset of classical cognitive problems. It is generally agreed that Alzheimer's patients have deficits of odor discrimination early in the course of illness, followed by increasing deficits of odor detection as the disease advances. This dysfunction appears to be specific to odorous stimuli, as gustatory and visual discriminations remain intact. The fact that odor discrimination is impaired in early stages of Alzheimer's disease, in the absence of frank anosmia, language deficits, or generalized cognitive decline, is consistent with a clinical syndrome of olfactory agnosia. Such a picture is akin to the visual agnosias classically arising from ventral temporal lesions that impair visual object recognition. In this presentation I will briefly review the literature regarding olfactory dysfunction in Alzheimer's disease and present some new preliminary imaging data from our lab (also see Howard, Li et al., abstract at this meeting) suggesting that neural representations of odor quality are degraded in primary olfactory structures that are early targets of neuropathology in this disorder. Our findings suggest that the use of olfactory imaging techniques may provide a novel diagnostic biomarker of disease onset in Alzheimer's disease.

What Can Pathology Tell us About Physiology?

Taste Function after Middle Ear Surgery

Masafumi Sakagami

Department of Otolaryngology, Hyogo College of Medicine, Nishinomiya, Japan

The chorda tympani nerve (CTN) controls taste in the anterior two thirds of the tongue on each side and runs close to the ear drum. CTN is frequently damaged by traction, stretching and cutting during surgical procedures. Because many surgeons consider hearing improvement to be the most important postoperative result, taste disturbance has rarely been focused on in the last four decades. In the present study, we examined the changes of CTN function before and after middle ear surgery using questionnaire and electrogustometry (EGM). The following results were obtained for the past ten years. In the preservation of CTN, (1) younger patients, especially 10 years old, had a higher rate of recovery of taste function (80-90%) than middle-aged and older patients (40-50%); (2) taste disturbance and tongue numbness were more frequently found in patients with preservation of CTN than in those with section of CTN; (3) the patients with non-infectious diseases had postoperative symptoms and elevation of EGM threshold more frequently than did those with infectious diseases; (4) the operation on the second side is recommended after the taste function on the first side recovers to normal level in case of bilateral otosclerosis; (5) the CTN function deteriorated on the diseased side as much as the healthy side in elderly patients and we do not have to pay as much attention to the CTN on elderly patients as on young and middle-aged patients. In section of CTN, (1) most of the patients with unilateral or bilateral section did not complain of taste disturbance within 1-2 years after surgery; (2) fungiform papillae became atrophic long time after section of CTN. These findings help explain the potential complications to the patients before surgery. Surgical video will be presented regarding manipulation of CTN.

What Can Pathology Tell us About Physiology?

Temporal Lobe Epilepsy and Temporal Lobe Resection: Influences on Olfactory Function

Richard L. Doty

University of Pennsylvania Smell and Taste Center, Philadelphia, USA

Temporal lobe epilepsy (TLE) and temporal lobe resection (TLR) damage limbic-related structures important for olfaction, including the entorhinal cortex, amygdala, and hippocampus. Olfactory testing is a unique probe of such pathology, given that olfactory afferents project ipsilaterally from the nose to the cortex without first synapsing in the thalamus. In this presentation the results of a recent study of ours are described in which the influences of unilateral TLE and TLR on olfactory function were rigorously determined in a large number of patients and matched controls. State-of-the-art tests of odor identification, detection, discrimination/memory, and odor event-related potentials (OERPs) were employed. Each side of the nose was separately assessed in patients with left- or right-side epileptic foci. Regardless of focus side, TLE was associated with significant bilateral loss of the ability to identify and detect smells, with somewhat greater loss occurring on the right than on the left side of the nose for odor identification. Relative to controls, male TLE subjects exhibited poorer UPSIT scores than female TLE subjects. Shorter P2 latencies and larger N2 OERP amplitudes were observed bilaterally that were more marked for patients with left hemisphere foci. TLR resulted in additional declines in psychophysical indices of function as well as in N2 OERP amplitudes. All of these measures were unrelated to the amount of tissue resected. In the TLE patients, significant correlations were present between UPSIT scores and volumes several olfactory-related CNS structures. This study demonstrates how olfactory dysfunction reflects central pathology within regions of the temporal lobe and clarifies the nature of the olfactory changes that occur in epilepsy and temporal lobe resection.

What Can Pathology Tell us About Physiology?

What Happens if One Part is Missing – Interactions Between the Chemical Senses

Antje Welge-Luessen

University Hospital Basel, Basel, Switzerland

There is ample evidence that chemical senses interact. Simultaneous taste stimulation influences olfactory perception and vice versa. Moreover, trigeminal and olfactory sensory inputs interact as well as trigeminal and gustatory sensory inputs. In everyday life, most stimuli stimulate more than one chemical sense simultaneously. Looking at patients with defined disorders and localised losses of parts of the chemical senses, these interactions and their impact on the perception as such can be studied. Based on clinical findings and experimental studies this talk will explore the interactions of the chemical senses. Moreover, the implications if parts of the chemical senses are either destroyed or lacking are elucidated.

Epidemiological Studies of Taste and Smell

Symposium on Epidemiological Studies of Taste and Smell

Howard J. Hoffman[1], Karen J. Cruickshanks[2] and Barry Davis[3]

[1]Epidemiology and Biostatistics Program, DSP, NIDCD, NIH, Bethesda, USA, [2]School of Medicine and Public Health, University of Wisconsin, Madison, USA and [3]Taste and Smell Program, DSP, NIDCD, NIH, Bethesda, USA

NIDCD is interested in fostering epidemiological research of taste and smell disorders, since impairments of the chemosenses may have important implications for health. There have been few attempts to measure the prevalence of olfactory and taste dysfunction in populations and there are many methodological challenges to be addressed. These challenges differ from those faced in studies based in the clinic or research laboratory. Two years ago at the annual meeting of the Association for Chemoreception Sciences, the Institute convened a Workshop to assess the feasibility of developing quick taste and smell tests that would be suitable for use in large, nationally-representative health examination surveys, such as the National Health and Nutrition Examination Survey (NHANES). This symposium will include presentations from recent and continuing epidemiological studies and discuss some of the methodological challenges of measuring function in field studies in children and adults, needed tools and resources, and barriers to progress.

Epidemiological Studies of Taste and Smell

Olfactory Impairment in Adults: The Epidemiology of Hearing Loss Study and the Beaver Dam Offspring Study

Carla R. Schubert[1], Karen J. Cruickshanks[1], Claire Murphy[2], Charles W. Acher[1], Guan-Hua Huang[3], Barbara E.K. Klein[1], Ronald Klein[1], Michael B. Miller[4], F. Javier Nieto[1], James S. Pankow[4] and Ted S. Tweed[1]

[1]University of Wisconsin, Madison, USA, [2]San Diego State University, San Diego, USA, [3]National Chiao Tung University, Hsinchu, Taiwan and [4]University of Minnesota, Minneapolis, USA

Olfactory function may be important for environmental and nutritional safety and enjoyment. Population-based epidemiologic studies of olfaction are needed to understand the magnitude of the health burden, identify modifiable risk factors and develop and test prevention and treatment strategies. However, measuring olfaction in large studies is challenging and requires repeatable, efficient methods which can measure change over time. Experiences from two large cohort studies, the Epidemiology of Hearing Loss Study (EHLS) and the Beaver Dam Offspring Study (BOSS) will be shared. In both studies, the San Diego Odor Identification Test (SDOIT) was used to measure olfaction.[1] Subjects were asked to identify eight common household odors (such as coffee and chocolate). Olfactory impairment was defined as correctly identifying less than 6 out of 8 odorants after two trials. EHLS participants (n=2491) were age 53-95 years at the time of the first measurement (1998-2000) and participants in the on-going BOSS range in age from 21-84 years. The prevalence of olfactory impairment in the EHLS was 25% overall, more common in men than women and increased with age.[1] Five years later (2003-2005), olfaction was measured a second time in the EHLS participants. The majority of EHLS participants (84%) were classified the same. Among the unimpaired at the baseline 12.5% became impaired while 31% of those impaired at baseline improved to unimpaired. Factors associated with change will be discussed as will new data from the BOSS cohort.

[1]Murphy C, Schubert CR, Cruickshanks KJ, et al., Prevalence of Olfactory Impairment in Older Adults. JAMA 2002;288: 2307-2312. NIH AG11099 and AG021917.

Epidemiological Studies of Taste and Smell

Assessing the Prevalence of Olfactory Dysfunction in Pediatric Populations

Pamela Dalton[1], Julie Mennella[1], Beverly Cowart[1,2], Edward Pribitkin[1,2], Kyle Fisher[2] and James Reilly[3]

[1]Monell Chemical Senses Center, Philadelphia, USA, [2]Thomas Jefferson University, Philadelphia, USA and [3]Nemours A.I. duPont Hospital for Children, Wilmington, USA

Although several potential etiologies for smell loss (i.e., head trauma, allergic rhinitis, enlarged adenoids) are common among children, studies

evaluating the prevalence of olfactory dysfunction in this population are rare. Several challenges present to the clinician or researcher hoping to evaluate odor identification ability in young children. Children are likely to be unfamiliar with many of the odor stimuli used in adult tests and have limited ability to read and identify labels among the choice alternatives, which is the typical adult response option. Consequently, specialized forms of olfactory tests must be developed for this population. Based on the format of the San Diego Children's Odor Identification Test, we are developing a short form odor identification test utilizing standardized odor stimuli in which participants match 6 odors to pictures of the odor source. The pilot version of this test is being administered to children between the ages of 3-16 as part of the pre-surgical intake evaluation at the Nemours A.I. duPont Hospital for Children and as part of basic research studies at the Monell Center. The hospital study population is broad and includes children undergoing ENT surgery as well as control subjects (children undergoing general surgery), with approximately 50 children per week eligible for evaluation. To improve correct interpretation of the results, stimulus familiarity is evaluated by having their parent/guardian complete the test and answer a short questionnaire about the child's experience with the various odor stimuli. The challenges faced in studying this population as well as extrapolation to larger scale populations will be discussed.

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Epidemiological Studies of Taste and Smell

Individual Differences in Olfaction: Genotype–Phenotype Associations

Charles J. Wysocki[1], Danielle R. Reed[1], Yehudit Hasin[2] and Doron Lancet[2]

[1]Monell Chemical Senses Center, Philadelphia, USA and [2]Weizmann Institute of Science, Rehovot, Israel

There is considerable variability in individual perception of odor. Some of this variation may result from the genes that are expressed in the olfactory epithelium. Many human olfactory receptor (OR) genes are non-functional, which suggests that these genes do not encode proteins that participate in olfactory transduction processes, which may result in specific anosmia. However, some OR genes are fully functional in some groups of people while at the same time they are pseudogenes in other people. We have been focusing on a subset of OR genes that are known to be variable across people; these genes are called segregating pseudogenes. To evaluate the extent that these segregating pseudogenes contribute to variation in olfactory sensitivity we have tested over 300 people for sensitivity to ~ dozen odorants and have analyzed the segregating pseudogenes from the DNA of the participants. Initially, we have focused on OR7D4, previously implicated in the perception of androstenone, and OR11H7P, suggested to be involved in the perception of isovaleric acid. For the most part: specific anosmias appear to be independent of each other; they do not appear to be gender-specific; they may be related to ethnic/racial group. We will discuss the relationships between genotype and phenotype and present findings on relationships among the various phenotypes under study.

Epidemiological Studies of Taste and Smell

Epidemiological Studies of Taste and Smell: Invited Discussant

Claire Murphy[1,2]

[1]San Diego State University, San Diego, USA and [2]University of California, San Diego, USA

Epidemiological data for estimating the prevalence of chemosensory disorders have been less available than such data for other modalities. Several reasons for this will be discussed: the time-consuming nature of many existing tests, stimulus delivery in a large-scale study, and the rationale for inclusion in a large-scale epidemiological study. The expense of mounting such a study is a significant factor and thus, the opportunity to include measures of chemosensory function in ongoing community-based studies has greatly facilitated the collection of recent data. A number of challenges still exist for future studies, including cross-cultural issues in stimulus design, testing of special populations, and the optimal analyses of population-based chemosensory data.

Epidemiological Studies of Taste and Smell

Measuring Taste Impairment in Epidemiologic Studies – The Beaver Dam Offspring Study

Karen J. Cruickshanks[1], Carla R. Schubert[1], Derek J. Snyder[2], Linda M. Bartoshuk[3], Charles W. Acher[1], Clint T. Baldwin[4], Guan-Hua Huang[5], Barbara E.K. Klein[1], Ronald Klein[1], F. Javier Nieto[1], Michael B. Miller[6], James S. Pankow[6] and Ted S. Tweed[1]

[1]University of Wisconsin, Madison, USA, [2]Yale University, New Haven, USA, [3]University of Florida, Gainesville, USA, [4]Boston University, Boston, USA, [5]National Chiao Tung University, Hsinchu, Taiwan and [6]University of Minnesota, Minneapolis, USA

Gustatory function may play an important role in determining diet and nutritional status and therefore indirectly impact health. There have been few attempts to study the spectrum of taste function and dysfunction in human populations. Epidemiological studies are needed to understand the impact of taste function on public health, identify modifiable risk factors and develop and test strategies to prevent clinically significant dysfunction. However, measuring taste function in epidemiological studies is challenging requiring repeatable, efficient methods which measure change over time. Insights gained from translating lab-based methods to a population-based study, the Beaver Dam Offspring Study (BOSS) will be shared. In this study, a generalized labeled magnitude scale (gLMS) method was used to measure taste intensity of filter paper disks saturated with salt, sucrose, citric acid, quinine, or 6-n-propylthiouracil and a gLMS measure of taste preferences was administered.[1] An inexpensive camera system to capture digital images of fungiform papillae and a masked grading system to measure the density of fungiform papillae were developed. Adult children of participants in the population-based Epidemiology of Hearing Loss Study in Beaver Dam, Wisconsin are eligible for this study. The parents were residents of Beaver Dam and 43-84 yrs of age in 1987-88; offspring range in age from 21-84 yrs in 2005-2008. Methods will be described and preliminary results about the distributions of taste function in the BOSS cohort will be presented.

[1]Bartoshuk L.M, et al., Psychophysics of sweet and fat perception in obesity: problems, solutions and new perspectives. Philos Trans R Soc Lond B Biol Sci 2006;36:1137-48. NIH AG021917.

Epidemiological Studies of Taste and Smell

Prevalence of Prop Tasters among Children in the ALSPAC Study, Bristol, England and Associations with Genetic Profile and Nutritional Intake

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Kingdom and [4]Department of Community Dentistry and Behavioral Science, University of Florida, Gainesville, USA

Objective: To confirm the genetic basis for bitter tasting in a large population and assess associations with dietary intake and health outcomes. *Methods:* Children from the Avon Longitudinal Study of Parents and Children (ALSPAC) were invited to a research clinic when they were 10 years old. Prior to the visit they recorded (with parental help) all food/drinks consumed over 3 separate days in a diary. This diary was checked and expanded in discussion with a nutritionist in the clinic. Afterwards, a PROP (bitter tasting compound) taste test was administered using a standardised protocol. The child recorded intensity of the taste on a general visual analogue scale (analysed on a linear scale 0-10). The diet diaries were used to assess nutrient and food group intakes. DNA samples had been gained from these children in previous contacts and were analysed for genetic variation in *TAS2R38* – a gene associated with bitter tasting ability. Tasting ability (taster/non-taster) was predicted from the genetics and tested against the PROP results.

Results: A total of 4,795 children (from 13,988 children in the original cohort) completed the PROP taste test. It was repeated in 168 children with a correlation of 0.62 ($r^2 = 0.39$). Genetic information was available for 4,178 of these children. An association between bitter compound tasting ability and *TAS2R38* variation was confirmed. Predicted 'non-tasters' had a median score of 3.75 [IQR 5.2]; predicted 'tasters' median score was 8.07 [IQR 2.5]. Associations with differences in dietary intake of both food and nutrient will be presented together with certain health effects.

Conclusions: The genetic basis of bitter tasting was confirmed in this large group of children. These findings may be very important in explaining differences in relation to dietary intake.

Epidemiological Studies of Taste and Smell

Surveying Food/Beverage Liking: A Tool for Epidemiological Studies to Connect Chemosensation With Health Outcomes

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Public health agencies such as the Centers for Disease Control acknowledge potential roles that "taste" preferences play on chronic disease risk including obesity. Chemosenses drive selection of highly liked foods from a food supply abundant in fat, sugar and salt at the expense of less-preferred/less-available foods like fruits and vegetables. Functional variation in chemosensation arises from genetics, development, environmental exposures and aging. Emerging research has shown that chemosensory variation impacts chronic diseases via food choice, particularly in relation to intakes of fat, sugars, vegetables and alcohol, with implication for health promotion efforts. We contend that assessing dietary risk via food/beverage liking holds promise for linking chemosensation with health outcomes in population-based studies. Surveying liking is a time-efficient, cognitively simple task versus typical intake measures (eg, frequency surveys, dietary records), which are tedious to complete and labor-intensive to interpret. Because of cognitive issues of memory and restraint on intake, individuals under or over report intakes, leading to inaccurate conclusions about diet-disease relationships. Laboratory- and community-based studies of adults demonstrate that surveys of preference for fat and sweet likely reflect habitual intake, as they explain variability in adiposity and adiposity-related health outcomes such as blood pressure. Statistical models show that bitter taste phenotype explains variability in intake and adiposity via food and beverage preference. Inclusion of surveys of food/beverage liking may increase the ability to determine how chemosensory variation moderates chronic disease risk, with the potential to inform prevention efforts at a public health level.

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Epidemiological Studies of Taste and Smell

Perspectives on Future Community-Based or Nationally-Representative Epidemiological Research Studies of Olfactory and Taste Impairment

Howard J. Hoffman and Barry Davis

NIDCD, NIH, Bethesda, USA

Epidemiological studies of olfactory and taste disorders are needed as both may have important implications for health. There have been few attempts to measure the prevalence of olfactory and taste dysfunction in populations and there are many methodological challenges to be addressed. These challenges differ from those faced in studies based in the clinic or research laboratory. The studies to be described in this session represent translational research, in that the tests employed were initially developed for use with patients in the clinic or laboratory and subsequently modified for use in testing subjects from large community, population-based samples. This symposium includes presentations from existing epidemiological studies and will discuss some of the methodological challenges of measuring function in field studies in children and adults, needed tools and resources, and barriers to progress. As part of the NIH Blueprint for Neuroscience Research, a "Toolbox" of brief exam measures in the sensory domain (also in cognition, emotion, and motor domains) is being developed for future use in clinical trials and epidemiologic studies. In addition to the NIH Toolbox, other promising approaches are under development that may be utilized in future large, population-based studies, such as the U.S. National Health and Nutrition Examination Survey (NHANES). Our objective is to improve the chemosensory (smell and taste) health of individuals and the public through epidemiologic research that measures important aspects of smell and taste function while also assessing potential risk factors and other conditions associated with taste or smell disorders or impairments. This research may identify prevention strategies or suggest promising areas for future clinical trials of treatment interventions.

Peripheral Taste Functions

Insulin Activates Enac and Regulates Salt Intake in Mice

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Diabetes is a profound disease that results in a severe lack of regulation of systemic salt and water balance. Following from our early work on the endocrine regulation of salt taste at the level of the epithelial sodium channel (ENaC), we have begun to investigate the role of insulin in the regulation of salt taste. We have characterized behavioral responses to NaCl using a mouse model of acute hyperinsulinemia. Insulin-treated mice show significant avoidance for NaCl at lower concentrations than the control group using short-term taste assays. Interestingly, these differences between groups were abolished when amiloride (100 μM) was added into NaCl solutions suggesting that insulin was regulating ENaC. To test for the ability of insulin to alter ENaC function, we performed patch clamp recording on isolated mouse taste cells (TRCs). In fungiform and vallate TRCs that exhibit functional ENaC currents (e.g. amiloride-sensitive Na^+ influx), insulin (5-20 nM) caused a significant increase in Na^+ influx at -80 mV (EC_{50} = 6.1 nM). The insulin-enhanced currents were inhibited by amiloride (30 μM). Similarly, in ratiometric Na^+ imaging using the Na-sensitive dye SBFI, increasing extracellular Na^+ from 0 to 140 mM elicited an increase in Na^+ influx in a subset of TRCs. Insulin treatment (20 nM) enhanced the Na^+ movement in TRCs consistent with its action in electrophysiological assays. The ability of insulin to regulate ENaC function is dependent upon the enzyme PI3-kinase since treatment with the inhibitor LY294002 (10 μM) abolished insulin-induced changes in ENaC currents. Our results are consistent with a role for insulin

in maintaining functional expression of ENaC in mouse TRCs and may be an example of the ability of the gustatory system to respond to nutritional challenges.

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Peripheral Taste Functions

Transport of Glutamate and GABA on the Taste Buds that Express GAD 67, a Glutamate Decarboxylase

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Our recent research further substantiates the hypothesis that GAD67, an isoform of glutamate decarboxylase (GAD, EC:4.1.1.15) which produces γ-aminobutyrate (GABA) from L-glutamate, plays a key role in the taste mechanism. We have found that GAD67 exists in the typeIII taste bud cells by using GAD67/GFP knock-in mouse, immunohistochemical and RT-PCR methods [Nakamura et al., AChemS (2007) 104 & press release 10]. The antibody against GABA stained the type III taste buds, which suggested that GAD67 was enzymatically active. Our recent research also found the presence of GABA receptor subtypes (α1, α5, β2, β3, γ3, R1a, R1b, R2) in the taste buds; thus, both $GABA_A$ and $GABA_B$ receptors may play roles in GABA signal transduction. These data also suggested the possibility that GAD67might be a key enzyme for the taste signal pathway, since its substrate and product are a known umami component and a ligand for chloride ion channel, one of the GABA receptors, respectively. In this study, we have investigated the transporting system of both glutamate and GABA within taste bud cells. By using immunohistochemical and RT-PCR methods, the expression of glutamate transporter and GABA transporter on the mouse taste buds were examined. We found that glutamate transporter subtypes, GLAST, GLT-1, and EAAC1, and GABA transporter subtypes, GAT1, GAT3, and GAT4, were expressed in the mouse taste buds. The results suggest that taste buds are capable of importing glutamate and of exporting GABA. The produced GABA may act as a ligand to GABA receptors; however, whether or not GABA acts from inside or outside of the cells needs to be determined. Our present results offer additional evidence for the hypothesis that GAD67 may be the key enzyme for the taste signal transduction mechanism.

Peripheral Taste Functions

Response to Serotonin Agonist in Mouse Type II Taste Cells

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Among the various candidate neurotransmitters at the taste bud level, serotonin (5HT) has been detected in mammalian taste cells (Yee et al., 2001) as well as the 5HT1A receptor (Kaya et al., 2004). Serotonin is released from type III taste cells in response to taste stimulation (Huang et al., 2005) but its precise target has not been studied. Since serotonin modulates the detection threshold of bitter and sweet stimuli in Human (Donaldson et al., 2006), we hypothesize that serotonin acts on Type II cells which possess the transduction machinery for bitter, sweet and umami compounds. We isolated circumvallate taste cells from transgenic mice expressing GFP from the TRPM5 promotor to identify type II cells. Results showed that a subset of the TRPM5-GFP cells respond to BP554 (10μM), a potent agonist of the 5HT1A receptor. In addition, a few non-TRPM5-GFP cells also responded to BP554 and to the bitter tastant denatonium. On the contrary, taste cells responding to 55mM KCl (likely type III cells) never responded to the 5HT1A agonist. Our results suggest that type II cells possess the 5HT1A receptors allowing a communication between III cells and Type II cells. Further experiments will determine if 5HT affects responses to sweet and bitter stimuli.

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Peripheral Taste Functions

Sourness-Suppressing Peptides in Beef

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Objective: Sourness-suppressing peptide has been found in conditioned pork loin 1). This study was performed to identify the sourness-suppressing peptides in beef and to clarify their mechanism of sour taste suppression.

Methods: Peptide fraction was prepared from bovine loins vacuum-cooked at 60 degrees for 6 hours after conditioning at 0 degrees for 14 days. This peptide fraction was applied to ODS column on HPLC, and peptides in beef were separated. Some major peptides increased during postmortem aging and cooking were identified and synthesized. The sourness-suppressing activity of peptide was evaluated by sensory evaluation and electro-physiological methods.

Results: HPLC analysis showed that many peptides increased during postmortem aging and cooking. One major peptide was isolated and identified APPPPAEVPEVHEEVH (peptide-B) with MW 1734, which was clarified to be derived from troponin T in bovine loin. The synthesized peptide-B suppressed the sour taste by sensory evaluation. For further evaluation, cRNA of human ASIC2a (human acid-sensing ion channel 2a) was injected and expressed in *Xenopus* oocyte. The peptide-B (0.1%) partially inhibited the lactic acid-induced current in oocyte at pH 4.5. The mechanism of sourness suppression by the peptide was speculated to comprise inhibition of the interaction of sour taste substances with its ion-channel.

Conclusion: A sourness-suppressing peptide (peptide-B) was also identified from conditioned beef as well as pork, and its activity was clarified by sensory evaluation and electro-physiological methods. *1) Okumura, T., Yamada, R., and Nishimura, T, "Sourness-suppressing peptides in cooked pork loins", *Biosci., Biotechnol. Biochem.*, **68**, 1657-1662, 2004.

Sex (& Taste), Drugs (& Taste), & Rock and Roll (& Taste)

Multimodal Sensory Integration of Courtship Stimulating Cues in *Drosophila Melanogaster*: Gustation, Olfaction and a Whole Lot More

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Finding a mating partner is a critical task for an organism. It is in the interest of males to employ multiple sensory modalities to search for females. In *Drosophila melanogaster*, vision is thought to be the most important courtship stimulating cue at long distance, while chemosensory cues (gustation and olfaction) are used at relatively short distance. When visual cues are not available, olfaction and mechanosensation/hearing are the critical cues that allow the male to detect the presence of a female in a large arena and initiate courtship. Once initiated, gustation maintains and amplifies behavior and drives it to completion if the target is appropriate (i.e. a virgin female). How does a male decide to terminate courtship toward an

inappropriate (unreceptive female ormale) target? Males have compounds on their cuticles that are aversive to other males. Chemical cues transferred to females by males during mating change both her behavior and her attractiveness to other males leading to reduced courtship of previously mate females. The intrinsic salience of all of these cues is likely to be modulated by learning.

Sex (& Taste), Drugs (& Taste), & Rock and Roll (& Taste)

Consumption of Palatable Foods Inhibits Pain-Related Behaviors

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Eating and escaping from injurious stimuli are both important to survival. We asked which of these behaviors would trump the other. The clear answer is that rats continue to eat during noxious stimulation of mild and even moderate intensity, forgoing escape and withdrawal reactions. Such defense of feeding from interruption by noxious stimulation is mediated by the medullary raphe, a region critical to morphine analgesia. Withdrawals from painful stimuli are suppressed in rats fed *ad libitum* while they are eating chow or chocolate chips or while they are ingesting water, sugar, or saccharin solutions delivered intraorally. Thus the suppression of withdrawals from noxious stimulation during ingestion is independent of hunger, appetite and caloric content. Within the context of freely-available, high fat food, the defense of eating from interruption may contribute to obesity. However, rats interrupt ingestion of non-preferred solutions, or of solutions associated with nausea or illness, to withdraw from noxious stimulation, a possible substrate for illness-associated anorexia. In sum, these findings suggest that obesity and anorexia are simply opposite answers to the same action selection problem, whether to continue or interrupt eating available food.

Sex (& Taste), Drugs (& Taste), & Rock and Roll (& Taste)

Sugar and Fat and Drugs, Oh My!

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Concepts of food addiction have evolved from the idea that specific components of food are addictive, to the idea that the palatability or taste of food activates addiction-related neuronal processes. Sweet tasting foods, for instance, have been shown by others to activate reward-related pathways in the brain and, when consumed intermittently and excessively, to induce neuronal and behavioral changes in rats that may model addiction-like alterations in humans (1). Our work with intermittent excessive (binge) consumption of fat has shown similar outcomes, i.e. behavioral, pharmacological, and neuroanatomical alterations that may be relevant to addiction. Behaviorally, rats with brief intermittent access to an optional source of fat: a) consume more in a brief period of time (binge) than do rats with more regular access to the same fat, b) escalate intake across time to a greater extent than do controls, c) consume more when extended access is provided, d) increase progressive ratio responding for fat, and e) exhibit 'addiction'-like behavior for cocaine. Pharmacologically, the binge rats respond to dopamine receptor blockade differently than do controls. Neuroanatomically, the binge rats show enhanced FOS expression in the anterior cingulate, a region of the brain thought to be involved in the development of addiction-related behavior (2). Taken together, the available data suggest that intermittent excessive consumption of fatty foods, like intermittent excessive consumption of sweets, may induce addiction-like alterations in behavior and neurobiology. Whether this is due to pre-ingestive stimulation of taste pathways and/or post-absorptive actions has not yet been determined. 1. Avena, et al., 2003, Neurosci Biobehav Rev; 2. Kalivas, Volkow, 2005, Am J Psychiatry.

Sex (& Taste), Drugs (& Taste), & Rock and Roll (& Taste)

Tasting Sounds: Tone-Related Anticipatory Activity in the Gustatory Cortex of Awake Rats

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Electrophysiological recordings of gustatory cortical (GC) neurons in anesthetized animals have provided fundamental insights into the organization of taste responses. The role of GC in taste processing, however, remains largely mysterious. From experimental and theoretical work comes the suggestion that cortex, rather than simply coding for the identity of stimuli and their physical properties, may cooperate with other forebrain areas to interpret sensory information in relation to task requirements and internal states. I will provide evidence in support of this framework and present a series of experiments where the same tastes are presented in contexts in which cognitive states such as attention and expectation vary. Specifically, I will compare neural responses to stimuli delivered by the experimenter at random times with activity evoked by the same tastes when self-administered following auditory cues. Differences in the temporal profile of GC responses will be discussed, with a particular emphasis on the anticipatory activity triggered by auditory cues. Finally, to better understand the brain dynamics observed in these two conditions, I will relate the results of experiments in which neural activities from GC and two sources of top-down modulatory inputs – amygdala and orbitofrontal cortex – were simultaneously recorded. The results of these experiments lead to the suggestion that GC anticipatory activity may be the result of systems-wide interactions in which top down influences modulate GC responsiveness to meaningful environmental cues.

Poster Session I: Tues July 22

Genetic Composition of Mouse Lines Selectively Bred for High and Low Saccharin Intake

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Inbred mouse strains differ in their responses to sweet taste stimuli, in part, due to allelic variation of the *Tas1r3* locus. However, analysis of hybrids between the C57BL/6ByJ (B6) and 129P3/J (129) strains suggests that other genetic loci are also involved. To confirm the existence of such loci, we crossed B6 inbred mice with 129.B6-*Tas1r3* congenic mice. Despite the genetic identity at the *Tas1r3* locus, mice from the F2 generation varied in consumption of 20 mM saccharin. Beginning from the F2 generation, we started selective breeding of lines with high and low saccharin intake, which resulted in a phenotypical divergence between these two lines. This demonstrates that genes other than *Tas1r3* also affect saccharin consumption. To find positions of these genes, we have genotyped mice from the 4th and 8th generations of selective breeding with markers on chromosomes (Chr), linked to saccharin intake in segregating crosses between progenitor strains. In the S4 generation there was a significant divergence of the High and Low lines in frequencies of alleles in all these chromosomal regions. Consistent with a greater phenotypical difference between the lines in S8 compared with S4, line divergence in allele frequencies in these regions increased in S8. At the locus on Chr1, all S8 mice from the High line were

homozygous for B6 alleles, and all S8 mice from the Low line were homozogous for 129 alleles. For all other loci alleles segregated within the selected lines. We expect that the phenotypical selection will reach its limit when all loci under selection will be fixed in a homozygous state. Our approach of selective phenotype-based breeding coupled with genotyping is an efficient way for high-resolution mapping of genes involved in taste responses to saccharin.

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Poster Session I: Tues July 22

Psychogenomics of Umami Taste Perception and Human TAS1R Genes

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Monosodium glutamate (MSG) elicits a unique taste in humans often labeled savory or umami. *TAS1R1* and *TAS1R3*, two members of the *TAS1R* class of taste G-protein coupled receptors (GPCRs), have been hypothesized to function in combination as a heteromeric glutamate taste receptor in humans. In this study, we completely sequenced the coding regions of genomic *TAS1R1* and *TAS1R3* for 48 individuals of a single large pedigree who had been phenotyped for their responses to MSG, monopotassium glutamate (MPG), and mixtures of MPG with 5' ribonucleotides. Subjects were tested repeatedly with both forced-choice and magnitude rating measures of umami taste. In our small sample population we found that *TAS1R3*, the common subunit to the *TAS1R* sweet and umami taste heteromer receptors, contained more variations than did *TAS1R1*, contrary to earlier reports. Additionally, we found six rare nonsynonymous single nucleotide polymorphisms (nsSNPs) that have been previously reported. We will present the degree to which the variations and haplotypes of these *TAS1R* genes predict variations in umami taste perception.

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Poster Session I: Tues July 22

A TAS2R9 Variant is Associated with Dysglycemia in Humans

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TAS1R- and TAS2R-type taste receptors are expressed in both the gustatory and digestive systems,where they play important roles in the detection of sweet- and bitter-tasting stimuli. In the gut, these receptors can modulate the secretion of hormones important for the control of insulin biosynthesis and release. Thus, differences in taste receptor efficacy could impact glucose homeostasis. We show that an allele of *TAS2R9* (T560, encoding Val187) is associated with dysglycemia in humans. We conducted an association analysis of haplotype-tagging single nucleotide polymorphisms linked to all *TAS1R* and *TAS2R* genes in the Amish Family Diabetes Study. We identified a significant association of the *TAS2R9* T560 allele in non-diabetic Amish individuals with indicators of glucose and insulin dysregulation, including insulin resistance, decreased glucose absorption and higher insulin and glucose area-under-the-curve during an oral glucose tolerance test. TT homozygotes in the Amish also showed an increased risk of type 2 diabetes. TAS2R9 is expressed in human enteroendocrine cells, consistent with a normal role in the regulation of incretin secretion. Together, these findings indicate that a TAS2R9 receptor variant negatively impacts glucose homeostasis.

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Poster Session I: Tues July 22

Novel TAS2R SNP Associations with Taste Sensation, Liking or Intake for Alcoholic and Bitter Non-Alcoholic Beverages

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Of 38 *TAS2R* genes, functional variation has only been demonstrated for *TAS2R38* and *TAS2R43/44*. We have reported that the propylthiouracil bitterness phenotype (PROP) predicts the bitterness of scotch, beer, coffee and grapefruit juice (Lanier et al., 2005) and alcohol intake (Duffy, Peterson et al., 2004), consistent with data that *TAS2R38* haplotype predicts alcohol intake (Duffy, Davidson et al., 2004). Here, we expand our examination of bitter receptor genetics in relationship to orosensory phenotype and ingestive behaviors to include new *TAS2R* SNPs in a laboratory-based study of adults. Data were analyzed with analysis of covariance; significance criterion $p \leq 0.05$. First, we examined genetic effects on self–reported alcohol intake and sensory (bitterness/sweetness) and hedonic ratings of blended scotch whisky. We identified a novel *TAS2R16* SNP not previously associated with intake, and confirmed the putative relationship with *TAS2R38* haplotype in a new cohort. Neither genotype explained variability in bitterness/sweetness or liking of the Scotch, suggesting the ability of PROP bitterness to predict scotch bitterness is not specific to *TAS2R38*. Second, we identified several SNPs that may help explain variation in the bitterness of coffee and grapefruit juice. Variability in coffee bitterness was explained by SNPs on *TAS2R3*, *TAS2R4* and *TAS2R5*. For grapefruit juice, SNPs on *TAS2R48* and *TAS2R60* associated with increased bitterness with concomitant decreases in sweetness and liking. In summary, it appears that *TAS2R38* variation is only one of several examples of functionalvariation in bitter receptor genetics with the potential to influence ingestive behaviors and health outcomes via oral sensation and hedonic response to beverages.

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Poster Session I: Tues July 22

Do Polymorphisms of the *TAS1R3* Gene Influence Long-Term Sugar Intake and Weight Gain in Mice?

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Tas1r3 is a polymorphic gene that codes for two alternate forms of a sweet taste receptor (T1R3) in mice. Mouse strains with the *Tas1r3*-Sac-b allele (e.g., C57BL/6 and FVB/N) show higher daily intake of 10% sucrose and fructose solutions than do strains with the *Tas1r3*-Sac-d allele (e.g., 129P3 and AKR) during 2-day tests. We asked whether the strains with the *Tas1r3*-Sac-b allele would continue to show higher daily sugar intakes over a 40-day test, and if so, whether the high sugar intake would lead to greater weight gain. To this end, we offered four strains of mice (C57BL/6, FVB/N, 129P3 and AKR) one of five test solutions (water, 10% sucrose or fructose, 34% sucrose or fructose), together with water and lab chow. We measured fluid intake and weight. There were three main findings. First,

the strain differences in daily sugar intake were inconsistent over time. For instance, the C57BL/6J strain consumed more of the 10% sucrose solution than the 129P3 strain during the initial 2 days, but the relative daily intake of both strains became equivalent across the subsequent 38 days. Second, all strains gained more weight on the sucrose than on the fructose solutions. Third, there was no clear relationship between sugar intake and weight gain. For instance, the AKR strain had the lowest intake of sucrose, but it nevertheless gained the most weight and accumulated the most fat. Likewise, the FVB strain had the highest intake of the sugar solutions, but it experienced the smallest weight gain. Taken together, our findings indicate that polymorphisms of *Tas1r3* do not influence 40-day patterns of sugar intake and weight gain in mice. Future studies will explore the apparent disconnect between sugar intake and weight gain.

Poster Session I: Tues July 22

T1R3 Knockout Mice Learn to Prefer Flavors Paired with Intragastric Sucrose Infusions

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Sweet taste in the mouth is mediated by the T1R2+T1R3 taste receptor. Deleting either receptor subunit substantially reduces sweetener preferences in mice. In 24-hr tests, however, T1R3 knockout (KO) mice developed strong preferences for concentrated sucrose solutions that generalized to dilute sugar solutions in subsequent tests. This suggests that these KO mice, like wildtype (WT) mice, learn flavor preferences based on the post-oral effects of sugar. Yet, these KO mice are missing T1R3 receptors in the gut that are important for intestinal sugar detection and absorption. This study asked whether T1R3 KO mice show impaired flavor conditioning following intragastric (IG) sucrose infusions. T1R3 KO and C57BL/6J WT mice were fitted with chronic IG catheters and housed 24 h/day in infusion cages with ad lib chow. On alternate days they drank a flavored solution (e.g., grape 1% Intralipid) paired with matched IG infusions of water, and a different flavored solution (e.g., cherry 1% Intralipid) paired with IG infusions of 16% sucrose. (Intralipid, a stable soybean oil emulsion, was used as a solvent because it stimulates strong fluid intake in both KO and WT mice.) Following 6 one-bottle training days, the mice were given a two-bottle test with both flavors paired with the matched infusions. The KO and WT mice consumed similar amounts of the sucrose-paired flavor (12.6 vs. 13.3 g/day) and strongly preferred it, by 92% and 90%, to the water-paired flavor. Therefore, although T1R3 KO mice fail to prefer sucrose in initial oral tests, they can learn strong flavor preferences based on post-oral actions of the sugar. These data indicate that T1R3 receptors in the gut are not required for the post-oral rewarding effects of sucrose.

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Poster Session I: Tues July 22

A Major QTL on Mouse Chromosome 17 Influencing Consumption of Calcium, Saccharin and Other Taste Compounds

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The BTBR T^+ *tf*/J (BTBR) strain has among the highest preferences for calcium of 40 inbred strains tested. In two-bottle choice tests, BTBR mice drank ~10x more 50 mM $CaCl_2$ than did NZW/LacJ (NZW) mice. To determine the genetic variation underlying this difference, 610 F_2 mice received two-bottle choice tests and were genotyped for 625 SNP markers spanning the entire genome. Linkage analyses identified a QTL on proximal chromosome 17 with remarkably strong linkage to preferences for 50 mM $CaCl_2$ (LOD = 45) and 2 mM saccharin (LOD = 101) as well as several other taste compounds. The NZW alleles of this QTL dominantly decreased $CaCl_2$ consumption and increased saccharin consumption. Therefore, we have begun to develop a congenic strain set in which the chromosomal region underlying the QTL has been introgressed from the NZW strain onto the BTBR background. In the N_5 generation (with ~96.9% BTBR background), we have distinguished 7 haplotype groups. One haplotype group (n = 15) has only a ~3 cM NZW fragment introgressed. It has significantly lower preferences for 50 mM $CaCl_2$ (14% vs. 41%) and higher preferences for 2 mM saccharin (49% vs. 81%) than does a group homologous for BTBR over the region of interest (n = 27). These results reveal the existence of a gene (or genes) on chromosome 17 with a strong influence on calcium and saccharin consumption. A promising candidate is *Itpr3*, an inositol trisphosphate receptor implicated in taste perception. The congenic mice will provide an invaluable resource for cloning and functional characterization of the calcium consumption-related gene(s).

Poster Session I: Tues July 22

A Genetic Analysis of Saccharide Taste in *Drosophila Melanogaster*

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The ability to perceive the healthfulness of a food by taste is critical for survival. Taste discrimination among nutritious and toxic substances leads to the acceptance or rejection of a potential food source, respectively. *Drosophila melanogaster* have taste responses very similar to humans and other mammals. Carbohydrates are a major food type for both mammals and flies. *Drosophila* concentration-response ranges for saccharides are within the perception ranges of humans. There is also extensive genetic homology between flies and humans. Using an assay that measures taste reactivity (proboscis extension assay) we have screened 18 inbred lines for their responses to three saccharides; sucrose, fructose, and glucose to isolate lines that exhibit extreme taste-response behavior. The inbred lines have exhibited wide variability in taste response to the tested saccharides. We have selected two lines for further study and have found that they are profoundly different in their taste responses to sucrose in an ingestive preference assay (free roaming ingestion choice test) as well as the taste reactivity test. We will cross these lines and use Quantitative Trait Loci (QTL) analysis to search for genes whose variability correlates with their saccharide taste perception and ingestion.

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Poster Session I: Tues July 22

The Chemoreceptor Gene Family of the Waterflea *Daphnia Pulex*: Many GRS but No ORS

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Daphnia pulex is the first aquatic invertebrate to have its genome sequenced. *Daphnia* is thought by some to be the sister group to terrestrial insects and the availability of a draft genome sequence has allowed us to investigate the chemoreceptor gene repertoire of this arthropod. Here we describe the chemoreceptor superfamily in *D. pulex*, finding six lineages of Grs, for a total of 58 genes. These 58 Grs form a major species-specific cluster of 49 genes, a smaller cluster of 5 genes, as well as a highly divergent singleton (Gr58), each with their own distinctive gene structure. The final three genes, Grs55-57,

share distinctive amino acid motifs and cluster with the sugar receptors of insects, and may illuminate the origin of this distinctive subfamily. These chemoreceptor genes presumably mediate the many ''taste'' and ''smell'' functions of this freshwater crustacean. Consistent with the prediction of Robertson et al., (2003), we find no evidence of Ors. This includes the basal and highly conserved ortholog of the unusual DmOr83b protein implicated in partnering with each of the specific Ors in individual olfactory sensory neurons. While it is always possible that this entire Or gene lineage was lost at some point in the history of *Daphnia pulex*, we think it more likely that the insect Or lineage is indeed a relatively recently expanded gene lineage concomitant with the evolution of terrestriality in the insects or their hexapod ancestors. We present EST and tiling array support for the predicted gene models, and preliminary expression data comparing gene expression between the sexes, which points to a female biased set of gustatory receptors.

Poster Session I: Tues July 22

Taste Genetics and Food Choices in Carnivores

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In species of Carnivora, hypercarnivorous behavior is often characterized on the bases of dental and cranial morphological specializations. Knowledge of the interrelationships among structure and function of the sweet receptor, the animal's behavioral sensitivity to sweeteners, and its diet choice are of potential importance to the reconstruction of the phylogenetic relationships among members of this Order. Previously, studies demonstrated that the known insensitivity to sweeteners shown by Felidae could be explained by the loss of the T1R2 receptor in this order. To explore the molecular and evolutionary events that lead to a loss of sweet taste function in hypercarnivores, we have sequenced the *Tas1r2* gene in 33 carnivorans and tested sweet taste responses in many of these. The sequencing results show that 1) the 247-bp microdeletion in exon 3 seen in cats is restricted to Felidae; 2) with the exception of cats and the linsang, no deletions or stop codons were found in other carnivorans, suggesting that some hypercarnivores in this Order have likely evolved toward their hypercarnivory via the process of convergent evolution. The structural alterations that led to malfunction of the sweet receptor in these hypercarnivores most likely occurred independently, and as such, these alterations are not identical (e.g., cats and linsang). The behavioral data show that genet, mongoose, meerkat, ferret and red panda prefer some sugars over water. In contrast, lion, Pallas' cat, and otter do not respond to any of the sugars tested. To some extent, these behavioral responses are consistent with their dietary choices. The results of this research program will provide novel insights into the nature and function of taste receptor genes and how their variation affects taste perception and food preference.

Poster Session I: Tues July 22

The Difference of Expression of Htas2rs in Japanese Live in Kanto and Kansai Area Around 20 Years Old

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We developed a new human taste evaluation method that analyzed hTAS2Rs by RT-PCR used for scraping smear of the tongue (SCREP). Unlike conventional gustometry, SCREP was the taste evaluation methods that do not require a taste sense of a subject. The results of SCREP resembled results of biopsy. The difference of normal subjects and taste disorder subjects was clear using SCREP. The Japanese eating habits can be divided into two areas. One is Kanto area around Tokyo and another is Kansai area around Osaka. In this study, expression characteristics of hTAS2Rs in Kanto and Kansai area were measured by SCREP. The subject, normal person that the people were not appealed of taste disordered, 18-25 years old, Kanto that the people lived in around Tokyo, Kansai that the people lived in Osaka and Okayama were recruited by Showa Women's University. The expression characteristics of taste receptors were measured by SCREP about these subjects. As a result, the taste receptors as hTAS2R9, 10, 16 and 48 were expressed over 45% subject lived in Kanto and Kansai area. These were marked no difference between Kanto and Kansai area. It is suggested that these receptors are could be contributed to Japanese common taste. In contrast, hTAS2R1, 7, 45 and 49 marked difference in expression characteristics between Kanto and Kansai area. The expression frequency of these taste receptors were 20-60% in Kanto area,that was higher than Kansai area. It is suggested that these receptors are could potentially be viewed as make difference of Kanto and Kansai specific taste. Atlast, hTAS2R3, 4 and 8 were difference between Osaka and Okayama, both in Kansai area. It is suggested that these receptors are could potentially be viewed as make a area specific taste.

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Poster Session I: Tues July 22

Genetic Alteration of Gurmarin Sensitivity is Associated with Formation of both Receptor and Neural System for Sweet Taste in Mice

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Gurmarin (Gur) is a peptide that selectively suppresses sweet taste responses in rodents. The inhibitory effect of Gur differs among tongue regions and mouse strains. Recent studies demonstrated that co-expression levels of genes controlling sweet receptors (T1r2/T1r3 heterodimer) versus Gα-protein, gustducin, are much lower in Gur-insensitive posterior circumvallate papillae than in Gur-sensitive anterior fungiform papillae. In C57BL, sweet-responsive fibers of the chorda tympani nerve resulted in two distinct groups: Gur-sensitive and Gur-insensitive. We previously produced a *dpa* congenic strain (*dpa*-CG) whose genetic backgrounds are identical to Gur-weakly-sensitive BALB except gene(s) controlling Gur-sensitivity derived from C57BL. Here, we investigated the potential link of Gur sensitivity with the co-expression for T1r2/T1r3 receptors and gustducin by comparing those of taste tissues of Gur-sensitive (C57BL, *dpa*-CG), and Gur-weakly-sensitive (BALB) strains. The results indicated that co-expression ratios among T1r2, T1r3 and gustducin in the fungiform papillae were significantly lower in Gur-weakly-sensitive BALB mice than in Gur-sensitive C57BL and *dpa*-CG mice. Furthermore, we investigated if such changes in taste cells of *dpa*-CG mice would lead to formation of two distinct nerve fiber groups or an intermediate type. The results demonstrated that *dpa*-CG mice possess two distinct Gur-sensitive and Gur-insensitive groups, although they exhibited lower response frequencies to sweet compounds than C57BL mice. These results suggest that genetic alteration of Gur sensitivity may be associated with formation of both receptor and neural system for sweet taste in mice.

Poster Session I: Tues July 22

The Effect of Polymorphisms in 4 HTAS2R Genes on Prop Bitterness Perception

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The bitter taste receptor TAS2R38 accounts for the majority of perceptual variation to the thyroid toxin 6-*n*-**prop**ylthiouracil (PROP). However, there are clearly other factors in addition to TAS2R38 polymorphisms that account for individual differences in PROP perception. The two most common alleles of TAS2R38 are AVI and PAV, after the Alanine/Proline, Valine/Alanine, Isoleucine/Valine polymorphisms at residue positions 49, 262, and 296 respectively. The AVI receptor is weakly activated by PROP and the PAV receptor is strongly activated. While most subjects with the AVI/AVI diplotype are either weakly or unresponsive to PROP, occasionally AVI/AVI subjects find PROP strongly bitter. We hypothesize that another hTAS2R receptor is rescuing this function due to an allele they possess that codes for a receptor that responds to PROP. hTAS2R4 has been shown to respond to PROP *in vitro* and it along with hTAS2R3 and hTAS2R5 are related to hTAS2R38 by sequence and proximity on chromosome 7. Additionally hTAS2R1 appears to be a paralogue of TAS2R38. We report here whether AVI/AVI subjects who respond strongly to PROP have specific alleles of TAS2R1, 3, 4, or 5 that account for their rescue of sensitivity to PROP.

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Poster Session I: Tues July 22

Tastant-Evoked Fos Expression in the Nucleus of the Solitary Tract of Mice Lacking P2X Receptors Important for Taste Transmission

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ATP is an essential signaling molecule for transmission of taste information from taste buds to the gustatory nerves. Genetic deletion of the ionotropic purinergic receptor subunits P2X2 and P2X3 (DKO mice) eliminates essentially all gustatory neural responses to all tastants including monosodium glutamte (MSG). To further investigate gustatory-related phenomena in these DKO mice, we examined taste-evoked Fos-like immunoreactivity (FLI) in the nucleus of the solitary tract (nTS) following taste stimulation by voluntary consumption. Water-deprived (22h) mice were allowed access to either water or 150mM MSG for 20-30 min. and then survived an additional 60 min before perfusion fixation with paraformaldehyde. Brains were processed for immunoreactivity for c-fos using the Oncogene Ab-5 antibody. In both wildtype (WT) and DKO mice, compared to water, consumption of MSG increases the number of FLI cells in nTS. This finding is surprising in that there is little gustatory neural response to MSG in the DKO mice. Nonetheless, DKO mice that drank MSG tend to have fewer FLI cells in nTS than do the WT controls. These findings suggest that tastant-related information may activate cells in the nTS despite greatly diminished or absent gustatory neural input. These effects may be due to non-gustatory orosensory or post-ingestive cues.

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Poster Session I: Tues July 22

Differences in Synaptic Characteristics of Rostral Nucleus of the Solitary Tract (RNST) Neurons with Input from the Chorda Tympani and Glossopharyngeal Nerves

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Gustatory information transmitted by chorda tympani (CT) and glossopharyngeal (IX) nerves has to pass through the first central synapse in the taste pathway in rNST. Little is known about the functional characteristics of this synapse. We have characterized this synapse by stimulating the solitary tract (ST) to evoke excitatory postsynaptic currents (EPSCs) in second order neurons. Monosynaptic connections between CT and IX nerve inputs were identified by anterograde tracing and measures of EPSC latency variances (jitter <300ms). 70% of rNST neurons synapsing with the CT had all-or-none EPSCs while 70% of rNST neurons with glossopharyngeal input had graded responses to increasing stimulus intensities. All-or-none stimulus recruitment indicates activation of single afferent axons or unitary synapses. rNST neurons with CT and IX input exhibit frequency dependent depression of EPSC amplitude. Variance-mean (V-M) analysis was used to analyze synaptic transmission and modulation (Trends Neurosci. 23:105, 2000). V-M analysis indicates that CT and IX synapses differed in release possibility, quanta size and number of releasing sites. These results suggest that second order rNST neurons respond to afferent input with different patterns of EPSCs that would influence transmission of gustatory information.

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Poster Session I: Tues July 22

A Method to Visualize Synaptic Organization of Gustatory Afferents onto Individual Projection Cell Dendrites in the Nucleus of the Solitary Tract

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The gustatory portion of nucleus of the solitary tract (NTS) is the primary target of both the chorda tympani and glossopharyngeal nerves and as such is the first stage in central processing of gustatory information. However, very little is known about synaptic organization and possible convergence of these inputs onto individual cells in the NTS. In order to address this issue, we have developed an approach combining tract-tracing, high-resolution confocal microscopy, and electron microscopy. Gustatory projection cells and their dendrites are retrogradely filled by tracer injection into the parabrachial nucleus, and the chorda tympani and glossopharyngeal nerves are anterogradely filled with differing fluorophores. Confocal microscopy is used to visualize axonal and dendritic processes in three-dimensions, and instances of close apposition between an axon and a dendrite are documented. These appositions are putative synaptic sites between gustatory axons and projection cells. In order to confirm that close apposition of two fluorophores indicate synapses, the same tissue is processed for DAB visualization of all labeled profiles, and prepared for electron microscopy. Individual dendritic segments that contain previously identified putative apposition are reconstructed and the synaptic contacts confirmed. With this approach, we will be able to study the organization of the chorda tympani and glossopharyngeal nerves onto individual gustatory projection neurons, noting innervation patterns, convergence of input, and the target cell morphology.

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Poster Session I: Tues July 22

FM1-43 as a Tool to Examine Presynaptic Activation in the Taste System

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FM1-43 is a lipophilic dye that can be used to label synaptic vesicles upon exocytosis, and is a powerful tool to examine synaptic events in *in vitro* preparations. Despite its potential, this technique has not been used previously to examine synaptic events in the taste system. Briefly, neuronal stimulation results in fusion of synaptic vesicles to the plasmalemma and release of neurotransmitter. After exocytosis, vesicles are recycled by endocytosis. In the presence of FM 1-43, a newly endocytosed vesicle will incorporate the dye into its lipid membrane, and these vesicles will fluoresce intensely. Remaining extracellular dye is then washed away, leaving dye only in the vesicles. During the next cycle of stimulation, in the absence of extracellular dye, the vesicle-associated dye is released and washed out. This property allows for both visualization of loaded synaptic boutons and monitoring of vesicle unloading during subsequent stimulation. We have applied this technique to the vagal lobe of the goldfish, equivalent to the nuc. solitary tract in mammals. In this system, primary gustatory afferent fibers carry taste information from the oral cavity to the vagal lobe, a highly organized structure in the medulla. These fibers synapse onto secondary neurons in the laminated sensory portion of the vagal lobe. We loaded primary afferent synaptic boutons with FM 1-43 in the presence of DNQX, which blocks postsynaptic responses thereby permitting labeling of only primary afferent nerve terminals. This technique can be a powerful tool to pharmacologically examine the effects of agonists/antagonists of putative presynaptic receptors between primary afferent taste nerves and their target cells in the brainstem.

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Poster Session I: Tues July 22

Effects of Paired-Pulse Electrical Stimulation of the Chorda Tympani Nerve on Taste-Responsive Cells in the Nucleus of the Solitary Tract of the Rat

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Previous studies of the nucleus of the solitary tract (NTS) in the rat have shown that stimulation of the chorda tympani (CT) nerve, both electrical and chemical, results in inhibition that can alter the responses to the basic taste qualities. In the current study we electrically stimulated the CT nerve using a paired-pulse (0.1 ms pulse duration, interpulse interval = 10-2000 ms) paradigm while recording from single taste-responsive cells in the NTS. Electrophysiological responses to taste stimuli (0.1 M NaCl, 0.01 M HCl, 0.01 M quinine and 0.5 M sucrose) were recorded in separate trials. Paired-pulse electrical stimulation of the CT was applied in blocks of 100 trials (1-0.25 Hz). The effects of CT stimulation were assessed in 52 NTS cells of which 36 were taste-responsive. Paired-pulse electrical stimulation of the CT was presented to 45 cells. The majority of cells (34 of 45; 75.6%) demonstrated paired-pulse attenuation to stimulation of the CT. The distribution of peak paired-pulse attenuation was bimodal with modes at 10 ms and 50 ms. Paired-pulse attenuation that peaked late was associated with a prolonged time course and was observed in cells with longer latencies of response to CT stimulation (>10 ms). Conversely, early peak attenuation decayed rapidly and was observed in cells with shorter latencies. Moreover, cells that responded with the shortest latencies to CT stimulation responded with high firing rates to relatively few taste stimuli. Results suggest that CT input can evoke two types of inhibitory influences with different time courses in different groups of cells. This type of input may correlate with and potentially determine the tuning of taste-responsive cells in the NTS.

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Poster Session I: Tues July 22

Analysis of the Morphology, and Biophysical Properties of Solitary-Parabrachial Projecting Neurons with Known Afferent Input

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Taste information relays from the rostral nucleus of solitary tract (rNST) to the parabrachial nucleus (PBN). The morphology and biophysical properties of these rNST-PBN relay neurons identified by retrograde labeling were studied in rat brainstem slices. Afferent input to these rNST-PBN projecting neurons was identified by terminal field labeling of chorda tympani (CT), lingual branch of the trigeminal (LV) and lingual-tonsillar branch of the glossopharyngeal (IX) nerves. Lucifer yellow containing pipette solution filled the neurons during recording for later morphometric analysis. rNST-PBN projecting neurons expressing a hyperpolarization-activated transient potassium current (I_{KA}) were observed more frequently in the medial part of the rNST, CT and IX terminal fields. In contrast, rNST-PBN neurons in the lateral part rNST (LV) rarely expressed I_{KA}. Morphometric analysis revealed that approximately 80% of the rNST-PBN neurons in medial rNST were multipolar (three or more primary dendrites) while bipolar (two primary dendrites) and multipolar neurons were evenly distributed in lateral rNST. Thus, medial rNST-PBN neurons with putative gustatory input express I_{KA} and differ in morphology from neurons in lateral rNST. Neurons expressing I_{KA} potentially change the pattern of neural discharges important in rNST gustatory processing.

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Poster Session I: Tues July 22

Temporal Characteristics of Responses to Salty and Sour Tastants with Changes in Intensity in the Nucleus of the Solitary Tract

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In central gustatory structures, multisensitivity across taste qualities along with incremental changes in responses with changes in stimulus concentration can confound the message conveyed by response magnitude (evoked spike count). The purpose of the present study was to investigate whether the temporal firing characteristics of taste responses in the nucleus of the solitary tract (NTS) can disambiguate information about taste quality and intensity. Single neuron responses to NaCl (0.6M, 0.1M, 0.01M) and HCl (0.06M, 0.01M, 0.001M) and their undiluted binary mixtures were recorded from the NTS of anesthetized rats. To assess the contribution of the temporal characteristics of the response to the discrimination among tastants, a family of metrics that quantifies the similarity of two spike trains in terms of spike count and spike timing was used. As expected, results showed that the response magnitude (spike count) produced by different taste qualities and different concentrations overlapped in most cells, implying that information conveyed by spike count is imprecise. Multidimensional scaling analysis (MDS) was applied to the taste responses using a measure of similarity based on the temporal characteristics of taste

responses. Tastants representing different taste qualities (NaCl or HCl) and intensities formed distinct individual clusters (clouds) in this ''temporal coding'' taste space. Furthermore, the clusters of different taste qualities most often occupied different sides of the taste space and were arranged logically from high to low concentrations. Thus, the temporal structure of taste responses in the NTS can convey information about both taste quality and intensity without confusion.

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Poster Session I: Tues July 22

Integration of Taste Information in the CNS of the Moth *Heliothis Virescens*

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We are using heliothine moths as model organisms for studying chemosensory coding as well as appetitive and aversive learning and memory. The goal is to understand how the brain handles gustatory and olfactory information in order to identify and memorize particular taste and odour qualities. In this study we investigated the gustatory pathways as an approach to understand the neural integration in taste coding and how taste information contributes as a reinforcer in associative learning and memory formation. Extracellular recordings from taste receptor neurones on the antenna and proboscis have shown responses to sucrose, quinine and water. The two compounds sucrose and quinine, known to be phagostimulatory and aversive respectively, elicit responses in separate receptor neurones. Staining with fluorescent dye revealed axonal projections in the lateral and dorsal SOG/tritocerebrum. Intracellular recordings from the SOG combined with fluorescent staining revealed interneurones responding to sucrose, quinine, water and tactile stimuli. Most neurones were selectively exited by sucrose, others selectively exited or inhibited by quinine. In addition some neurones were exited by both tastants. The stained neurones from different brain preparations were reconstructed and registered into a standard brain atlas in order to investigate the spatial relationship between them. Most interneurones are confined locally within the SOG/tritocerebrum area with dendritic arborisations contralateral to the axonal projections. Other neurones project into the deutocerebrum, protocerebrum as well as into the connectives leading to the thoracic ganglia. These results show that in addition to separate neural pathways mediating phagostimulatory and aversive information, a third pathway mediate mixed information.

Poster Session I: Tues July 22

Synaptic Pruning from Central Chorda Tympani Axons During Postnatal Development

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During normal development, the terminal field of the rat chorda tympani (CT) nerve displays a remarkable reorganization through postnatal days 15 (P15) and P50. We examined whether circuitry level changes accompany the decrease in CT terminal field volumes in the nucleus of solitary tract (NTS). Our electron microscopic studies of synaptic organization focused in the NTS where the CT and glossopharyngeal nerves overlap at adulthood. Biotin dextran amine was used to reveal the CT terminals in NTS of rats (n=3 for each age) at P15, P25, P35 and at adulthood (>P50). Several morphometric measures of labeled terminals were examined, including synapse length, terminal cross section area, and prevalence of multiple synapses. None of these measures changed throughout development, suggesting that these parameters of CT terminal morphology mature early. Similarly, the density of CT axon fibers did not change, suggesting that axons did not retract from the region examined. However, the volumetric density of synapses formed by labeled CT terminals declined nearly 50% between P15 and P50, with the largest decline occurring after P35. This indicates that synapse reorganization occurs even in a region from which CT axons do not retract with age. Furthermore, the reduction in CT synapse density was not due to proliferating neuropil because the volumetric density of all synapses within the same regions did not decline significantly. Finally, the decrease in GABAergic targets of CT synapses suggested that the developmental loss of CT synapses might be more selective for inhibitory targets. These data suggest that in addition to a retraction of immature projection fields during postnatal development, synaptic pruning and target reorganization characterize maturation of CT axons.

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Poster Session I: Tues July 22

Competition Derived Developmental Plasticity of the Gustatory System

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Neural competition among multiple inputs during development can affect the growth and organization of circuits in many sensory systems. We aim to explore the role of competition in development of the rat gustatory nerve terminal fields in the nucleus of the solitary tract. The gustatory system has three distinct and partially overlapping inputs: the greater superficial petrosal (GSP) nerve, the glossopharyngeal (IX) nerve, and the chorda tympani (CT) nerve. The terminal field volume and degree of overlap of these nerves is greatest early in development and decreases as animals age to adulthood. We sectioned the GSP and IX at postnatal day 15 (P15) to assess the effects of lack of competition from these nerves on the development of the CT terminal field. After 35 days post nerve section, a time before GSP and IX regain function to transmit taste information, the CT was labeled with biotinylated dextran amine and subsequently visualized with Strepdavidin Alexa Fluor 488. Terminal field volumes were assessed using confocal microscopy and image analysis software. The results show that sectioning the GSP and IX at P15 results in a CT terminal field volume at adulthood much greater than that of control animals. This indicates that the CT terminal field does not reorganize and prune back as it does in normal development when competitive influences from GSP and IX are removed. These studies provide evidence that competition between individual inputs to the gustatory system plays a role in setting up the mature organization of the terminal fields.

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Poster Session I: Tues July 22

Pheromone Cues and Sensory Neurons that Mediate Pup Suckling in the Mouse

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Suckling is the defining behavior in mammals. It involves a stereotyped sequence of actions in pre-weanling infants, beginning with head scanning nipple-search behavior, leading to robust attachment. A role for olfaction in suckling has been studied in lagomorphs and a rabbit nipple-search pheromone identified, but its bioactivity is not conserved in rodents. We therefore aimed to characterize the role of olfaction in mouse suckling, by combining a behavioral assay for nipple-search behavior with recordings of neonate olfactory neuron activation by active stimuli. We found that newborn mice display stereotyped behavior in identifying the nipple of a lactating female, and this can be impeded by washing the nipple. Using mice that are

genetically deficient for olfactory subsystems, we show that the main olfactory epithelium (MOE) is necessary for robust suckling, but mice lacking a functional vomeronasal organ display normal nipple-search behavior. We have identified several natural sources that promote nipple-search, including mothers saliva. Interestingly, these cues are missing in virgin saliva. We then recorded the calcium influx in MOE neurons from newborn mice in response to stimulation by saliva. We show that while around 1% of neurons respond to both stimuli, a similar number respond to virgin saliva only and 0.6% is activated by lactating female saliva only. This odor response profile is not significantly different in adult MOE, suggesting the cessation of suckling is not due to changes in odor detection. We conclude that nipple-search behavior in mice is promoted by one or more olfactory cues detected by the MOE and that these are present in biologically relevant sources, including mothers saliva. Future work will characterize the nature of the cue and the circuitry mediating its perception.

Poster Session I: Tues July 22

Medial Amygdala Activation in Response to Artificial Main Olfactory or Chemosensory Input in Male Hamsters

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In male hamsters mating behavior is dependent on chemosensory input from main olfactory and vomeronasal systems, whose central pathways contain cell bodies and fibers of gonadotropin-releasing hormone (GnRH) neurons. In sexually-naïve males, vomeronasal organ (VNX) but not main olfactory-lesion, impairs mating behavior. Intracerebroventricular (icv)-GnRH restores mating deficits in sexually-naïve VNX males and enhances medial amygdala (Me) activation by chemosensory stimulation. In sexually-experienced males, VNX does not impair mating and icv-GnRH suppresses Me activation. Thus, main olfactory input is sufficient for mating in experienced- but not naïve-VNX males. We tested whether GnRH enhances access of main olfactory input to the amygdala using icv-GnRH and pharmacological stimulation of the main olfactory bulb (MOB). In sexually-naïve intact males, MOB stimulation produced significant activation in MeAv and MePv. MePv activation is also characteristic of chemosensory stimuli from potential competitors and predators. In sexually-naïve VNX males, in which GnRH facilitates mating, GnRH enhanced activation by MOB stimulation in posterodorsal medial amygdala (MePd), a region rich in androgen resceptors and activated by conspecific reproductive chemosignals. Conversely, in sexually-experienced VNX males, animals that do not require GnRH to mate properly after VNX, there is a depression in activation due to GnRH and stimulation in MePd, similar to their response to natural chemosensory stimulation. MeP is rich in steroid receptors and many chemosensory behaviors are steroid dependent. Thus, it is likely that steroid receptors facilitate stimulation of MeP by different types (conspecific) of chemosensory stimuli and may be selectively suppressed in MeP by others (heterospecific).

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Poster Session I: Tues July 22

Noradrenergic System in the Basolateral Amygdala During Acquisition of Conditioned Odor Aversion in the Rat: Focus on Alpha2-Adrenergic Receptors

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Conditioned odor aversion (COA) results from the association between the olfactory memory trace of an odorized-tasteless solution (conditioned stimulus, CS) and subsequent toxicosis. Previous studies have shown that the basolateral amygdala (BLA) is involved in the control of the olfactory memory trace during COA. More recently, pre-CS but not post-CS or pre-test blockade of β- and α_1-adrenoceptors in the BLA disrupted COA, thus suggesting that the noradrenergic system is involved, at least in part, in the processes that control the memory trace of the CS during acquisition of COA. In order to precise the importance of noradrenaline (NA) release in the BLA on this memory process, the present experiment investigated the effect of BLA presynaptic α_2-adrenoceptors activation during acquisition of COA. Male Long-Evans rats bilaterally implanted with cannulae aimed at the BLA were exposed to CS-toxicosis pairing using a 15 min ISI. Rats received infusions of 3 ng of the selective α_2-agonist UK 14,304 or vehicle 10 min before the CS presentation. Results showed that infusions of UK enhanced COA performances and resistance to extinction as compared to the control. This result is unexpected in reference to previous studies showing that activation of presynaptic α_2-adrenoceptors inhibits NA release in the CNS and impairs inhibitory avoidance learning. Therefore, the effect of UK may result from i) the activation of post-synaptic α_2-adrenoceptors in the BLA or/and ii) the fact that although reduced, the level of NA release in the BLA is enough for activation of post-synaptic α and β adrenoceptors, thus enabling the olfactory memory trace to last enough for its association with the delayed US.

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Poster Session I: Tues July 22

The Alarm Pheromone Increases Anxiety in Male Rats: Pharmacological Evidence Using Anxiolytics

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We previously reported that the alarm pheromone released from perianal region of male donor rats induced wide variety of responses in male recipient rats, such as aggravation of stress-induced hyperthermia, increment of defensive and risk assessment behaviors, and enhancement of the acoustic startle reflex (ASR). In addition, it was demonstrated in our previous study that the alarm pheromone increased Fos expression in brain structures such as the amygdala and the bed nucleus of the stria terminalis (BNST). These results suggest that the alarm pheromone increases anxiety in recipient rats. In this study, we examined whether anxiolytics can antagonize such pheromone effect using the ASR as an index. We used following drugs: a benzodiazepine, diazepam (0, 0.2, 0.7, and 2.0 mg/kg); a serotonin-1A receptor partial agonist, buspirone (0, 0.7, 2.0, and 5.0 mg/kg); a non-selective monoamine oxidase (MAO) inhibitor, phenelzine (0, 15, and 30 mg/kg); and a corticotropin-releasing factor subtype 1 receptor (CRF1) antagonist, CP-154,526 (0, 10, and 30 mg/kg). As seen in our previous study, the alarm pheromone enhanced the ASR in 0-mg/kg drug-injected (vehicle-pretreated) recipient rats. Such pheromone effect was dose-dependently attenuated by pretreatment with diazepam, phenelzine, or CP-154,526, whereas the pretreatment of buspirone had almost no effect on the alarm pheromone-induced enhancement of the ASR. These results suggest that the effect of the alarm pheromone is regulated via the GABAergic, monoaminergic, and CRFergic systems, whereas the serotonergic system may be less likely involved in such pheromone effect.

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Poster Session I: Tues July 22

Presence of the Main Olfactory Epithelium but not the Vomeronasal Organ is Necessary and Sufficient for Maternal Behavior in *Mus Musculus*

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The main olfactory epithelium (MOE) has commonly been associated with the processing of nonsocial odors whereas the vomeronasal organ (VNO) is believed to detect pheromones. Recent research has suggested that some pheromonal compounds are detected by the MOE and others are processed by both the VNO and the MOE. Maternal behaviour is believed to be pheromonally mediated and therefore provides a means to investigate the role of both systems in the processing of pheromones. We removed the MOE of one group of pregnant CD1 mice with ZnSO4, surgically removed the VNO of a second group, removed the MOE and VNO of a third group and a fourth group had sham treatments only. Measures of pup survival, pup retrieval and nursing behavior revealed that mice with an intact MOE had significantly more maternal behaviors than mice without an intact MOE. The presence or absence of the VNO made no difference to the level of maternal behavior. This work supports the hypothesis that the MOE is the primary mediator of maternal behavior in mice.

Poster Session I: Tues July 22

Effects of Vomeronasal Organ Removal on Medial Amygdala Response to Reproductive, Competitive and Predator Stimuli

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Species-specific chemosensory signals convey information important for reproductive and social communication. These signals may be detected by receptors in the vomeronasal organ (VNO) and/or by the main olfactory system. The information is then relayed to the medial amygdala (Me), an area that receives convergent chemosensory input from the main and accessory olfactory systems. The Me of male mice is activated by chemosensory stimuli from females and males of their own species (conspecific) and of other species (heterospecific), especially by intrinsically salient stimuli from conspecific and predator species. Known connections to hypothalamic subnuclei suggest that anterior medial (MeA) and ventral posterior medial (MePv) amygdala nuclei are related to defensive behavior and the dorsal posterior MeP (MePd) nucleus to reproductive behavior. Male mice exhibit increased FRAs expression to salient chemosensory signals, with the pattern of expression differing depending on the category of stimulus presented. Preliminary results indicate that removal of the vomeronasal organs (VNX), eliminates characteristic patterns of response in medial amygdala in mice; suggesting the vomeronasal system is necessary for normal chemosensory processing of salient chemosensory stimuli in the mouse medial amygdala. Concurrent experiments examine the role of oxytocin in MeA/P chemosensory processing, and characterize the phenotypes of cells activated by chemosensory stimuli.

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Poster Session I: Tues July 22

Primary Olfactory Areas Mediating Ferret Odor-Induced Stress Responses in Rats

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Exposures to predator odors are very effective methods to evoke a variety of stress responses in rodents. We have previously found that ferret odor exposure leads to changes in endocrine hormones (corticosterone and ACTH) and behavior. To distinguish the contributions of the main and accessory olfactory systems in these responses, studies were designed to block these two systems independently. Male Sprague-Dawley rats were treated with 10% zinc sulfate (ZnSO4) or saline. Injection of ZnSO4 destroys the nasal epithelium and renders rodents anosmic for approximately 4 days, while leaving the accessory olfactory areas intact. After testing for anosmia, rats' behavioral responses to a control or ferret odor towel were determined in a defensive withdrawal paradigm. The next morning, ferret or control odor towels were placed in the rats' home cages for 30 minutes to examine the endocrine responses. Saline treated and ZnSO4 rats visited the ferret towel less than the control odor towel. Not surprisingly, loss of the sense of smell had behavioral effects in ZnSO4 rats exposed to control odor, with a reduction in visits to the towel stimulus observed. Both saline and ZnSO4 treated rats exposed to the control odor exhibited low levels of corticosterone and ACTH, while rats exposed to ferret odor had significantly elevated levels. This suggests that blocking main olfactory processing does not block the endocrine response to ferret odor exposure. However, because of the unclear effects on behavior, we cannot conclude that the main olfactory system does not play a role. Studies are currently underway to examine the effect of vomeronasal organ removal to further distinguish the roles of the accessory and main olfactory areas in the effects of ferret odor exposure.

Poster Session I: Tues July 22

Interspecies Chemical Communication through the Accessory Olfactory System in Mammals

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Olfaction is an ancient sense able to elicit profound cognitive responses and adaptable behavioral changes. The main olfactory neuroepithelium, located in the nasal cavity, harbors sensory neurons thought to detect odorants, a very diverse class of chemicals which are collectively associated with the sense of smell. The accessory olfactory system is thought to be responsible for the detection of pheromones, a class of chemical cues released by conspecifics that trigger genetically preprogrammed behaviors and neuroendocrine changes. In rodents, this system includes the vomeronasal organ (VNO), a tubular structure in the nasal cavity, and the acessory olfactory bulb, a station from which signals generated in response to pheromones are relayed to higher order areas in the brain such as the amygdala and the hypothalamus. We show here that predator odors, which elicit a range of innate behavioral changes indicative of fear in rodents, are able to activate a subset of VNO neurons as well as the accessory olfactory bulb and groups of neurons in the medial nucleus of the amygdala and in the ventromedial nucleus of the hypothalamus. Genetic ablation of VNO function leads to a loss in the activation of those brain areas and to severe impairment in the appearance of fear behaviors in the mouse, suggesting that the accessory olfactory system is involved in the detection of predator odors and provides functional inputs to the brain areas activated in the presence of these substances. Our results indicate an expanded role of VNO function beyond pheromone detection.

Poster Session I: Tues July 22

Vomeronasal Organ is Involved in Detection of Androstenone in Mice

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Specific anosmia to androstenone (AND) affects about 50% of adult humans (Amoore, 1977; Labows & Wysocki, 1984). An animal model for this phenomenon has been developed using inbred strains of mice CBA/J (CBA) and NZB/B1NJ (NZB) (Wang et al., 1993; Voznessenskaya, Wysocki, 1994). Using a Y-maze paradigm we estimated sensitivity of NZB and CBA mice to AND. CBA mice could detect AND at a concentration 2000-fold more diluted then NZB mice (Voznessenskaya et al., 1995). In a more recent study we investigated the role of main olfactory system (MOS) and vomeronasal organ (VNO) in the detection of AND. Three basic experimental approaches were used: behavioral, vomeronasal removal (VNX) followed by histochemical verification and immuynohistochemical. VNX caused a 4-16-fold decrease ($p<0.01$) in sensitivity to AND in highly sensitive CBA mice (n=10), but did not affect AND thresholds in NZB mice (n=10). The data obtained indicate the involvement of the VNO and MOS in the detection of androstenone. We observed a specific pattern of Fos-positive cells in main olfactory bulb of CBA mice (n=6) but not in NZB (n=6) mice in response to AND stimulation. AND stimulation caused activation in the accessory olfactory bulb in both strains of mice indicating the involvement of the VNO in AND detection. Patterns of Fos-positive cells were recorded in response to androstenone stimulation (0.1% w/v) in VNO receptor tissue of both strains of mice. We observed activated cells in basal and apical zone of CBA mice. In NZB mice activation was observed only in the apical zone. Different distributions of activated receptor cells in CBA and NZB mice may explain in part differences in sensitivity to the odorant.

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Poster Session I: Tues July 22

Exposure to Stress Affects Reception of Sex Pheromones in House Mouse

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We studied the influence of long term exposure to stress on reception of sex pheromones in male mice. Three basic approaches were used: behavioral, immunohistochemical and hormone assay. Test subjects were adult male mice of different social status. Patterns of Fos- positive cells were recorded in vomeronasal organ (VNO) receptor tissue in response to stimulation with bedding from receptive females. Plasma testosterone and corticosterone was detected using ELISA technique. Non-invasive monitoring of glucocorticoid (GC) metabolites in feces was performed. Expression of GC receptors (GR) in VNO was investigated immunohistochemically. Patterns of sexual behavior were recorded for experimental and control animals. Exposure to cat odor significantly ($p<0.05$, n=10) decreased number of mountings with intromissions, number of attempted mountings and number of nasal contacts. Pattern of activation in receptor epithelium of male VNO was recorded in response to exposure of receptive female bedding. We observed activated cells in basal and apical zone of VNO receptor tissue regardless of differences in plasma testosterone level. Exposure of male mice to cat odor for 10 days completely blocked the response of VNO receptor epithelium (n=8) and was coupled with significant increase of plasma corticosterone. Similar effect we observed when males were exposed to different type of stress - low temperatures (4C, 2 hours). Using antibodies against GR (M-20, SC) we showed that GRs are expressed in receptor VNO tissue. Taking into consideration that angrogen receptors are not expressed (Chichet et al., 2007) in VNO we may explain the observed effects by the presence of GRs in VNO receptor tissue. The data obtained indicate that GC may play an important role in pheromone reception in VNO.

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Poster Session I: Tues July 22

Neuroendocrine Effects of Goldfish Pheromones on Male Goldfish (*Carassius Auratus*)

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Goldfish (*Carassius auratus*) use reproductive hormones as endogenous signals to synchronize sexual behavior with gamete maturation, as well as exogenous signals (pheromones) to synchronize spawning interactions between conspecifics. We examined the effect of two known goldfish pheromones, 17α, 20β-dihydroxy-4-pregnen-3-one (17, 20β-P) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), on the neuroendocrine systems of male goldfish. Exposure to 17, 20β-P for 4h increased plasma androstenedione (AD) levels in male goldfish, whereas $PGF_{2\alpha}$ did not have a similar effect. Further examination by real-time quantitative (RT-PCR) revealed that exposure to $PGF_{2\alpha}$ for 4h significantly increased salmon GnRH (sGnRH) mRNA levels in the telencephalon and cerebellum of male goldfish whereas 17, 20β-P did not show a similar effect. It is interesting that these two goldfish pheromones show differential effects in the neuroendocrine systems in that 17, 20β-P is more influential in the periphery whereas $PGF_{2\alpha}$ is more effective in the central nervous system.

Poster Session I: Tues July 22

Olfactory Imprinting and Homing Ability in Four Pacific Salmon

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For a better understanding on the olfactory imprinting and homing ability in salmon, three different analyses have recently been applied using four Pacific salmon, pink, chum, sockeye, and masu salmon. The first approach investigated whether the artificial water prepared by the amino acid composition of natal streams had attractive effects on selective homing movements of mature fish from the four species in a two-choice test tank. The results demonstrated that the artificial natal stream water reconstituted by the amino acid composition of natal stream had attractive effects on the selective homing movement in chum, sockeye and masu salmon, but not in pink salmon. The second approach was electrophysiological and behavioral analysis on imprinted sockeye salmon which were exposed to either L-proline (Pro) or L-glutamic acid (Glu) for two weeks during parr-smolt transformation (PST). These fish were then examined with both electro-olfactogram response (EOG) and selective homing movement during the spawning period two years later. The results demonstrated that Pro- or Glu-exposed fish revealed higher EOG than control fish as well as a significant selectivity for Pro in the two-choice test tank during the spawning period. The third approach was molecular biological analysis on the isolation of odorant receptor (OR) in four Pacific salmon by nested polymerase chain reaction (PCR) and by 3' and 5' rapid amplification of cDNA ends (RACE). This resulted in the isolation of two OR gene candidates. These results will be discussed in relation to the possible role of amino acid in the natal stream water for olfactory imprinting and homing mechanisms among four Pacific salmon, with special reference to the evolutionary aspects.

Poster Session I: Tues July 22

Changes in Expression of Soig and Odorant Receptor Genes in Olfactory Epithelium During Olfactory Imprinting and Homing Migration in Salmon

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Anadromous salmon start downstream migration after the imprinting of odors from their natal river, and they return to the same home stream by recalling these odors. For the timing of olfactory imprinting, some previous researches suggested with the use of artificial odorants that juvenile coho salmon learn the odors of their home stream during parr-smolt transformation (PST). In order to elucidate the mechanism of olfactory imprinting and homing, we recently isolated two kinds of gene, SOIG (sockeye salmon olfactory system imprinting related gene) and odorant receptor genes (ORs; LSSOR1 and 2 was isolated from lacustrine sockeye salmon, CSOR1 and 2 was isolated from chum salmon), from the olfactory epithelium of lacustrine sockeye salmon and chum salmon. However, the function of these genes remained unknown. Therefore, the function of SOIG and ORs were investigated by analyzing the expression levels of these mRNAs in the olfactory epithelium of salmon during the PST and homing migration using the real time PCR. In the analysis of lacustrine sockeye salmon during the PST and sexual maturation, SOIG, LSSOR1 and 2 mRNA levels peaked in the PST, and then tended to decrease toward the post-PST. On the other hand, LSSOR1 increased during sexual maturation in female only. During the downstream migration of chum salmon, SOIG mRNA levels peaked just prior to release, and decreased toward the estuary, although the changes of CSOR1 and 2 mRNA levels were not statistically significant. During homing migration, SOIG and CSOR1 mRNAs were elevated from the Bering Sea to the pre-spawning ground. Changes in expression levels of these genes in crucial periods of salmon lifecycles suggest that SOIG and ORs might have important roles in olfactory imprinting and homing migration.

Poster Session I: Tues July 22

Emx2 Regulates Odorant Receptor Choice in Vertebrates

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Vertebrate olfactory sensory neurons rely on a highly diverse repertoire of odorant receptors to achieve the extraordinary discriminatory capabilities of the sense of smell. Each neuron expresses only a single receptor gene and protein, chosen from approximately 1400 candidates in the mouse genome, and the molecular basis for this singular choice has long remained elusive. Here I identify the homeodomain transcription factor Emx2 as playing a central role in the regulation of odorant receptor diversity. Mice lacking Emx2 fail to express 80% of OR genes, while the remaining genes are over-represented among the sensory neuron population, thus skewing the repertoire towards a small subset of receptors. Examination of the complete expression profile for two OR gene loci reveals a continuous asymmetric requirement for Emx2 within a given locus, suggesting that Emx2 may mediate directly the enhancer function of *cis*-acting flanking sequences at each locus. Choice of a locus may represent an intermediate stage of regulation during the choice of a single receptor by the sensory neuron.

Poster Session I: Tues July 22

Stochastic Protocadherin Gene Expression Diversifies Olfactory Neurons

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The mechanisms that govern the formation of specific neural circuits are poorly understood but may include determined or stochasticmechanisms of gene expression. Determined mechanisms translate the position or birthdate of a specific neuron into coordinated patterns of gene expression that influence the unique morphology, electrophysiologic properties and patterns of synaptic connectivity of various neuronal subtypes. Recently, a second type of genetic mechanisms has been shown to regulate neuronal connectivity. Stochastic expression of olfactory receptor genes dictates an individual olfactory sensory neuron's response to odorants and also helps to establish its pattern of connectivity with second order neurons. Similarly, in the fly, the DSCAM gene family has been shown to contribute extensive diversity to many types of neurons using stochastic mechanisms. DSCAM diversity is required for several important aspects of neural circuit formation, includingdendritic tiling, axon branching and self recognition, however DSCAM diversityin the mouse is highly limited. Here, we show that a large family of protocadherin genes supplies extensive diversity to olfactory sensory neurons by stochastic mechanisms. Each individual olfactory sensory neuron expresses a unique combinatorial of protocadheirn genes, gene expression profiles are notcorrelated with olfactory receptor expression and expression is largely monoallelic, suggesting that protocadherins may serve as the mouse analog of the DSCAM gene family We have generated mice that lack protocadherin genes.These mice exhibit behavioral correlates of neurological defects, suggesting that stochastic protocadherin gene expression regulates the formation or function of specific neural circuits.

Poster Session I: Tues July 22

Odorant Receptor (or) Gene Choice is Biased and Non-Clonal in Two Olfactory Placode Cell Lines, and or RNA is Nuclear Prior to Differentiation of these Lines

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We have investigated two clonal mouse olfactory placode (OP) cell lines as a model system for studying endogenous odorant receptor (OR) regulation. Both lines can be differentiated into bipolar neurons with transcriptional profiles of mature sensory neurons. We show that single cells exhibit monogenic OR expression like sensory neurons *in vivo*. Monogenic OR expression is established in undifferentiated cells and persists through differentiation, but OR gene choice is not a clonal property of either cell line. Interestingly, OR RNA shifts from predominantly nuclear to cytoplasmic during differentiation of both cell lines. Finally, our data indicates that are stricted subset of OR genes and OR clusters are over-represented in cell populations, suggesting either a pre-existing intrinsic bias in OP founder cells or extrinsic influences arising from culture conditions.

Poster Session I: Tues July 22

Promotor-Motifs Governing the Spatial Expression Pattern of Olfactory Receptors

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Odorant receptors (ORs) of the OR37 subfamily are only expressed in olfactory sensory neurons (OSNs) which are segregated within a small area in the

center of olfactory epithelium. The encoding genes comprise highly conserved DNA motifs immediately upstream of the transcription start site which might be candidate elements for governing the spatial expression pattern. To scrutinize this hypothesis, transgenic mouse lines were generated which carry random integrated DNA constructs with the coding region of OR37C and the 5'-region including the conserved DNA motifs. In 6 out of 7 independent mouse lines, the transgene was found to be expressed in cells segregated in the characteristic clustered pattern. The number of transgene expressing OSNs varied considerably between the different lines. The transgene was expressed in a mutually exclusive manner and only one allele per neuron. The axons of transgene expressing OSNs in all mouse lines projected to the ventral domain of the olfactory bulb; those axons of OSNs located within the OR37 area generally co-converged with the axons of cells expressing the endogenous OR37C gene in the same glomerulus. Ectopically positioned transgene expressing cells formed novel glomeruli. These results demonstrate that the major features of the special OR37 topography are recapitulated by the short transgene; thus, indicating that the conserved DNA elements are indeed involved in controling the distinct expression pattern of the OR37 receptor types.

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Poster Session I: Tues July 22

Odorant Receptor Mis-Expression Alters Organization and Function of the Olfactory System

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Mammalian odorant receptors (ORs) play a critical role in the functional formation of the olfactory system. Each olfactory sensory neuron (OSN) expresses just one OR from ~1300 OR genes. OSNs expressing the same OR project their axons to two stereotypic foci in the olfactory bulb. Neither the mechanism of OR gene regulation nor the role OR plays in axon guidance is clear. We have developed a genetic approach based on the tetracycline transactivator system to explore both OR gene regulation and its role in axonal targeting. Although an olfactory sensory neuron is functionally capable of supporting the expression of multiple ORs, several levels of control exist to ensure that each neuron normally expresses only a single odorant receptor. Surprisingly, this regulation also prevents the expression of transgenes consisting of only OR-coding sequence driven by a synthetic promoter (TetO repeats). Thus the OR coding sequence is a target for the intrinsic feedback inhibition. Notably, we can overcome this suppression by expressing the same transgenic ORs precociously in immature OSNs thereby establishing a generic method to express any OR in ~90% of OSNs. Previous studies have established an instructive role of ORs that is important in formation of a functional olfactory map in the olfactory bulb. Thus as well as revealing new information about the expression of ORs, these mice provide important data about OSN targeting in the bulb.

Poster Session I: Tues July 22

Positive Selection Shapes the Function of an Odorant Receptor for Sex-Steroid Derived Odors in Primates

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Odorant receptors are among the fastest evolving genes in animals. The number of odorant receptor genes and pseudogenes varies enormously among species. However, little is known about the selection pressure that has shaped the functions of odorant receptor orthologs in different species. We have previously demonstrated a link between the *in vitro* function of a human odorant receptor, OR7D4, and *in vivo* olfactory perception of two steroidal ligands, androstenone and androstadienone, chemicals that are shown to affect physiological responses in humans. We asked whether the response of OR7D4 to androstenone and androstadienone is conserved in primate evolution. Orthologs of *OR7D4* and another closely related receptor, *OR7D1*, were cloned from different primate species. Ancestral reconstruction allowed us to reconstitute additional putative OR7D4 orthologs in hypothetical ancestral species. Functional analysis of these orthologs revealed an extremely diverse range of OR7D4 function in various primate species *in vitro*. We detected evidence for positive Darwinian selection acting on limited amino acid residues of OR7D4 throughout primate evolution. Functional analysis of the nonsynonymous changes in the subset of Great Ape lineage revealed that positively selected sites caused dramatic changes in receptor function *in vitro*. Our results support the idea that positive selection has exerted influences on the dynamic functional evolution of OR7D4 in primates.

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Poster Session I: Tues July 22

Activity-Dependent Elimination of the Olfactory Sensory Neurons Expressing Multiple Odorant Receptors

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A fundamental belief in the field of olfaction is that each olfactory sensory neuron (OSN) expresses only one odorant receptor (OR) type. Here we report that coexpression of multiple receptors in single neurons does occur at a low frequency. This was tested by double *in situ* hybridization in the septal organ in which 90% of the sensory neurons express one of nine identified ORs. Using a combination of MOR256-3 (expressed in 50% of the neurons) and Mix 8 probes (expressed in 40% of the neurons) labeled either by digoxigenin and fluorescein, we found that 0.2% (22 out of 10460) of the sensory neurons from four-week old mice was doubled-labeled. Notably, the coexpression frequency using the same probe combination was nearly ten times higher (30 out of 1444 or 2.0%) in newborn mice, suggesting a reduction of the sensory neurons expressing multiple ORs during postnatal development. In addition, such reduction depended on the neuronal activity, since it was prevented by four-week sensory deprivation via neonatal naris closure (the frequency was 1.5% at four weeks or 45 out of 2976). Furthermore, multiple mechanisms may underlie the process of eliminating the OSNs expressing multiple ORs including apoptosis. Impairment of apoptosis in Bax null mice resulted in a relatively high coexpression rate of 1.6% (54 out of 3404) in young adults. Finally, the high coexpression frequency was restored following four-week naris closure performed in young adult mice (60 out of 2884 or 2.0%) suggesting maintaining the singular expression pattern also requires activity. The results indicate that activity induced by sensory inputs plays a role in ensuring the one cell-one receptor rule in a subset of olfactory sensory neurons.

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Poster Session I: Tues July 22

Phenotypic Plasticity in Expression of Chemoreceptor Genes in *Drosophila Melanogaster*

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Drosophila melanogaster presents an excellent model for assessing environmental effects on behavior. To assess to what extent transcription of the chemosensory repertoire responds to changing conditions, we constructed cDNA expression arrays that represent 50 *Odorant binding protein (Obp)*, 56 *Odorant receptor (Or)*, and 59 *Gustatory receptor (Gr)* genes, 4 genes that encode other antenna-specific proteins, 17 genes encoding components of neurotransmitter pathways, and 4 control genes. We compared transcriptional profiles under different environmental and physiological conditions, including transcript abundance between adults and larvae; males and females; mated and non-mated flies; young (5 days) and old flies (6 weeks); adult flies exposed to different odorants (benzaldehyde, ethanol); larvae exposed to different odorants; and, flies raised in groups and in isolation. All experiments were done with isogenic *Canton S(B)* flies with sexes separately (except for larvae). Transcripts for *Obp*s, *Or*s and *Gr*s were detected with high sensitivity after normalization and background correction with virtual absence of dye effects. There was excellent concordance between fluorescent signal intensity and transcript abundance detected by RT-PCR. While expression of many *Or* and *Gr* transcripts was not detectable, some generated strong signals and *Obp* transcripts generated signals that exceeded those observed with *Or*s and *Gr*s by an order of magnitude. We observed considerable variation in transcript abundance among chemoreceptor genes located within chromosomal clusters. In addition, transcript abundance showed a high degree of plasticity under different environmental and physiological conditions, indicating a great ability of flies to adapt expression of their chemosensory repertoire to changes in their environment.

Poster Session I: Tues July 22

Manipulation of Odorant Receptor Expression in Olfactory Sensory Neurons

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Olfactory discrimination depends on a large number of odorant receptor genes and differential ligand-receptor signaling among neurons expressing different receptors. In this study, we demonstrated that cultured olfactory sensory neurons express endogenous odorant receptors. Lentiviral vector mediated gene transfer allows successful ectopic expression of odorant receptor. Primary olfactory sensory neurons express characteristic signaling molecules and therefore provide a system to study receptor function and transcription regulation within its intrinsic cellular environment. We showed that the ectopically expressed mouse I7 is functional in the cultured olfactory sensory neurons. In addition, we observed a transcriptional suppression of endogenous receptors when ectopic I7 is constitutively expressed in the cultured olfactory sensory neurons. When two different odorant receptors are ectopically expressed simultaneously, under independent control of ubiquitous exogenous promoters, both receptor proteins show no reduction in their expression within the same olfactory sensory neurons up to 7 days in vitro.

Poster Session I: Tues July 22

Proximal Promoter Contribution to Patterned or Expression

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Odorant receptor (OR) genes exhibit highly restricted expression patterns within the olfactory epithelium. Cells expressing any particular OR are limited to distinct domains along the dorsal-medial to ventral-lateral regions of the epithelium. DNA sequences that contribute to this patterning remain unclear. In this study we sought to identify elements responsible for restricted expression of M71 and M4, two ORs that reside in largely non-overlapping domains. We generated chimeric promoter OR transgenes with a junction point at a conserved O/E protein binding element upstream of *M71* and *M4* transcription start sites. Transgene ORs were tagged with IRES-Tau-LacZ and flanked by chicken HS4 insulator sequences to control locus-specific effects on expression. Expression of each construct was examined in 129/B6 transgenic mice and compared to their tagged parental constructs. The *M71:M4* hybrid transgene mimics endogenous *M71* patterns, while the *M4:M71* transgene expresses ventrally compared to native *M71* and *M4*. LacZ positive axons converge to ectopic glomeruli for both transgenes. To investigate effects of HS4 elements on expression domain, we generated transgenic mice in which *M71* or *M4* parental constructs were flanked by insulators and have initiated analysis of expression domains and targeting in these mice. This study demonstrates the first expression of OR genes within confines of insulator sequences, arguing against the need for long-range adjacent cis elements for OR expression or patterning. Our results suggests that short-range elements upstream of a proximal promoter OE site contribute to domain-restricted expression of OR genes.

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Poster Session I: Tues July 22

Unconscious Perceptual and Affective Processing of Odors in Anosmia Due to Right Orbitofrontal Injury

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Growing evidence suggests that faint undetectable odorants can elicit olfactory processing to the extent that they influence various aspects of human behavior. However, mechanisms underlying unconscious olfactory processing remain largely unclear. Towards that end, we investigated unconscious olfactory processing in a 32-year-old man (S.) with a restricted lesion in the right orbitofrontal cortex (OFC), who exhibited complete anosmia albeit no impairment on main neuropsychological tests. We presented neutral (9% rose oxide and 17% pinene) and unpleasant (1% trimethylamine and 1% valeric acid) odorants unirhinally to S. in an odor-detection task, in combination with functional magnetic resonance imaging (fMRI) and physiological recording. S. performed below chance on odor detection, confirming his complete anosmia. Nevertheless, consistent with his right-sided lesion, detection accuracy was higher for odors delivered to the left than the right nostril. Furthermore, fMRI results paralleled the behavior, revealing greater activity elicited by left-delivered odors in left olfactory OFC. Unpleasant versus neutral odors delivered to the left nostril led to heightened skin conductance responses and enhanced activity in left anterior OFC, supporting unconscious affective processing. Interestingly, detection of neutral odors exceeded that of unpleasant odors (also exclusive to the left stimulation), suggesting that unconscious affective processing may interfere with ongoing odor detection. These findings thus demonstrate perceptual and affective aspects of unconscious olfactory processing in complete anosmia, and present the intriguing possibility that right OFC is critical (and the left OFC not sufficient) for olfactory awareness.

Poster Session I: Tues July 22

Functional Role of α7-Nicotinic Acetylcholine Receptors in Mouse Models of Schizophrenia

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Patients with schizophrenia have polymorphisms in the human α7-nicotinic acetylcholine receptor (α7) promoter, decreased expression of α7 in the hippocampus, olfactory hallucinations, and difficulties in odor discrimination. Investigations using mouse models of schizophrenia have demonstrated similar polymorphisms for α7 and decreased hippocampal expression; however, it is not known if there are similar deficits in the olfactory system in animal models. Here we characterize α7 expression in the glomerular layer of the olfactory bulb (OB) and determine the ability of mouse strains with altered α7 expression (wild-type – WT and α7 heterozygous knockouts – HET) to discriminate odors. [^{125}I]α-bungarotoxin (α-BGT) autoradiography was used to measure α7 protein in the OB of multiple strains of mice: C57, C3H, and DBA. The amount of α7 expression was quantified using modified software (GLOM-MAP). For odor discrimination, mice were trained on a go-no go odor task with an olfactometer and the MLPEST program was used for determination of threshold for odor discrimination. α-BGT expression was highest in C57 followed by DBA and C3H mice. For the discrimination task using an aldehyde odor pair, WT C57 and C3H discriminated an entire log unit lower compared to the HET C3H. These data suggest that differences in α7 expression in the adult mouse OB may contribute to the decreased ability to discriminate similar odorants. Thus, by characterizing the relationship between olfactory function and α7 expression in the OB of mice, we may provide a new tool to elucidate the mechanism of olfactory dysfunction in patients with schizophrenia.

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Poster Session I: Tues July 22

Anosmia as Early Predictor of Parkinson Disease: Tyrosine Hydroxylase Immunoreactivity in the Human Olfactory Bulb and Anterior Olfactory Nucleus

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Anosmia is one of the earliest symptoms of Parkinson's disease. We try to characterize the neurological changes in the olfactory bulb and anterior olfactory nucleus underlying olfactory deficit. Changes in dopamine, i.e., tyrosine hydroxylase (TH) immunoreactivity, are evaluated. Post-mortem material from 11 Parkinson and 17 control patients has been used. Horizontal sections were obtained using a freezing sliding microtome. Immunohistochemistry consisted of primary monoclonal antibody against TH (1:200; Diasorin, Stillwater, MN) and an anti-mouse secondary antibody (1:2000; Vector, Burlingame, CA). Our results indicate the presence of TH-like immunoreactive cell bodies in glomeruli, external plexiform layer, stratum album of the olfactory bulb and anterior olfactory nucleus. Non-quantitative comparisons among them are still inconclusive regarding immunoreactive cell bodies in Parkinson as compared to control. The literature describe an increase of dopaminergic elements in the olfactory bulb of Parkinson patients. This contrast with the loss of dopaminergic cells in the substantia nigra. This fact has to be correlated also with the progressive decrease in the number and structural integrity of glomeruli with aging. This work tries to address this apparent controversy. Next we want to compare changes in dopamine with cytopathological markers as alpha-synuclein-immunopositive Lewy bodies by means of double immunoflurescence.

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Poster Session I: Tues July 22

Advanced Time-Series Analysis of MEG Data as a Method to Explore Olfactory Function in Healthy Controls and Parkinson's Disease Patients

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Objectives To determine whether time series analysis of magnetoencephalography (MEG) data is suitable to study brain activity related to olfactory information processing, and to find out if this method may also serve to detect differences in olfaction-related brain activity between Parkinson's disease (PD) patients and controls.

Methods: Whole head 151-channel MEG recordings were obtained in 21 controls and 20 PD patients during a 10-minute olfactory stimulus paradigm, consisting of 10 alternating rest-stimulus cycles (30 s each). The olfactory stimulus, phenylethylalcohol (40% v/v) was delivered by a Burghart olfactometer. Artifact-free 6.5 s epochs were selected from each 30 s rest and stimulus epoch. Overall relative spectral power and local, interhemispheric and intrahemispheric synchronization likelihood (SL; a measure of functional connectivity between brain areas) were calculated for delta, theta, low alpha, high alpha, beta and gamma frequency bands.

Results: Controls showed increased theta band power (F [1,383] = 5.93, p = .015), and decreased beta band power (F [1,383] = 5.98, p = .015) after the odor stimulus. PD patients showed a decrease in overall low alpha power (F [1,366] = 5.59, p = .019). In controls, the odor stimulus induced increased interhemispheric delta band SL (F [1,383] = 4.84, p = .028) and decreased local beta band SL (F [1,411] = 4.59, p = .033). The response in PD patients was significantly different and involved a decrease in intrahemispheric high alpha band SL (F [1,375] = 9.64, p = .002).

Conclusion: MEG is a suitable method to detect olfactory responses using both spectral power and SL. Using SL, but not spectral power, we found differences in olfaction-related brain activity between PD patients and controls.

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Poster Session I: Tues July 22

Difference in fMRI Olfactory Activation Patterns in Elderly Subjects with High and Low Task Performance

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Olfactory function is impaired in older adults. The underlying cortical substrate for age-related differences in performance in an odor paradigm was

investigated in the present study employing fMRI. Seventeen healthy elderly subjects (68.2 ± 3.0 y.o.) participated in the study and completed 2 functional runs, one for encoding and one for retrieval. Odors were presented in a continuous flow of air in synchronization with the subject's inspiration and were followed by a 10s rest. Fifty odors were presented during an encoding run and 100 odors, i.e. 50 old and new odors were presented during the retrieval run. Subjects performed a detection task during the encoding run and a recognition task during the retrieval run and gave responses through a button press. Performance on the memory task was coded with hits, misses, correct rejections, false alarms and discriminability index d'. Subjects were separated in two groups according to their performance on d': one group was composed of 11 subjects with d'>0.1 (high-performers), and the other group was composed of 6 subjects with d'<0.1 (low-performers). FMRI images were preprocessed with Statistical Parametrical Mapping (SPM2, Friston et al., 1995) and processed with AFNI deconvolution algorithm (Cox 1996). Six subjects were randomly chosen from the group of high performers and an ANOVA was run in AFNI between 6 high and 6 low performers. Activation in temporal lobe and frontal areas was associated with measures of memory performance. This study suggests that patterns of cortical activation in frontal and temporal areas are associated with degree of impairment.

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Poster Session I: Tues July 22

Association Between fMRI Brain Activation and Neuropsychological Performance in Individuals at Risk for Alzheimer's Disease

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Deficits in olfaction and memory occur during the normal aging process and are more marked in Alzheimer's disease (AD). The ε4 allele of the apolipoprotein E gene is associated with an increased risk for the development of AD. Individuals with the ε4 allele (E4+) demonstrate deficits in olfactory memory performance prior to a general decline in cognitive functioning. The current study examined associations between neuropsychological test performance and brain activation during a cross-modal olfactory recognition memory paradigm in older adults (E4+, n=18 and E4-, n=21). Before scanning, participants were presented with 16 odors. During 2 functional runs, names of odors presented before scanning (target) or not presented (foil) were shown. Participants discriminated between targets and foils using a button box. A region of interest (ROI) analysis was conducted and fit coefficients corresponding to recognition memory performance (e.g., hits, misses, false alarms, and correct rejections) were correlated with post-scanning performance on learning trials from the California Odor Learning Test (COLT) and California Verbal Learning Test (CVLT). Correlations between ROI and CVLT and COLT learning trials demonstrate allele-associated differences in the relationship between fMRI activation and performance. For the E4+ individuals, CVLT performance was correlated with frontal lobe activation, suggesting compensatory activity, recruitment of additional neuronal populations, or alterations in the frontal-temporal circuits. For E4- individuals, COLT performance was correlated with the mesial temporal lobe, which is consistent with the hypothesis that areas activated during retrieval are some of the same areas activated during encoding.

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Poster Session I: Tues July 22

Relationship of Olfactory and Neurocognitive Deficit in Alzheimer's Disease

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It is unknown whether Alzheimer's disease (AD)-related reduction in central olfactory system neural activity, as measured by functional magnetic resonance imaging (fMRI), is correlated with indices of odor perception and dementia. To investigate this question, 12 AD patients and 13 healthy non-demented senior controls underwent fMRI while being exposed to each of three different concentrations of lavender oil (0.10%, 0.32% & 1%). All the participants were administered the University of Pennsylvania Smell Identification Test (UPSIT), the Mini-Mental State Examination (MMSE), the Mattis Dementia Rating Scale-2 (DRS-2), and the Clinical Dementia Rating Scale (CDR). Blood oxygen level-dependent (BOLD) signal changes within the primary olfactory cortex, hippocampus, and insula were drastically reduced in AD patients relative to controls (ANCOVA with age as covariance, $p < 0.01$), and correlated significantly with UPSIT scores (p's < 0.001, with age as a confounding factor). More importantly, the olfactory BOLD signal in these structures significantly correlated with MMSE, DRS-2, and CDR measures of dementia (p's < 0.03, with age and educational level as confounding factors). These findings demonstrate the sensitivity of olfactory fMRI in testing the olfactory and functional cognitive decline of AD and the influence of odorant concentration on brain activation in this devastating and prevalent neurodegenerative disease.

Poster Session I: Tues July 22

Relationship of Functional Activation Deficit and Anatomical Atrophy in the Primary Olfactory Cortex and Hippocampus of Alzheimer's Disease

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The objective of this study is to investigate the relationship of atrophy in olfactory structures to functional deficit in Alzheimer's disease (AD) by examining the correlation of olfactory fMRI activation with local atrophy in the primary olfactory cortex (POC) and hippocampus. Twelve AD patients and twenty age-matched normal controls (NC) received standardized smell and neurocognitive tests. All subjects underwent high resolution MRI and an olfactory fMRI study with a variable odor intensity paradigm on a 3T system. Volumes of POC and hippocampus were measured by manual segmentation. The areas outlined were saved as ROIs for subsequent fMRI activation calculation. The average volumes of the POC and the hippocampus in AD group were reduced by 39% and 44% respectively, compared to those of NC group. There was a high correlation of POC atrophy with hippocampus ($P<0.001$). A much greater reductions of fMRI activation in the corresponding structures (98% in POC and 95% in hippocampus) were demonstrated. The activation reduction and local atrophy in these two regions was significantly correlated ($P=0.008$ for POC and $P=0.033$ for hippocampus). We revealed that POC suffers prominent local atrophy as hippocampal area which was demonstrated previously in AD. The fMRI

activation in the atrophic areas specific to AD reduced dramatically with marginal signal recovery using stronger odor intensity. These results provided neuropatholoigcal and neurofunctional bases for olfactory deficit in AD.

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Poster Session I: Tues July 22

Olfactory Imaging Probes of Limbic Dysfunction in Alzheimer's Disease

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At the present time, the clinical diagnosis of Alzheimer's disease (AD) can only be confirmed at autopsy. With the development of new preventative and therapeutic interventions on the horizon, the identification of pre-clinical, non-invasive diagnostic biomarkers is becoming increasingly critical. Given that the sense of smell is frequently diminished in patients with AD, often prior to the emergence of overt clinical symptoms, we tested the hypothesis that the early accumulation of neuropathological lesions in limbic brain regions might disrupt olfactory function and thereby account for the perceptual impairments in smelling. To this end we used olfactory functional magnetic resonance imaging (fMRI) techniques as a diagnostic probe of limbic integrity in Alzheimer's patients. We scanned patients with early-stage AD (n = 6) and age-matched control subjects (n = 4) during an olfactory paradigm of fMRI cross-adaptation, in which they made sequential sniffs and smelled pairs of odors that were either similar or different in odor quality. We found that the volume of piriform cortex responding to odor quality was significantly reduced by 85% in AD patients vs. controls, despite a lack of significant group difference in odor-activated cortex per se. The effect of odor quality on fMRI cross-adaptation in piriform cortex was also impaired in AD. These findings demonstrate the feasibility of our experimental design in the context of AD and suggest that the use of olfactory fMRI as a diagnostic bioassay may facilitate the detection of pre-clinical stages.

Poster Session I: Tues July 22

ENaC Expression in Primate Taste Bud Cell Types

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The mammalian epithelial sodium channel (ENaC) family is comprised of four members. Alpha and Delta ENaC generate sodium-permeable pore-forming subunits, whereas Beta and Gamma ENaC are accessory subunits. To better understand the function(s) of ENaCs in taste bud physiology, we evaluated the expression of all four ENaC subunits in non-human primate (macaque) taste buds. Using taste buds and lingual epithelial cells isolated by laser capture microdissection, Alpha, Beta, and Gamma ENaC were expressed in both taste buds and lingual epithelial cells by RT-PCR analysis. Within the taste bud, Alpha ENaC was expressed in most taste cells, including TRPM5 cells (sweet, bitter, umami) and PKD2L1 cells (sour) by double label in situ hybridization analysis. Unlike Alpha ENaC, Delta ENaC was specifically expressed in taste buds, and not lingual epithelium, by RT-PCR analysis and localized to a subset of TRPM5 cells expressing T1R1 (umami) but not T1R2 (sweet) or T2Rs (bitter) by double label in situ hybridization analysis. Based on the molecular and histological expression profiles of Alpha and Delta ENaC, we conclude that Alpha ENaC is expressed in taste buds as well as lingual epithelium and is expressed in many taste cell types, whereas Delta ENaC is expressed in taste buds but not lingual epithelium and is expressed in the umami taste cell population in primates.

Poster Session I: Tues July 22

Maillard Reacted Peptides (MRPS) Modulate Human Salt Taste and the Amiloride-Insensitive Salt Taste Receptor

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During the ageing and/or cooking process formation of MRPs enhances food flavor and taste. MRPs isolated from naturally aged products also function as salt taste modifiers. To test the effect of MRPs on salt taste, MRPs were synthesized by reacted a peptide fraction (1000-5000 Da) purified from soy protein hydrolysate with galacturonic acid(GalA), glucosamine($GlcNH_2$), xylose(Xyl), fructose(Fru) or glucose(Glc). The effect of MRPs was investigated on human salt taste and on the chorda tympani taste nerve responses to NaCl in S.D rats, wildtype and TRPV1 KO mice. MRPs produced a biphasic effect on human salt taste in the presence of amiloride and on the CT responses in rats and wildtype mice in the presence of NaCl+benzamil (Bz), enhancing the NaCl response at low concentrations and suppressing it at high concentrations. The effectiveness of MRPs as salt taste enhancers varied with the reacted sugar: GalA=$GlcNH_2$>Xyl>Fru>Glc. The concentrations at which MRPs enhanced human salt taste were significantly lower than the concentrations of MRPs that produced enhancement in the CT response. Elevated temperature(>38°C), resiniferatoxin, capsaicin and ethanol produced additive effects on the NaCl CT responses in the presence of MRPs. Elevated temperature and ethanol also enhanced human salt taste. SB-366791 inhibited the Bz-insensitive NaCl CT responses in the absence and presence of MRPs. TRPV1 KO mice demonstrated no Bz-insensitive NaCl CT response in the absence or presence of MRPs. The results suggest that MRPs modulate human salt taste and the NaCl+Bz CT responses by interacting with TRPV1t.

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Poster Session I: Tues July 22

The Taste System can Discriminate Mixtures of Water Dissolved Minerals Varying on Cations Proportion

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The taste of water depends on the total dissolved solids (TDS) namely the quantity of minerals dissolved in water (Teillet et al., 2007). However, it remains unclear whether the Human taste system is able to discriminate between two water samples with the same TDS but different proportions of minerals. In other words, has our gustatory system the ability to reflect a difference in taste quality due to a difference in the proportion of minerals in mixture? We addressed this question using 7 water samples containting a mixture of Na^+, K^+, Ca^{2+}, Mg^{2+}, HCO_3^-, SO_4^{2-} and Cl^-. These 7 samples

contained the same total amount of dissolved solids but varied in ions proportion. 62 subjects compared these 7 samples following a pair comparison procedure. Differences between the samples were evaluated using the binomial law and a Bonferroni correction. The results indicated that the panel discriminated the sample including a higher amount of Na^+ from those including a higher amount of Mg^{2+} or K^+ and the sample with a higher proportion of K^+ from the one boosted in Ca^{2+}. These findings evidenced that, beyond total dissolved solids variations, our taste system is able to differentiate between the proportions of cations in mixture. As a consequence, the taste of water appeared to be driven both by the total amount of dissolved solids but also by their respective proportion. We thank ANRT (CIFRE n°372/2006) for financial support. Teillet E, Urbano C, Cordelle S, Schlich P. (2007) A study of the sensory perception of tap waters versus bottled mineral waters using a combined sorting, descriptive and hedonic task carried out by 389 French consumers. 7th Pangborn Sensory Science Symposium. Minneapolis, USA.

Poster Session I: Tues July 22

Organic Salts, Organic Acids, and Benzamil Differentially Modulate The Responses of Rat Geniculate Ganglion Neurons to Salt

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We recorded single-cell responses from geniculate ganglion (GG) neurons of anesthetized male rats to determine the influence of anion size (chloride, acetate, gluconate), acidity (citric acid), temperature (cooling from 35-15°C and warming from 15-35°C), and 1μM benzamil on salt responses in different neuron types. We used artificial saliva (15mM NaCl, 22mM KCl, 3mM $CaCl^2$, 0.6mM $MgCl^2$) as the rinse solution and solvent for all stimuli. Simultaneous with GG recordings, we recorded stimulus-evoked summated potentials (electrogustogram; EGG) from the tongue to signal when the stimulus contacts the taste receptors and the response begins. Artificial saliva elevated the spontaneous firing rates of all neurons, especially Acid-generalists (N=3). Benzamil suppressed NaCl (0.1, 0.3M) responses by NaCl-specialists (N=7) and Sucrose-specialists (N=3) neurons, but not by Acid-generalist neurons. NaCl-specialist and sucrose-specialists neurons responded to Na-salts in a concentration dependent manner, but were unresponsive to citric acid at all concentrations and only weakly responsive to KCl at 0.3 and 0.5M. In contrast, Acid-generalist neurons responded to all salts and to citric acid in a concentration dependent manner. The responses by NaCl-specialist neurons to NaCl, but not to Na-acetate or Na-gluconate were reduced when presented as a mixture with citric acid; in contrast, citric acid increased Acid-generalist responses to all salts. Moreover, NaCl-specialist and Acid-generalist neurons were excited by cooling and inhibited by warming, albeit at different thresholds. The current findings provide evidence that taste neurons are differentially modulated by salts, pH, benzamil, and temperature, all of which serve as tools for neuron classification.

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Poster Session I: Tues July 22

Sodium Depletion Alters Amiloride-Sensitive Salt Taste in Humans

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Previously, we observed that an amiloride sensitive pathway is important for humans to perceive NaCl as salty after adaptation to NaCl. Amiloride blocks Na transit through the epithelial sodium channel (ENaC). In many tissues, ENaC activity is regulated by aldosterone, a sodium conserving hormone stimulated by renin activity. We hypothesized that aldosterone also affects ENaC activity in the human tongue. To investigate this possibility we depleted 17 subjects of sodium by administering a potent diuretic, furosemide (40 mg), in the evening and the following morning. The state of sodium depletion was confirmed by increases in serum aldosterone concentration ($p < 0.01$) and plasma renin activity ($p < 0.01$), and reduction of body weight ($p < 0.01$). Prior to furosemide administration, in the afternoon after the 2nd dose of furosemide as well as two days later (recovery), subjects provided magnitude estimates of the component taste qualities (sweet, salty, sour and bitter) of 125 mM NaCl after adaptation to 100 mM NaCl. Adapting and trial solutions were presented without and with amiloride (10 μM). Prior to furosemide, subjects characterized 125 mM NaCl as predominantly salty and amiloride reduced the saltiness by 32% ($p < 0.01$). After sodium depletion by furosemide, subjects still characterized 125 mM NaCl as predominantly salty, but amiloride failed to reduce the saltiness ($p = 0.4$). Two days later (recovery) amiloride again reduced the saltiness of 125 mM NaCl ($p < 0.05$). These data support the hypothesis that increased aldosterone, induced by sodium depletion, alters the cellular mechanism responsible for salt taste, although the direction of the effect is unexpected. Thus, human salt taste, responsive to hormonal stimulation, may participate in sodium homeostasis.

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Poster Session I: Tues July 22

Nacl Dominates Hamster Taste Responses to Electrolyte Mixtures

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NaCl taste in rodents is transmitted to the brain by two classes of chorda tympani (CT) peripheral neurons: the amiloride-sensitive Na^+/Li^+-specific and the electrolyte generalists. We have shown that NaCl suppresses quinine·HCl behavioral taste responses and CT neural steady-state responses in hamsters. Either blocking lingual epithelial Na^+ channels (ENaC) with amiloride or substituting KCl for NaCl eliminates this neural mixture suppression. Blocking ENaC also creates NaCl-KCl behavioral equivalence. To examine further this "salty-bitter" taste suppression, multi-unit hamster CT (n = 6) neural responses were recorded to binary combinations of 50 mM NaCl with [mM] quinine·HCl [3, 10], KCl [50,100], $MgSO_4$ [10, 30], denatonium benzoate [10, 30], citric acid [1, 3] or acetic acid [1, 3]. Behaviorally, hamsters cross-generalize the 4 ionic bitter stimuli, which do not generalize to the 2 acids. When mixed with NaCl, steady state CT responses to each ionic bitter stimulus were completely suppressed. There was no mixture suppression after blocking Na^+-specific activity with 30 μM amiloride. Thus, ENaC-related transduction is associated with suppression of neural responses to multiple ionic bitter stimuli. CT responses to the acids were not suppressed in NaCl-acid mixtures. In hamsters, acid-sensitive CT fibers fall among the electrolyte generalists. However, rat CT electrolyte generalists, which all respond to NaCl, include distinct subgroups that respond strongly to either quinine or citric acid (Breza et al., 2007) and thus, potentially provide pathways for the distinct effects of NaCl reported here. These results suggest that, before transmission to the primary afferent, specific and dynamic modulatory interactions occur among taste modalities within a taste bud.

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Poster Session I: Tues July 22

Electrophysiological Responses of the Chorda Tympani Nerve Following 24-H Dietary Sodium Deprivation

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Previous studies show that during dietary Na^+ deprivation, there is a decrease in chorda tympani nerve (CT) activity which may be necessary

for increased NaCl intake. Recent studies from our laboratory show that 2 days of dietary Na^+ deprivation increase licking responses to concentrated NaCl solutions and reduce CT responses to NaCl. Furthermore, amiloride, an epithelial Na^+ blocker, suppressed CT responses in control rats as expected, but had virtually no effect on CT responses to NaCl in Na^+-deprived rats. Physiological compensatory responses such as a decrease in urinary Na^+ output are activated rapidly after just 24-h of dietary Na^+ deprivation. Therefore, we hypothesized that peripheral gustatory changes may occur during this time frame as well. Accordingly, the goal of the current study was to determine whether 24-h dietary Na^+ deprivation decreases CT responses to NaCl and to assess CT amiloride-sensitivity. We recorded whole nerve electrophysiological activity from the CT in response to lingual application of NaCl (75, 150, 300, 450, 600 mM) and to NaCl mixed with 100 μM amiloride. Preliminary data indicate that 24-h of dietary Na^+ deprivation does not alter CT responses to NaCl, as responses of Na^+-deprived rats were similar to controls at all concentrations. Moreover, amiloride suppressed CT responses to NaCl regardless of treatment. These results suggest that although 24-h of dietary Na^+ deprivation induces compensatory mechanisms to limit the loss of Na^+, it is not sufficient to affect gustatory signaling which may allow for increased consumption of NaCl.

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Poster Session I: Tues July 22

Sodium-Deficiency Induced by Spironolactone Elicits Discriminative Ability for Hypotonic Salty Solutions in C57Bl/6 Mice

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Sensory evaluation tests by human being have been used for palatable salty enhancers or substitutes. However, there are no suitable evaluation methods by animal behavioral studies. We though that behavioral studies should be conducted under restrictive conditions such as preferable concentration or sufficient salt appetite to evaluate palatable tastant. A water-deprivation or a sodium-deprivation elicits salt appetite. In this study we investigated the preference for hypotonic NaCl solutions by sodium-deprived C57BL/6 mice fed a NaCl-deficient diet containing mineralocorticoid receptor antagonist spironolactone. In short-term two-bottle choice test, the sodium-deprived mice preferred 0.03 M NaCl solution to 2% sucrose solution although the sodium-repleted mice preferred 2% sucrose solution strongly. The sodium-deprived mice ingested 0.15 M NaCl significantly more than 0.03 M NaCl, and ingested 0.03 M NaCl significantly more than 0.015 M NaCl, but the sodium-repleted mice showed no significant difference in the same tests. These concentrations were sufficiently higher than the threshold of NaCl because both groups of mice could distinguish 0.015 M NaCl from deionized water. Furthermore, the significant preference for 0.03 M NaCl to 0.015 M NaCl was observed every test in 5 days in a row, because the small amount of sodium was insufficient to normalize the sodium-deprived conditions during the test. Brief access tests focusing on the initial determinants of licking responses showed that the number of licking increased in sync with the intensity of saltiness in test solution. These results suggest that the mice that can discriminate stably such a small difference of saltiness might be useful to evaluate a compound seed having a little enhancing effect on the salty taste.

Poster Session I: Tues July 22

Chemical Specificity of Rodent Geniculate Ganglion Neurons Characterized by their Responses to KCl and Citric Acid

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We determined the chemical specificity of geniculate ganglion (GG) neurons by electrophysiological recording of single cell responses to lingual application of the basic taste stimuli, as well as 0.1 M MSG and 0.1 M KCl in anesthetized male rats. Simultaneous with GG single-cell recordings, we recorded stimulus-evoked summated potentials (electrogustogram; EGG) from the tongue to signal when the stimulus contacts the taste receptors and the response begins. During recording, the tongue was adapted to 35° C artificial saliva (15 mM NaCl, 22 mM KCl, 3 mM $CaCl^2$, 0.6 mM $MgCl^2$) instead of water. We recorded the responses from 56 GG neurons and like our prior studies they separated into two major groups of narrowly (n = 24; 43%) and broadly tuned (n = 32; 57%) neurons. Narrowly tuned neurons consisted of 14 sucrose-specialists (25%) and 10 NaCl-specialists (18%). In general, these neurons were unresponsive to KCl, although 2/24 responded weakly to KCl and citric acid. In contrast, 29/32 (91%) broadly tuned GG neurons responded robustly to KCl and 31/32 (97%) responded robustly to citric acid. We propose that narrowly tuned GG neurons receive input mostly from Type II, receptor cells in fungiform taste buds. In contrast, broadly tuned GG neurons responded to citric acid and KCl, reflecting synaptic input from Type III, presynaptic cells (Tomchik et al., 2007; Kataoka et. al, 2008). Overall, artificial saliva elevated the spontaneous firing rate of several neurons. As a consequence, we observed a few instances of stimulus-induced inhibition, mostly in narrowly tuned neurons. Although highly preliminary, inhibition may sharpen contrast between stimulus categories.

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Poster Session I: Tues July 22

Mechanisms of Sea Hare Ink as a Feeding Deterrent Against Predatory Fish

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Chemical defenses are widespread in nature, yet their mechanisms for deterrence are generally poorly understood. The ink secretion of sea hares (*Aplysia californica*) is a mixture of ink from the ink gland and opaline from the opaline gland that protects sea hares from a diversity of predators. Its mechanisms against crustacean predators include use of deterrent compounds that are aversive or unpalatable, as well as attractive molecules (amino acids) that stimulate predators' appetitive responses. The aim of the current studies was to use chemical, behavioral, and electrophysiological techniques to determine the mechanisms by which the ink secretion of sea hares acts on predatory fish. Feeding and squirting behavioral assays determined that ink is a strong deterrent to seven species of fish, and one component of the secretion, hydrogen peroxide, is a mild deterrent to two species. Sea catfish (*Ariopsis felis*) and bluehead wrasse (*Thalassoma bifasciatum*) were chosen for further analysis based on their utility as model organisms. Electrophysiological analyses demonstrate that ink and opaline, but not hydrogen peroxide, are highly stimulatory to the olfactory and gustatory systems of the sea catfish. Bioassay guided fractionation of whole ink identified aplysioviolin as unpalatable to bluehead wrasse. Further analysis is underway to

analyze the electrophysiological responses to presentations of aplysioviolin and other ink components both in the presence and absence of amino acid stimulants.

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Poster Session I: Tues July 22

Hydrogen Peroxide and other Components in the Ink of Sea Hares are Chemical Defenses Against Predatory Spiny Lobsters Acting Through Non-Antennular Chemoreceptors

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When attacked by a predator, sea hares release two chemically defensive secretions: ink and opaline. These secretions, which are mixed in the mantle cavity and then pumped towards the attacker, are complex and can act through diverse mechanisms, including aversion and phagomimicry. We investigated how ink, opaline, and hydrogen peroxide (H_2O_2), which is produced when the two are mixed, affect the chemosensory behavior of the sympatric predator *Panulirus argus*. Our results show that all three are effective defenses. Opaline possesses as yet unidentified chemicals that inhibit feeding and evoke a strong aversive response that includes a very characteristic rubbing of the mouthparts and, in some cases, tail flipping. H_2O_2, while able to evoke the aversive response, has a much weaker effect on feeding. Ink does not evoke aversion and also has a weak effect on feeding. Ablation experiments show that the aversive effect is not mediated by the antennules. This is consistent with the observation that the secretions are released when the sea hare is in the grasp of the predator, when the latter is using its non-antennular chemoreceptors. To understand how the secretions defend the sea hares, we are currently performing experiments to identify the receptor types involved in mediating these behaviors, focusing on H_2O_2 and the aversion response. Our results so far show that, as expected, antennular chemoreceptors are generally unresponsive to H_2O_2, and thus we are currently focusing on the mouthparts and gills, both important sites of chemoreception in decapod crustaceans.

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Poster Session I: Tues July 22

Evidence that Planktivorous Fishes Aggregate to Dimethylsulfoniopropionate (DMSP)

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The use of natural chemicals as olfactory or gustatory cues by marine fishes is not well understood. Recently, we documented that the abundance of some marine fishes vary according to local concentrations of the chemical dimethylsulfoniopropionate (DMSP). Many species of marine algae produce DMSP as a secondary metabolite. DMSP is released from algal cells by lysis and zooplankton grazing, and therefore, its distribution in the ocean is commonly associated with areas of high primary productivity and foraging activity. While DMSP has been studied intensively for its role in oceanic sulfur cycles and global climate regulation, here we tested whether planktivorous reef fishes aggregate to controlled deployments of DMSP over coral reef habitat in the wild. We released DMSP (10_7^- M) over the fringing coral reefs of Curaçao, Netherlands Antilles. We found that the density of three planktivorous fishes increased significantly at DMSP release sites. Brown chromis (*Chromis multilineata*) numbers increased nearly four times background levels ($c_r^2 = 9.66$, $P = 0.002$, $n = 8$), and creole wrasse (*Lepticus parrae;* $c_r^2 = 25.6$, $P < 0.001$, $n = 8$) and boga (*Inermia vitatta*) were also observed in greater numbers at DMSP release sites. The attraction of schooling planktivorous fishes to DMSP in a natural setting suggests that this chemical provides a sensory link between algal production and reef fish behavior. Moreover, our finding that fish aggregated to experimental deployments of DMSP suggests a mechanism for how fish locate productive foraging patches and has important implications for our understanding of how chemically-mediated behaviors may be impacted by changing global temperatures.

Poster Session I: Tues July 22

The Sensory Basis for Ecological Paradigms on Wave-Swept Shores

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The rocky intertidal has provided significant examples of predators mediating prey populations, but still elusive is how predators locate preferred prey in this dynamic habitat. In seminal studies, Murdoch (1969) predicted that numerically dominant, predatory whelks (*Acanthinucella spirata*) stabilize populations by switching prey species in response to relative abundances. Moreover, Paine (1966) found strong trophic cascades and community-wide impacts initiated by seastar predation on mussels. At sites along the southern California coast, we revisited these ecological paradigms through lab and field experiments. Our results contrasted with those of Murdoch, showing strict preference of *A. spirata* for barnacles (*Balanus glandula*), regardless of alternative prey densities. The efficiency of whelks in finding live barnacles within a bed was explained by tenets of optimal diet theory. Specifically, whelk ability to exploit barnacle prey depended on an insoluble proteinaceous cue. A protein complex of ~200 kDa was extracted from *B. glandula*, purified, and placed in acid-washed, heat-treated barnacle tests. The extracted protein caused arrestment and feeding in *A. spirata*, but there was no significant effect of equivalent preparations from alternative prey (mussels, *Mytilus spp.*; turban snails, *Tegula funebralis*). Further experiments examined the interaction between seastars (*Piasaster ochraceus*) and mussels. The force exerted by tube feet on rocky substrata was enhanced significantly by protein additions from mussel prey, but not from alternative, non-prey (control) species. Combined results identify contact proteins as essential determinants of major trophic interactions within wave-swept shores.

Poster Session I: Tues July 22

Investigating Olfactory Foraging in Wandering Albatross (*Diomedea Exulans*) Using a Global Positioning System (GPS)

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Wandering albatross *(Diomedea exulans)* are pelagic seabirds that forage over thousands of square miles of open ocean for live prey and carrion. These birds have long been thought to hunt, in part, by smell, due to their large olfactory bulbs and a tendency to be attracted to fishy-smelling odors. Weimerskirch et al., recently used GPS monitoring coupled with stomach temperature recorders to examine predictions related to area-restricted search (ARS) in freely ranging wandering albatross. This instrumentation

provides high precision location data (GPS, 10 second sampling rate) along with measurements of both prey mass and timing of prey ingestion. We are now extending this approach to test predictions related to olfactory search. Models of odor transport predict that prey odors should disperse laterally and downwind of the source and acquire an irregular and patchy concentration distribution due to turbulent transport. For a seabird foraging over the ocean, olfactory search should therefore involve straight, crosswind flight to optimize the probability of encountering a plume, followed by upwind, zigzag flight to localize the prey. By contrast, birds approaching prey by sight would be expected to fly directly to a prey item, irrespective of wind direction. We confirm these predictions in freely ranging wandering albatrosses. We found that initial olfactory detection was implicated in nearly half (46.8%) of all flown approaches preceding prey capture events, accounting for 45.5% of total prey mass captured by in-flight foraging. These results suggest that the sensory basis of prey detection needs to be considered when developing models for area-restricted search at large spatial scales of the open ocean. We are currently extending these results to look at individual variation in foraging behavior.

Poster Session I: Tues July 22

Chemical Cues and the Keystone Species Hypothesis

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Sensory systems provide critical filters that enable organisms to detect and recognize valuable resources. Trophic cascades, structuring populations and communities, are determined to a large degree by trait-mediated interactions that rely on sensory inputs. Certain molecules serve as chemosensory stimuli and play keystone roles in determining outcomes of predator-prey dynamics at multiple trophic levels. Here, we investigated the potential contributions of surface-adsorbed proteins as signal molecules within wave-swept, rocky intertidal habitats. As indicated by initial results, barnacles (*Balanus glandula*), were constrained to produce a high molecular weight, insoluble, glycoprotein complex for cuticle/shell formation. These compounds evoked settlement by conspecific larvae in field assays, and thus, could operate as seminal cues for recruitment. Moreover, the same substances triggered predation by a numerically dominant whelk species (*Acanthinucella spirata*) on barnacle juveniles and adults in lab and field experiments. Such proteins, therefore, influence simultaneously demographic processes that would enhance, or diminish, barnacle populations. As dominant competitors for space, the relative balance between barnacle recruitment and predation mortality may have strong, cascading direct and indirect effects on community dynamics. Hence, surface-adsorbed proteins could play keystone roles within rocky intertidal habitats.

Poster Session I: Tues July 22

Isolation of a Candidate Receptor that Responds to a Chemical Defense Compound

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Chemical signaling plays an important role in ecological interactions, such as communication and predator-prey dynamics. Since sessile species cannot physically escape predators, many contain compounds that deter predation; however, it is largely unknown how predators physiologically detect deterrent chemicals. Few studies have investigated ecologically relevant aversive taste responses in any predator. Our objective was to functionally identify a chemoreceptor that may be responsible for an aversive behavioral response in a heterologous expression system. We previously showed that zebrafish (*Danio rerio)* reject artificial diets laced with sponge chemical defense compounds, suggesting that zebrafish can recognize deterrent compounds relevant to coral reef systems. Transcripts made from a whole adult zebrafish cDNA library were expressed in a heterologous system, *Xenopus laevis* oocytes, and tested for chemoreceptor activation via electrophysiology, using the cystic fibrosis transmembrane conductance regulator (CFTR) as a reporter. Oocytes expressing gene sequences from the library and CFTR exhibited an electrophysiological response to formoside, a sponge-derived defense compound. This bioassay was utilized to functionally screen the zebrafish library for a chemoreceptor that responds to formoside. One candidate clone has been isolated using this functional assay and is currently being sequenced. This response requires CFTR, suggesting that the clone does not encode a ligand-gated ion channel. Furthermore, the response is enhanced by the co-expression of β_2-adrenergic receptor, which may increase functional expression of this protein. This clone may encode a receptor capable of interacting with deterrent chemicals, which would enable understanding of predator detection of chemical defenses.

Poster Session I: Tues July 22

Toxic Fruit Tunes Fly Smell

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Drosophila sechellia, an insular endemic specialist sibling of D. melanogaster, is only found on Morinda citrifolia fruit. We studied how the olfactory circuitry accommodated the shift to superspecialism on this smelly fruit, toxic for its sister species. Ripe morinda fruit contained high levels of hexanoic and octanoic acids and esters thereof. Antennal basiconic sensilla inhabiting one sensory neuron responding to hexanoates (AB3) were overexpressed on the costs of other sensilla types. In the antennal lobes the two glomeruli innervated by the two sensory neurons of the AB3 sensillum were enlarged. We found that the second neuron is also sensitive to a fruit compound missed in early screens, 2-heptanone. The brain thus contains two macroglomeruli tuned to the fly's only host. As an increase in number of sensory neurons and size of glomeruli does not necessarily correspond to a greater attractiveness of the corresponding ligands, we tested the behavioral response of flies to methyl hexanoate, heptanone and hexanoic acid singly and in ratio's found in the fruit in choice assays. They were tested against water and against each other. Single components were attractive at low and high concentration to D. sechellia, but repellent to D. melanogaster. Furthermore, mixtures were more attractive than single compounds for D. sechellia, but repellent for D. melanogaster. Finally, mixtures were attractive for D. sechellia, but repellent for D. melanogaster in a choice with single compounds. How overexpression of sensory neurons may cause such dramatic behavioral differences in the two sibling species is discussed.

Poster Session I: Tues July 22

Sexual Dimorphism and Seasonal Variation in the Harderian Gland of the Red-Sided Garter Snake

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The Harderian gland of the red-sided garter snake, *Thamnophis sirtalis parietalis*, is a secretory structure that plays a role in the vomeronasal system by

solubilizing semiochemicals, such as the otherwise insoluble female garter snake sexual attractiveness pheromone. Detection of the pheromone by the vomeronasal system is essential for male courtship of female garter snakes. Feeding, which occurs only in the summer, involves detection of prey chemicals by the vomeronasal system as well, and may require carrier molecules (proteins) to deliver prey proteins to the vomeronasal organ. Because only male snakes respond to the female pheromone and breeding occurs primarily in the spring and feeding in the summer, the morphology of the Harderian gland was expected to be sexually dimorphic and seasonally variable. We found this to be true. Harderian glands were larger, cell heights were greater, and lumen diameters larger in the summer than in the winter or spring. Whereas the acinar cell heights and lumen diameters of males increased significantly from winter to spring, those of females did not. Sexual dimorphism was most evident in the acinar cell heights and lumen diameters in the spring, with males having significantly greater cell heights and lumen diameters than females. Keywords: Harderian gland, sexual dimorphism, seasonal change, sex pheromone, vomeronasal organ.

Poster Session I: Tues July 22

The Identification of Attractive Volatiles in Aged Male Mouse Urine

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In many species, females prefer to mate with older (aged) males, possibly because older males are of higher genetic quality than younger males. Some animals, including mice, which rely heavily on chemical communication there is some indication that an animal's age can be determined by its scent. To determine the attractant(s) in aged male mouse urine, chemical and behavioral studies were performed. The urine donor mice were of the inbred strain C57BL/6J (B6). The chemical analysis of the urinary volatiles was performed using flame ionization detector-gas chromatography and gas chromatography mass spectrometry in conjunction with head space solid phase micro extraction. The most prominent differences involved significantly greater level of putative pheromones 3,4-dehydro-*exo*-brevicomin (DB), 2-*sec*-butyl-4,5- dihydrothiazole (BT), and 2-isopropyl-4,5-dihydrothiazole (IT), and lower level of 6-hydroxy-6-methyl-3-heptanone in aged mice relative to adult male mice ($p<0.001$; Friedman nonparametric ANOVA). We also demonstrate that the attraction of B6 male mouse urine odor to the conspecific female was greater in aged male by means of the odor preference test ($p<0.0001$; Wilcoxon test). Because DB, BT and IT were tight ligands of major urinary proteins, these volatiles were selectively excluded from mouse urine by the ultrafiltration (10kDa cut off). Hence, this attraction of aged urine odor was offset by the ultrafiltration of the adult and aged mice urine. Our results suggest that the aged male B6 mice develop an aging odor that is attractive to female mice in an experimental setting, and this attraction is at least, partly due to increasing putative mouse pheromone signaling

Poster Session I: Tues July 22

Urinary Volatile Biomarkers in Mouse Models of Lung Cancer

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To identify volatile biomarkers of lung cancer that may have diagnostic potential, we employed two mouse models, the Kras-induced (LKR) cell line and Lewis lung (LLC) cell line. Recipient mice were injected with cells (injected) or saline (control) and urines were collected daily until the developing tumors reached a size requiring sacrifice. Sensor mice, trained in a Y-maze, discriminated between mice with and without tumors at several stages during tumor growth. Surprisingly, mice trained to discriminate between urine odors of one of the tumor models generalized this response to the other model without further training. These results are consistent with the existence of volatile biomarkers shared by both cancer cell lines. The next set of studies was designed to identify the chemical basis for this discrimination. Urinary volatile organic compounds were analyzed with solid-phase-microextraction, followed by gas chromatography coupled with mass spectrometry. No individual compounds were identified in the injected mice that were not also present in the control mice. However, the amounts of several compounds were dramatically different between injected and control mice. Furthermore, principal component analysis and support vector machine analysis generated a high score for discriminating between tumor and control groups. Thus, in this mouse model, it was possible to identify mice with lung cancer tumors based on volatile biomarkers.

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Poster Session I: Tues July 22

Olfactory Phenotypes of BBS8-Null Mice

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Bardet-Biedl Syndrome (BBS) is a pleiotropic, heterogeneous human disease associated with polydactyly, renal anomalies, mental retardation, and retinal degeneration. These phenotypes are consistent with defects in cilia formation or function underlying the disease. Identification of the BBS8 gene, one of 12 currently implicated in the disease, led to the hypothesis that BBS is caused by basal body and/or cilia defects. Consistent with this idea and the critical role of cilia in olfaction, we previously showed that both BBS patients and BBS mouse models exhibit impaired olfactory function. To explore the nature of olfactory defects in BBS, we genetically ablated the mouse BBS8 gene. BBS8 expression is particularly abundant in olfactory sensory neurons (OSNs) and specific BBS8 antibodies reveal staining in the dendritic knob in a shell-like structure surrounding the basal bodies. BBS8 null mice have reduced olfactory responses to a number of odorants, as seen by electro-olfactogram recording. Immunohistochemical analyses of olfactory epithelium reveal a near-complete loss of cilia from OSNs, a disorganized dendritic microtubule network, and mislocalization of proteins normally enriched in cilia. Interestingly, although OSN numbers are largely normal, targeting of OSN axons to the olfactory bulb (OB) is aberrant; axons expressing the same receptor display reduced fasciculation and project to multiple targets in the OB. Using reagents that reveal the characteristic neuronal activity of each OSN, we observed altered activity in BBS8-null OSNs. We hypothesize that the dramatic reduction in cilia structures, the essential signaling platform for olfaction, may alter the uniformity of responses in populations of OSNs expressing the same receptor, thereby contributing to the observed axon targeting defects.

Poster Session I: Tues July 22

Molecular Mechanism of NCX Regulation Via Dynamic Interactions of CAM, OMP and Bex in Olfactory Sensory Neurons

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Calmodulin (CaM) plays a key regulatory role throughout the olfactory signal transduction cascade. Elevations in internal Ca2+ ([Ca2+]i) are returned

to baseline by the actions of PMCA and the Sodium/Calcium exchanger (NCX). PMCA is CaM-dependent but the influence of CaM on NCX is unknown. OSNs of OMP-/- mice show slower Ca2+extrusion kinetics and compromised NCX activity. We hypothesized that this is due to interactions among OMP, its partner protein Bex (which binds CaM), CaM, and NCX. We now present evidence that CaM and OMP participate in regulating NCX activity. Using the Biacore biosensor we analyzed the interactions of CaM and OMP with synthetic peptides derived from Bex and from the large intracellular loop of NCX1. In the presence of Ca2+, CaM binding to the XIP peptide of the auto-inhibitory domain of NCX1 is Ca2+dependent and high affinity (kd=20nM), implying Ca/CaM regulation of NCX1. OMP also interacts with XIP but is Ca2+ independent and lower affinity (kd=700nM). These data suggest two mechanisms by which OMP and CaM could regulate NCX activity. First, OMP may bind to NCX1 at the resting level of [Ca2+]i and be competed off by Ca/CaM upon elevation of [Ca2+]i. Alternatively, CaM may regulate NCX activity by its interaction with the OMP binding partner Bex which also binds Ca/CaM (kd= 280nM). Using fluorimetry we have demonstrated the interactions of dansyl-CaM with additional putative CaM binding site peptides from NCX. Furthermore, several of these peptides compete with Ca/CaM for the activation of cAMP-PDE, testifying to their functional significance. These data support our hypothesis that complex interactions among Bex, CaM and OMP modulate the activity of NCX, and other CaM binding proteins, to regulate olfactory signal transduction.

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Poster Session I: Tues July 22

A Physiological Role for Nasal MUPS in Chemical Communication

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Small hydrophobic, volatile molecules signal information about the outside world and some may provide pheromonal signals when present in biological fluids. Although there have been considerable advances in elucidating the nature of olfactory receptors and the transduction pathway, we know very little about the critical process for managing hydrophobic molecules in the hydrophilic nasal mucus. A specific class of lipocalin proteins synthesized and localized to the nasal mucosa, the Major Urinary Proteins (MUPs) 4 and 5 (nasal MUPs), selectively bind a number of small hydrophobic odorants and pheromones *in vitro*. However, the *in vivo* function of the nasal MUPs (nMUPs), remains unresolved. We hypothesized that nMUPs serve to selectively bind biologically meaningful hydrophobic molecules and are critical for ligand detection and subsequent behavior. We demonstrate that the mRNAs for nMUPs are regulated by hormonal state, show sexual dimorphism and display apparent compensation when one isoform is lost by genetic disruption. Habituation-dishabituation behavioral assays of null nMUP mutants indicate that these mice have higher olfactory detection thresholds to a subset of odorants. In accord with the behavioral assays, electrophysiological recording showed that deletion of a single nMUP gene led to reduced sensitivity to odorants. These results support a physiological role for nMUPs in chemical communication. Furthermore, physiological modulation of nMUPs may provide a mechanism to influence biologically relevant social behaviors

Poster Session I: Tues July 22

Expression of Proton Sensors in Olfactory Tissue

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Acid sensing ion channels (ASICs) are proton-gated cation channels that participate in a wide variety of physiological processes. Their expression in the olfactory bulb (OB) was documented some years ago, but their function therein remains obscure. In this study we used RTPCR to amplify and clone ASIC1a in the OB and OE (olfactory epithelium) of mouse and rat. Sequencing of all four clones identified an open reading frame of 526 amino acids. A few amino acid substitutions were noted, when compared with the ASIC1a sequences of mouse and rat brain, and the physiological significance of these is under investigation. Immunohistochemical analysis of mouse and rat OB confirmed the presence of ASIC1a immunoreactivity in all the major cell types: ensheathing cells, periglomerular cells, mitral cells and granule cells. Qualitative RTPCR was used to test for the expression of additional proton sensors in olfactory tissue. Transcripts for the following sensors were found to be differentially expressed in mouse and/or rat OB and/or OE: ASICs 1b, 2a, 2b and 3; PKD1L3 and PKD2L1; and the GPCRs GPR4 and OGR1 (GPR68). Subcloning and sequencing of the amplification products confirmed their identities. There was no detectable expression of hyperpolarization-activated cation channels (HCNs). The expression of such a diverse array of proton sensors in OB and OE suggests that changes in pH, perhaps transient in nature, play a more significant role in olfactory perception than is currently assumed. Alternatively, or additionally, these sensors may be involved in pH homeostasis in the OB and OE.

Poster Session I: Tues July 22

Expression of Connexin 57 in the Olfactory System in Mice

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Connexin 57 (Cx57), a member of gap junction-forming proteins, is recently shown to be expressed in horizontal cells in retina (Hombach et al., 2004; Euro J Neurosci 19:2633-2640) and is necessary for normal function of these neurons (Shelley et al., 2006; Euro J Neurosci 23:3176-3186). Here, I report expression of Cx57 in the olfactory system in adult mice. *In situ* hybridization revealed that Cx57 was expressed in layers that reside cell bodies of neurons and basal cells but not in the layer where cell bodies of supporting cells are located. Degrees of expression varied among Cx57 positive cells. In some regions, strong expression was found in the apical layers of neurons whereas signals in the immature neuron layer and basal layer were weak. There were areas that cells of the immature neuron layer and basal layer carried strong hybridization signals. It was common, especially in the olfactory bulb, to observe patched Cx57 positive cells neighboring with Cx57 negative cells. Detectable signals were also found in axon bundles and in some cells in the nonsensory epithelium and lamina proper. Western blot and real-time PCR data support the notion that the olfactory epithelium expresses Cx57. Thus, Cx57 is expressed in neurons, basal cells and some non-neuronal cells in the olfactory system. The function of Cx57 in olfactory transduction should be determined in future.

Poster Session I: Tues July 22

Calcium Store-Mediated Signaling in Sustentacular Cells of the Neonatal Mouse Olfactory Epithelium

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In the olfactory epithelium (OE), sustentacular cells manifest several glial-like functions. In the CNS, glial calcium signaling requires store release and regulates multiple cellular events including gene expression, proliferation, metabolism, ion/transmitter transport, and exocytosis. We tested the

hypothesis that sustentacular cells exhibit glial-like calcium signaling using a mouse OE slice model. We observed rapid, robust increases in intracellular Ca^{2+} in response to muscarinic and purinergic G-protein coupled receptor (GPCR) stimulation. Oscillatory Ca^{2+} transients were evoked in a subpopulation of sustentacular cells. Purinergic UTP-evoked increases in intracellular Ca^{2+} were elicited by release from intracellular stores and were not dependent on extracellular Ca^{2+}. The cytosolic Ca^{2+} chelator BAPTA-AM (100 μM) and the Ca^{2+}-ATPase inhibitor cyclopiazonic acid (10 μM) completely and irreversibly blocked purinergic-induced Ca^{2+} transients. PLC antagonists U73122 (100 μM) and neomycin (150 μM) inhibited the UTP-evoked Ca^{2+} transient. 2-aminoethoxydiphenyl borate (100 μM), an IP_3 receptor antagonist, inhibited the UTP-induced Ca^{2+} transients. Tetracaine (500 μM), an antagonist of the ryanodine (RYR) receptor also reduced the UTP-elicited Ca^{2+} transient. Collectively, these data suggest that GPCR activation of PLC, production of IP_3, activation of IP_3 receptors, release of Ca^{2+} from stores, and subsequent Ca^{2+} induced Ca^{2+} release from RYR receptors mediate the UTP-elicited increases in intracellular Ca^{2+}. Our findings indicate that sustentacular cells are not static support cells, and, like glia in the CNS, have complex Ca^{2+} signaling that could regulate multiple cell functions.

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Poster Session I: Tues July 22

Calcium Clearance from Olfactory Sensory Neurons - The Significance of PMCAs

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In mammalian olfactory sensory neurons, binding of odorants to the G protein-coupled receptors causes an increase in intracellular calcium via opening of cyclic nucleotide gated channels – a process mediated by Adenylyl Cyclase III. The calcium signal thus generated is gradually terminated by calcium clearance mechanisms like the Na^+/Ca^{2+} exchanger (NCX) and Plasma membrane Calcium ATPases (PMCA). Previously we showed through immunocytochemistry that PMCAs are expressed in mouse olfactory neurons. We now show the functional significance of PMCAs in clearing calcium in relation to the NCX and SERCA by using calcium imaging and curve-fitting techniques. Olfactory neurons from wild type and PMCA2 knockout mice (gift from Dr.Gary Shull) were treated with 5μM Fura-2AM and stimulated with either 60 mM KCl or 1mM IBMX / 30μM Forskolin to mimic odorant-signal transduction. The calcium clearance kinetics (rate constants) of these neurons were compared by curve-fitting the normalized fluorescence ratio values. PMCA2 knockout cells were significantly slower than the wild type cells in clearing calcium. Inhibiting PMCAs using 10μM Carboxyeosin (CE) significantly slowed down calcium clearance in both wild type and knockout cells. Inhibiting SERCA using 5μM CPA and NCX using Low Na^+ Ringer's also significantly reduced the rate constants for calcium clearance. On an average, PMCA inhibition reduced the rate constants for calcium clearance in the dendritic knob by 34%, NCX inhibition by 35% and SERCA inhibition reduced it by 30%. Also, the resting calcium level in the knockout cells was slightly but not significantly higher than the wild type cells. Our results indicate an important role for PMCAs in calcium clearance from OSNs along with NCX and SERCA pump.

Poster Session I: Tues July 22

Calcium Microdomains in the Chemosensory Cilia of Olfactory Receptor Neurons

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Odor transduction occurs in the cilia of olfactory sensory neurons (OSN), where odors activate olfactory receptors inducing a G_{olf}-mediated adenylyl cyclase activation. cAMP opens cyclic nucleotide-gated channels (CNGC) that mediate Ca^{2+} influx to the cilia. Ca^{2+} opens Ca^{2+}-activated Cl^- or Ca^{2+}-activated K^+ channels, leading to depolarization or hyperpolarization, respectively. Ca^{2+} is also involved in odor adaptation, regulating CNGC and enzymes of cAMP turnover. The fundamental roles of Ca^{2+} in odor transduction require fine spatial and temporal control of its ciliary concentration. Ca^{2+} is extruded from the cilia by a Na^+/Ca^{2+} exchanger (NCX) and a plasma membrane Ca^{2+} ATPase (PMCA). We investigated the existence of Ca^{2+} microdomains by measuring Ca^{2+} fluorescence in individual *Rana pipiens* olfactory cilia after cAMP photorelease. Cells were loaded with Fluo4 Ca^{2+} indicator and Ca^{2+} was monitored using a two-photon microscope in the line raster mode. Spatial analysis along the cilia revealed that Ca^{2+} increased in discrete regions, strongly suggesting sub-micrometer microdomains. In contrast, when OSNs were exposed to 1 mM IBMX (PDE inhibitor) or 10 mM cyclodextrine (cholesterol scavenger), Ca^{2+} increases became evenly distributed along the cilia. No Ca^{2+} increases occurred in 0-Ca^{2+} external solution. Fluorescence decay time constant for Ca^{2+} extrusion was slower after substituting external Na^+ with Li^+ (t=24.9±3.2 s) or when supplementing 50 mM carboxyeosin (t=36.1±4.5 s), compared to control solution (t=10.8±1.9 s), supporting the participation of both NCX and PMCA in Ca^{2+} removal from the cilia.

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Poster Session I: Tues July 22

Omp Deletion Alters Functional Maturation of Olfactory Sensory Neurons Revealed by Patch Clamp Recordings

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Olfactory marker protein (OMP) is expressed at high levels in mature olfactory sensory neurons (OSNs) of vertebrates. OMP expression begins at E14 in mice, and increases until 1 month postnatal when it reaches mature levels. Adult mice lacking OMP exhibit reduced behavioral sensitivity to odor and altered response properties in electro-olfactogram and single OSN recordings. Considering OMP's developmentally regulated expression, it is intriguing to test whether OMP protein is required for maturation, and if its deletion results in functionally immature OSNs. In this study, functional maturation of OSNs was investigated by using single cell patch clamp recordings in genetically labeled MOR23-GFP cells in P0, P7, and P30 mice with intact or deleted OMP expression (wt/MOR23-GFP vs. OMP-null/MOR23-GFP double mutant mice). Response properties (latency, rise time, amplitude, half width, decay time, and paired-pulse amplitude ratio) were analyzed. Cells from wt/MOR23-GFP exhibited changes in response properties with age. From P0 to P30, wt/MOR23-GFP cells developed shorter latency, faster activation, narrower half width, and faster decay. Compared

to the wt/MOR23-GFP cells from P30 animals, the OMP-null/MOR23-GFP cells from P30 animals had longer latency, slower activation and decay, resembling the wt/MOR23-GFP cells from P0 animals. These studies reveal that OSNs exhibit functional maturation postnatally, and highlight a potential role of OMP in development.

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Poster Session I: Tues July 22

Olfactory Marker Protein (OMP) is a Novel Modulator of Ca^{2+} Efflux in Olfactory Sensory Neurons (OSN)

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Ca^{2+} participates in essentially all eukaryotic signaling cascades. In OSNs Ca^{2+} entry following odorant stimulation is the first step in signal transduction. Odorant signal transduction occurs in the cilia of OSNs where it is initiated by odorant molecules interacting with olfactory receptors. The subsequent activation of G-protein coupled adenylylate cyclase results in elevated intracellular cAMP leading to opening of CNG cation channels and Ca^{2+} entry. The Ca^{2+}current is amplified by a Ca^{2+}- activated chloride channel. As the rise in intracellular Ca^{+2} is critical to the transduction process, there are also mechanisms that return Ca^{+2} to pre-stimulus levels. OMP is a 19kDa protein that is phylogenetically conserved and highly restricted to mature OSNs. Based on recent data, it has been hypothesized that OMP is a novel modulator of Ca^{2+} efflux, playing a key role returning intracellular Ca^{+2} to pre-stimulus levels, thereby preparing the OSN to respond to the next stimulus. We used optical recording methods and a voltage-sensitive dye to study the consequence of varying external Ca^{2+} concentration on the odorant responses of OMP-KO and WT mice. Relative to WT mice, odorant responses recorded from the olfactory epithelium (OE) of OMP-KO animals were unaffected by the changes in external Ca^{2+}. Whereas increasing or decreasing external calcium relative to normal levels had the effect of respectively increasing and decreasing the magnitude and timing of the OE's response in WT mice, no such effects were observed in OMP-KOs. OMP-KO animals maintained their typical response defects. We hypothesize that OMP and its partner protein Bex, interact with CaM, regulating multiple CaM regulated steps in the olfactory transduction cascade.

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Poster Session I: Tues July 22

Molecular Cloning and Molecular Histochemistry of Salmon Olfactory Marker Protein in the Lacustrine Sockeye Salmon (*Oncorhynchus Nerka*)

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Olfactory marker protein (OMP) is useful molecular marker for matured olfactory receptor cells in the vertebrates. It is generally accepted that anadromous Pacific salmon (*Oncorhynchus* spp.) imprint some odorants of their natal streams at the downstream migration, and use their olfaction for discriminating those streams during spawning migration. Despite the importance of the olfactory receptor cells for the olfactory imprinting, the expression of OMP is not well understood in salmon olfactory receptor cells. In this study, salmon OMP was characterized in the olfactory organs of lacustrine sockeye salmon (*O. nerka*) by molecular biological and histochemical techniques. Two cDNAs encoding the salmon OMPs were isolated and sequenced. These cDNAs contained a coding region encoding 173 amino acid residues and the molecular mass of this protein is calculated to be 19,387.11 and 19,581.17. Both amino acid sequences showed high homology (90%). The protein and nucleotide sequencing demonstrates the existence of a remarkable homology between salmon OMPs and other teleost OMPs. By in situ hybridization using a digoxygenin-labeled salmon OMP cRNA probe, signals for salmon OMP mRNA were observed preferentially in the perinuclear regions of the ciliated olfactory receptor cells. By immunohistochemistry using a specific antibody to salmon OMP, OMP-immunoreactivities were seen in the cytosol of those cells. Our results provide the first cDNA cloning of OMP in salmon olfactory organ, and indicate that OMP is useful molecular marker for detection of the ciliated olfactory receptor cells in Pacific salmon.

Poster Session I: Tues July 22

Possible Mechanism of Olfactory Sensitivity to Calcium in the Goldfish (*Carassius Auratus*)

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The goldfish has been shown to have acute olfactory sensitivity to the inorganic cations calcium and sodium. However, the cellular mechanisms responsible for this sensitivity are unknown. The current study investigated whether the olfactory sensitivity to calcium (Ca^{2+}) may be mediated by a Ca^{2+}-sensing receptor. Olfactory sensitivity of the goldfish to two substances known to act as agonists at the mammalian Ca^{2+}-sensing receptor, neomycin and gadolinium (Gd^{3+}), was assessed using the electro-encephalogram (EEG) recorded from the olfactory bulb with a 'suction' electrode. The effect of the ion-channel blocker, tetracaine, on this sensitivity was also assessed. Goldfish had high olfactory sensitivity to neomycin with an EC_{50} of 198 mM, similar to that of Ca^{2+} (75 mM). However, the I_{max} of the response was significantly less than that of Ca^{2+}. Sensitivity to Gd^{3+} (EC_{50}; 4.5 mM) was significantly less than that to Ca^{2+} and, again the I_{max} of the response was significantly less than that of Ca^{2+}. Tetracaine inhibited the olfactory responses to both Ca^{2+} and Na^{+} with IC_{50}s of 13.8 and 12.0 mM (1.0 mM stimulus), respectively. Taken together these results suggest that a Ca^{2+}-sensing receptor may be responsible for the olfactory response to calcium. However, its affinities for neomycin and Gd^{3+} are significantly different from that of the mammalian Ca^{2+}-sensing receptor so it is likely to be structurally different. It is possible that there are two different Ca^{2+}-sensing receptors in the goldfish olfactory epithelium, one of high affinity (neomycin-sensitive) and one of low affinity (Gd^{3+}-sensitive). Furthermore, the transduction mechanisms of both Ca^{2+} and Na^{+} sensitivities involve tatracaine-sensitive ion channels.

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Poster Session I: Tues July 22

Origins of Variability in Olfactory Perception

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Olfactory perception is highly variable. Other studies have identified many components contributing to this variability. However, the multitude of causes and effects in odor perception is best addressed by quantitatively measuring the performance in multiple olfactory tests in a large number of diverse subjects. Towards this goal we are presenting the results of a study in which olfactory detection thresholds for several odors and subjective

assessments of the intensity and pleasantness of more than 100 different odorous stimuli were recorded in at least two replicates. This experimental setup allowed us to quantify the relative importance of inter-trial and inter-individual variability. We will present a systematic study of the complex interactions of factors including gender, race, age, smoking habits, and body mass index on general olfactory acuity and on the perception of specific odors. We furthermore analyzed the correlations between performances in different tests. For example, we found a statistically significant correlation between the detection threshold for pentadecalactone and the perceived intensities for both pentadecalactone and galaxolide. We performed this type of analysis for all the 33,000 correlations between measurements contained in our data and discuss the properties of the olfactory system emerging from this analysis.

Poster Session I: Tues July 22

Influence of Visual Information and Test Paradigm on Clinical Olfactory Test Results

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The goal of this study was to determine if visual information and test paradigms affect clinical olfactory test results. Three hundred ninety-seven Japanese patients with complaints of olfactory dysfunction were administered both a new clinical olfactory test, the Odor Stick Identification Test for Japanese (OSIT-J), and the Japanese benchmark olfactory test, T&T olfactometry. Four different methods were used to administer the OSIT-J combining paradigms using word or picture-word alternatives with the four-plus alternative method based on a top-down strategy or the two-step identification method based on a bottom-up strategy. OSIT-J scores were compared for the different methods, referring to benchmark scores obtained with T&T olfactometry. OSIT-J scores using picture-word alternatives and the four-plus alternative method showed a stronger correlation with T&T olfactometry test scores than those using word alternatives and the two-step identification method, respectively. The average OSIT-J scores of the four-plus alternative method using picture-word alternatives were significantly higher than those using word alternatives in anosmic and severely hyposmic patients. The time required to administer the OSIT-J using both picture-word alternatives and the four-plus alternative method was the shortest of the four OSIT-J methods. Visual information and test paradigms may affect clinical olfactory test results. The OSIT-J method using picture-word alternatives and the four-plus alternative method may be the most suitable for clinical practice.

Poster Session I: Tues July 22

Decline in Odor Memory and Odor Identification Performance Across the Adult Lifespan

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The odor memory test (OMT) was developed to provide concurrent measures of odor memory and odor identification. It employs a four-alternative forced choice identification task with an old/new memory discrimination procedure. It is well established that odor memory and odor identification decline with age. The current study used the OMT to assess the rate of decline in olfactory memory and identification performance over the adult lifespan. Participants were assessed using the OMT and the University of Pennsylvania Smell Identification Test (UPSIT). The OMT requires participants to label/identify ten odors during an encoding trial. During a second phase following a retention interval, 20 odors are used in an old/new odor memory discrimination task combined with additional odor identification trials. As would be expected, performance for both odor memory and identification decline after the age 60. Unexpectedly, performance for odor identification declined more quickly than performance for odor memory. The results are discussed in terms of the olfactory and cognitive demands of odor memory and identification tests.

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Poster Session I: Tues July 22

Predictors of Prognosis in Patients with Olfactory Disturbance

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Objectives: Although olfaction is often compromised by such factors as head trauma, viruses, and toxic agents, the olfactory epithelium and sectors of the olfactory bulb have the potential for regeneration. This study assessed the degree to which olfactory function changes over time in patients presenting to a university-based smell and taste center with complaints of olfactory dysfunction and the influences of etiology, sex, age, smoking behavior, degree of initial dysfunction, and other factors on such change.

Methods: Well-validated odor identification tests were administered to 542 patients on two occasions separated from one another by 3 months to 24 years. Multivariable regression and Chi-square analyses assessed the influences of the variables on the longitudinal changes in olfactory test scores.

Results: On average, smell test scores improved modestly over time. Patient age, severity of initial olfactory loss, and the duration of dysfunction at first testing were significant predictors of the amount of the change. Etiology, sex, time between the two test administrations, and initial smoking behavior were not significant predictors. The number of anosmics and microsmics exhibiting statistically-significant improvement in function was 56.72% and 42.86%, respectively. However, only 11.31% of anosmics and 23.31% of microsmics regained normal age-related function over time.

Interpretation: Some recovery can be expected in a significant number of patients who experience smell loss. The amount of recovery depends upon the degree of initial loss, age, and the duration of loss. Etiology is not a significant determinant of prognosis, in contrast to what is commonly believed.

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Poster Session I: Tues July 22

Men Grasp Odors Better than Women Do

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Behavioral studies have shown that olfactory information processing is more efficient for women than men. However, investigation on this topic remains confined to differences in psychophysics thresholds and perceptual ratings. Herein we used kinematics to test gender effects on odor management when the sense of smell was recruited during an everyday task, a reach-to-grasp movement. Participants (10 F and 10 M) reached-to-grasp a small (e.g. strawberry) or a large (e.g. orange) target fruit in absence or in presence of an odor evoking either a small (e.g. strawberry) or a large (e.g. orange) fruit. By using a CyberGlove we measured both arm reach duration and hand motion when grasping[1]. For males reach duration increased when the 'size' of the odor did not match the size of the target [Large odor-Small target M = 1724 ± 145 ms vs. Small odor-Small target M = 1663 ± 146 ms,

t(9) = 4.131, p<.05. Small odor-Large target M = 1625 ± 146 ms vs. Large odor-Large target M = 1568 ms ± 137 ms, t(9) = 3.078, p<.05]. Further males exhibited a bigger thumb extension for the Large odor-Small target than for the Small odor-Small target [M = 14 ± 2 deg vs. M = 12.50 ± 2 deg, t(9) = 2.95, p<.05]. The mismatch between the 'size' of the odor and the size of the target did not alter females' movements. These interference effects demonstrate that males extract from odors representations that are highly detailed and able to elicit specific hand shaping behaviors. The present study adds substantially to the debate about gender differences in odor perception indicating that when odors have to-be-acted, a male advantage emerges.

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Poster Session I: Tues July 22

Estrogen Replacement Therapy Induces Functional Asymmetry on an Odor Memory/Discrimination Test

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The secondary afferents of the olfactory system largely project to the ipsilateral cortex without synapsing in the thalamus, making unilateral olfactory testing a useful probe of ipsilateral hemispheric activity. In light of evidence that lateralized performance on some perceptual tasks may be influenced by estrogen, we assessed left:right nostril differences in two measures of olfactory function in 14 post-menopausal women receiving estrogen replacement therapy (ERT) and 48 post-menopausal women receiving no such therapy. Relative to women not taking ERT, those receiving ERT exhibited better performance in the left nostril and poorer performance in the right nostril on an odor memory/discrimination test. Similar laterality effects were not observed for an odor detection threshold test. These results suggest that estrogen influences the lateralization of an odor memory/discrimination task and that hormone replacement therapy in the menopause may be an excellent paradigm for understanding lateralizing effects of hormones on some sensory processes.

Poster Session I: Tues July 22

The Effect of Response Alternatives on Odor Naming and Recall

Melinda S. Brearton, Nakulan Balasubramaniam, Briana Wallace, Erica J. Mannea, Konstantin A. Rybalsky, Jason M. Bailie, Blair Knauf, Lloyd Hastings and Robert A. Frank

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Previous episodic odor memory research has revealed that providing verbal cues for odors significantly affects recognition memory performance. Providing an accurate and three alternative odor labels during both an encoding trial and a subsequent retrieval trial resulted in a significantly higher rate of recognition memory performance compared to providing no verbal cues during the task. The current study investigated the source of the verbal label advantage. Young, healthy participants were randomly assigned to experimental conditions that varied the number of odor labels provided during a joint odor identification/odor memory task. Memory performance was measured using an old/new odor test that required participants to smell and identify ten odors during the encoding trial. After a ten minute retention interval, participants were presented with ten old and ten new odors, and asked to accurately identify and distinguish between old and new odorants. The number of labels had a significant effect on memory, ranging from nearly perfect remembering when four labels were provided to no effect of the labels as the number of labels increased. It was concluded that episodic memory for odors is affected by the number of response alternatives provided as labeling cues. The results indicate that the mere presence of verbal cues is not sufficient for improved memory performance.

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Poster Session I: Tues July 22

Influence of Encoding and Retrieval Support on Odor Recognition Memory

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Alzheimer's patients are typically diagnosed as hyposmic, having lost some but not all of their olfactory ability. The precise nature of this hyposmia is not well understood, partly due to gaps in our general knowledge of normal odor memory processes. The appropriate interpretation of atypical odor memory performance in disorders such as AD requires a better understanding of odor memory dynamics in healthy individuals. The present study compared recognition memory performance (episodic memory) as a function of varying procedures shown to improve odor identification (semantic memory) performance. One hundred healthy adults completed a combined odor identification and odor recognition memory task employing 20 common odors. Four different conditions were used to manipulate the availability of odor labels during the odor encoding and retrieval portions of the task. It was predicted that manipulations that improve performance on the odor identification task would support better episodic memory performance due to the verbal labeling effects of memory encoding and retrieval. An analysis of variance revealed that while accuracy of labeling, as well as consistency of label use, varied significantly depending on the presence of semantic cues, only the condition that employed odor labels during the encoding as well as the retrieval phases of the task produced significant improvement in recognition memory. The results offer evidence that the ability of verbal labels to aid odor memory requires cuing at both memory encoding and retrieval. This information about normal olfactory memory processes may prove to be useful to the assessment of olfactory memory deficits in patient populations.

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Poster Session I: Tues July 22

Impact of Olfactory Loss on Behaviors Associated with Eating, Food Purchasing, and Cooking

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Although many patients with olfactory dysfunction complain about their problems associated with eating, little is known about the impact of olfactory dysfunction on dietary behaviors. Therefore, the aim of this study was to examine the influence of olfactory loss on dietary behaviors such as eating, food purchasing, and cooking. Using a questionnaire, the dietary behaviors of a total of 90 patients (44 functional anosmia (A) and 46 hyposmia (H) discriminated by ''Sniffin' Sticks test'') aged from 31 to 81 years, were compared to those of 101 healthy subjects aged from 31 to 75 years. Patients with olfactory loss had problems related to eating (A: 59.1% and H: 37.0%), food purchasing (A: 45.2% and H: 33.3%), and cooking (A: 58.5% and H: 52.3%). Patients aged from 31 to 50 years complained more about eating problems associated with olfactory loss than the patients older than 51 years. In addition, patients reported a lower frequency of specific cooking techniques such as seasoning (p<0.01), boiling (p<0.05), and baking (p<0.05) than healthy

subjects. In conclusion, our findings demonstrated olfactory loss influences on eating, food purchasing, and cooking. It would be meaningful to establish strategies to reduce problems associated with dietary behaviors of patients with olfactory loss.

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Poster Session I: Tues July 22

Effects of Peppermint Scent Inhalation on Appetite Control and Caloric Intake

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Previous research indicates that inhalation of certain scents may reduce hunger levels. The present study evaluated hunger levels during peppermint inhalation vs. non-inhalation, in addition to actual food consumption and dietary evaluation (e.g., fat intake, caloric intake, vitamin and mineral intake, etc.) over a period of two weeks. In a within-subjects design, participants completed a peppermint inhalation condition (administered every 2 hours) and a non-inhalation condition. Each condition was performed for 5 days during separate weeks. During the protocol, participant rated their hunger level every two hours and completed a food diary listing everything they consumed for the two five-day periods. Results indicate participants consumed significantly fewer total calories, calories from saturated fat, total fat, and sugar during the peppermint inhalation condition. The fewer number of calories consumed equated to a weight loss of one pound per week. Participants also rated their hunger level significantly lower during peppermint inhalation. The primary implication of these results is that peppermint scent can be used as an effective adjunct to decrease appetite, decrease hunger cravings, and consume fewer calories, which may lead to weight reduction and greater overall health. This is particularly relevant to manufacturers of weight loss and diet supplement companies who are attempting to find a 100% natural adjunct to their products.

Poster Session I: Tues July 22

Using Odors to Treat Sleep Apnea: A Test of Feasibility

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Apnea; a repeated suspension of breathing during sleep, is a prevalent sleep disorder with significant impact on daily life as well as on general health. We combined the following information to generate a potential treatment: 1) It is largely held that odorants fail to wake humans from sleep. 2) Odorants modify respiratory patterns. Given this, we hypothesized that providing an odorant during apnea may "jumpstart" the respiratory pattern without waking the individual. To address this, we first set out to test the influence of different odorant regimens on patterns of sleep and sleep-respiration. Subjects slept in a stainless-steel-coated odorant non-adherent room where we measured an EEG, EOG, EMG, EKG, blood oxygenation, as well as overall and nasal respiration. Subjects wore a small nasal mask where we could deliver odorants in a controlled fashion, with no non-olfactory cues as to odorant onset and offset. One of the following odorants: lavender oil, vanillin or ammonium sulfide was presented to 30 sleeping subjects. We found no difference in the frequency of waking between odor and odorless periods ($t=1.84$ all $p>0.08$). Analysis of nasal respiration revealed that all three odorants had a similar influence on respiratory pattern; a decrease in inhalation magnitude and an increase in exhalation magnitude ($t=4.98$ all $p<0.0005$). In addition, no difference was observed in total respiratory volume, as measured by respiratory belt ($t=0.66$ $p>0.52$). These results suggest that odorant presentation during sleep modulates respiratory patterns by altering the ratio between nasal and oral respiration without affecting overall respiration volume. These results point towards feasibility of using odorants to treat sleep apnea.

*These authors contributed equally.

Poster Session I: Tues July 22

Modulation of the Voltage-Gated Potassium Channel, Kv1.3, by the Adaptor Proteins Grb10 and Nshc in the Olfactory Bulb

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Gene-targeted deletion of the *Shaker* channel Kv1.3 results in a super-smeller phenotype and an altered metabolism that is resistant to obesity. To further elucidate physiological means of channel suppression in the olfactory bulb (OB) via cellular signaling, we used immunocytochemical and immunoprecipitation (IP) approaches to demonstrate the functional and molecular targets of channel regulation by two adaptor proteins, neuronal Src homology and collagen (nShc) and growth factor receptor-binding protein 10 (Grb10), in the neurotrophin pathway. Co-transfection of Kv1.3, neurotrophin receptor tyrosine kinase B (TrkB), and either nShc or Grb10 in HEK 293 cells relieved brain-derived neurotrophic factor (BDNF)-induced current suppression of Kv1.3. IP and Western analysis revealed that nShc forms a protein-protein interaction with Kv1.3 that was independent of BDNF-induced phosphorylation of Kv1.3. Interestingly, Grb10 did not directly scaffold with Kv1.3, none the less, it decreased channel expression at the membrane surface, and concomitantly decreased the BDNF-induced phosphorylation of Kv1.3. To examine the possibility that the Src homology 2 (SH2) domains of Grb10 were directly binding to phosphorylated tyrosines in Kv1.3, we utilized channel point mutations to substitute multiple tyrosine residues with phenylalanine. Removal of tyrosines 111-113, 137, and 449 prevented Grb10 from decreasing Kv1.3 expression. Tyrosines 111-113 and 137 have also been shown to be important for BDNF-induced current suppression. Our findings indicate that SH2 containing, adaptor protein recognition motifs on the channel could serve as therapeutic targets to decrease the conductance state of the channel.

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Poster Session I: Tues July 22

A Computationally Faster Mitral Cell Model

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It is assumed that biophysically realistic neuron responses in computational models requires numerical solutions to differential equations. This appears especially true at the soma and in dendrites that contain active conductances making network modeling with complex cells computationally infeasible. While mitral cells backpropagate action potentials along their dendrites, lateral dendrites receive only inhibitory synaptic events from granule cells. Furthermore, the magnitude of synaptic inhibition decays along the lateral dendrite, indicating that synaptic events along the mitral cell lateral dendrites can be modeled as passive conductances. We therefore designed a mitral cell with a primary dendrite and soma, which calculate membrane dynamics via traditional differential equations, but we then transform inputs from the soma and granule cell synapses onto the lateral dendrites as a combination of a few exponential functions. Subthreshold dynamics in the lateral dendrites can therefore be quickly predicted until the next event, reducing the need for continuous computations across all compartments of the lateral dendritic tree. Superthreshold dynamics are modeled with

rules. We compare our computational mitral cell model with the models of Bhalla and Bower and Chen et al., with respect to dynamical behavior and increases in computational speed.

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Poster Session I: Tues July 22

Intrinsic Conductances Actively Shape Excitatory and Inhibitory Postsynaptic Responses in Olfactory Bulb External Tufted Cells

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The initial synapse in the olfactory system is from olfactory nerve (ON) terminals to postsynaptic targets in olfactory bulb glomeruli. Recent studies have disclosed multiple presynaptic factors that regulate this important linkage but less is known about the contributions of postsynaptic intrinsic conductances to integration at these synapses. The present study demonstrates voltage-dependent amplification of excitatory postsynaptic potentials (EPSPs) in external tufted (ET) cells in response to monosynaptic (ON) inputs. This amplification is mainly exerted by persistent Na^+ conductance (I_{NaP}). Larger EPSPs, which bring the membrane potential to a relatively depolarized level, are boosted by the low voltage-activated Ca^{2+} conductance (I_{LVA}). In contrast, the hyperpolarization-activated nonselective cation conductance (I_h) attenuates EPSPs mainly by reducing EPSP duration; this also reduces temporal summation of multiple EPSPs. Regulation of EPSPs by these subthreshold, voltage-dependent conductances can enhance both the signal-to-noise ratio and the temporal summation of multiple synaptic inputs and thus help ET cells differentiate high- and low-frequency synaptic inputs. I_h can also transform inhibitory inputs to postsynaptic excitation. When the ET cell membrane potential is relatively depolarized, as during a burst of action potentials, IPSPs produce classic inhibition. However, near resting membrane potentials where I_h is engaged, IPSPs produce rebound bursts of action potentials. ET cells excite GABAergic PG cells. Thus, the transformation of inhibitory inputs to postsynaptic excitation in ET cells may enhance intraglomerular inhibition of mitral/tufted cells, the main output neurons in the olfactory bulb, and hence shape signaling to olfactory cortex.

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Poster Session I: Tues July 22

Spatio-Temporal Activity of Neurons in the Insect Antennal Lobe: A Data Driven Computational Model

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Olfactory systems share several similarities across phyla; in particular, the first relay of olfactory information in the brain (vertebrates olfactory bulb, insects antennal lobe) is formed of units called glomeruli, in which the synaptic connections take place. The fruit fly *Drosophila* is a good model for how this system sets up first order representations of neural stimuli. With only 40 glomeruli flies can perceive and discriminate a wide array of odors. Previous studies have extensively described the input to the AL from olfactory receptor neurons when stimulated by a large variety of odorant molecular classes. Furthermore, the output from projection neuron has also been well characterized to the same sets of odorants. Thus we have a wealth of information that shows how ORN inputs are transformed into a spatiotemporal output across projection neurons by networks in the antennal lobe. However, little is known concerning the actual interaction of the neurons within the antennal lobe, i.e. which neurons are activating/inhibiting which other neurons to produce this output. We used computational modeling to address this question, using as input the known activity of the olfactory receptor neurons for various odors. The activity of the neurons (in arbitrary units) was then modeled using a set of differential equations as a function of the activity of the other neurons and of their connectivity to this neurons. The connectivity itself was systematically investigated within the frame of known anatomical relationships. Then, all the results obtained were compared to known output of the antennal lobe, using calcium imaging data. Therefore, from the known input of the antennal lobe, we were able to determine which simulated connectivity patterns give output activity compatible with real data.

Poster Session I: Tues July 22

Local Circuit Interactions within Olfactory Bulb Glomeruli Generate all-or-None Mitral Cell Network Activation

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Glomerular microcircuits in the olfactory bulb play a major role in structuring sensory signals as they pass to the cortex. Here we examined the output of mitral cells (MC) that results from local synaptic processing within glomeruli. The focus of our first studies, done in rat olfactory bulb slices, was on long-lasting depolarizations (LLDs) that can be evoked in MCs by olfactory nerve stimulation. During patch-clamp recordings from MCs, we found that LLDs were all-or-none synaptic events in single cells (n=9). They also appeared to happen in an all-or-none fashion across all cells affiliated with a glomerulus, based on a near-perfect correlation between LLD currents and local field potentials within glomeruli (correlation coefficient = 0.9±0.1, n=5). LLDs also appeared to control spiking within a glomerular network in an all-or-none fashion, based on a near-perfect correlation seen in spike responses during loose cell-attached recordings from MC pairs connected to the same glomerulus (n=4). In order to assess whether this behavior extended to *in vivo* conditions, we placed a multi-electrode array into the olfactory bulb to record signals from the mitral cell layer in awake-behaving mice passively receiving odorants. For 3 ''responsive'' single units, odor-responses plotted as histograms of spike rate per trial were unambiguously bimodal (sum of squared errors significantly lower than unimodal fits, F-test, $p<0.05$), with peaks that changed in amplitude but not position with odor concentration. This behavior is consistent with spike activity under natural conditions being controlled by the all-or-none LLDs. Our results suggest that the glomerular local circuit transforms olfactory signals in a very specific way, converting graded input into a glomerulus to network-wide, all-or-none output.

Poster Session I: Tues July 22

Intrabulbar Projection Neurons Modulate Olfactory Bulb Output

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In the mammalian olfactory system, intrabulbar projections (IBPs) mediated by External Tufted cells (ET-cells) specifically link isofunctional odor columns within the same olfactory bulb giving rise to an intrabulbar map thought to play a role in coordinating information flow through the bulb. To study the function of IBP neurons within the glomerular network we developed a ''hemibulb'' preparation that maintains IBPs intact enabling the select activation of ET-cells associated with specific glomeruli. Using P2-GFP mice, we recorded from P2 mitral cells (MT-cells) while selectively stimulating P2 ET-cells. Here we show that ET-cell activity evokes a slow

modulatory (SM) potential within MT-cells which is mediated by the glomerular network and consists of both excitatory and inhibitory components. Interestingly, the timing of the SM potential with respect to olfactory nerve (ON) stimulation can produce converse effects on MT-cell output. When ET-cell activity precedes ON stimulation, the MT-cell response is potentiated; when ET-cell activity follows ON stimulation the MT-cell response is inhibited. Thus, IBP neurons through their ability to both potentiate and inhibit MT-cell activity play a key role in shaping OB output.

Poster Session I: Tues July 22

Computational Investigation of the Interaction Between Synaptic Adaptation and Potentiation in Olfactory Cortex

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Segmentation of target odorants from background odorants is a fundamental computational requirement for the olfactory system and is thought to be behaviorally mediated by olfactory habituation memory. Data from our lab (DAW) have shown that odor specific adaptation in piriform neurons, mediated at least partially by synaptic adaptation between the olfactory bulb outputs and piriform cortex pyramidal cells, is highly odor specific, while that observed at the synaptic level is specific only to certain odor-features. Behavioral data (CL) show that odor habituation memory at short time constants corresponding to synaptic adaptation is also highly odor specific and is blocked by the same pharmacological agents as synaptic adaptation. Using previously developed computational models of the olfactory system (CL) we here show how synaptic adaptation and potentiation interact to create the observed specificity of response adaptation. The model analyzes the mechanisms underlying the odor specificity of habituation, the dependence on functioning cholinergic modulation and makes predictions about connectivity to and within the piriform neural network.

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Poster Session I: Tues July 22

Odorant-Evoked Glomerular Activity Patterns Indicate that Mice are not Small Rats

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Over the past decade, we have characterized the spatial representations of odorant chemistry in the rat olfactory bulb using [^{14}C]2-deoxyglucose as a metabolic marker of evoked activity and a large panel of systematically related and unrelated odorants as stimuli. Given the usefulness of transgenic mice for mechanistic experiments in olfaction, there has been interest regarding the extent to which our results generalize to mice. We now have mapped responses to 32 odorants in mice, and we find that while certain stimuli such as carboxylic acids, aromatics, and long-chained hydrocarbons and aldehydes give comparable patterns in rats and mice, many other odorants give activity patterns that are almost entirely distinct in the two species. In mice, as in rats, certain odorants that share molecular features (*e.g.*, bicyclic structures or ester bonds) evoke overlapping patterns, but the locations of the activated domains can differ in rats and mice. In rats, increasing carbon number within a homologous series of aliphatic odorants is generally associated with chemotopic progressions of activity within glomerular domains responding to the odorant functional group and/or hydrocarbon backbone. Such chemotopic progressions are not obvious in mice, which instead show more abrupt differences in activated glomeruli within the domains for odorants differing by a single methylene group. We conclude that whereas clustering responses to odorant features may be a general strategy for odor coding, the specific locations of certain domains may be unimportant. We further propose that the smaller size of the mouse olfactory bulb may obviate the benefit of nearest-neighbor relationships to give optimal sharpening of responses to closely related odorants.

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Poster Session I: Tues July 22

The Addition of GFP-Labeled Glomeruli as Fiducial Markers in a 3D Model of the Mouse Main Olfactory Bulb

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The surface of the main olfactory bulb contains a topographical map of OSN activation known as an odor map. The basic functional units comprising these odor maps are the glomeruli, which are neuropil each receiving axons solely from olfactory sensory neurons expressing the same odorant receptor. Odor maps, when measured from genetically inbred animals smelling the same odorant, have been shown to contain both global similarities and regional differences. A major step towards understanding odor coding in the olfactory bulb is to better characterize the source of the variation in these maps, whether it be true individual differences or techical issues. We have developed an accurate and sensitive method to map the location of these glomeruli to within biological variability. This method, however, is limited by the requirement that the olfactory bulb be accurately sectioned along a plane parallel to the lateral olfactory tract. Any deviation from this plane could result in a mapping error. We have improved on this technique by constructing two 3-D models of the glomerular layer from two strains of mice. With these 3D models, we are better able to align odor maps captured from the bulbs of different animals, allowing us to correct for individual differences in bulb size or for technical errors that may have occurred during the surgical preparation of the bulbs. To test our new fitting technique, we have bred a new strain of transgenic mice that coexpress the green fluorescent protein (GFP) with three different odorant receptors: P2, MOR23, and M72. We have mapped the location of these glomeruli in reference to each other, establishing these glomeruli as a constellation of fiduciary markers that could be used to compare regional differences in immediate early gene odor maps.

Poster Session II: Wed. July 23

Dipeptide Sweetener Interaction with the Sweet Receptor

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The T1R2+T1R3 sweet receptor is a remarkably broadly acting receptor, capable of responding to native and artificial sweeteners. In vivo and in vitro studies suggest that this single heterodimeric receptor is the primary or only sweet taste receptor. There are many sweet tasting compounds of diverse chemical structure that bind to and activate the sweet receptor. How binding at different sites leads to receptor activation, and how the domains of each T1R monomer contribute to binding, activation and signal transduction is the focus of our work. To address these goals we have developed ligand binding and activity assays, and used these techniques in concert with mutagenesis and molecular modeling to begin to understand this complex receptor.

This presentation focuses on how the small molecule-binding site of T1R2 interacts with the dipeptide sweeteners aspartame, neotame and alitame. This "canonical" binding site is found within the "venus fly trap module" (VFTM) of T1R2. We have used the differential sensitivity of the human and mouse sweet receptors to dipeptide sweeteners, along with heterologous expression assays, site directed mutagenesis of T1R2, molecular modeling and a novel STD NMR (Saturation Transfer Difference Nuclear Magnetic Resonance) based binding assay to physically and chemically characterize the interaction of dipeptide sweeteners with the VFTM of T1R2.

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Poster Session II: Wed. July 23

Using the Human Sweet Taste Receptor to Discover Sweet Enhancers

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We are using human taste receptors to identify modulators of sweet taste. Here we report the first identification of sweet taste enhancers using receptor-based assays and chemistry optimization. To identify and optimize novel sweet enhancers we developed a set of proprietary high-throughput screening assays using the human sweet taste receptor (T1R2/T1R3). We first used these assays to evaluate a panel of known sweeteners. The results show that the rank order of potencies for these sweeteners in the receptor assay correlates with their rank order of sweetness intensities as confirmed through taste tests. Additionally, the EC_{50} of sweeteners are approximately equivalent to their taste thresholds. We used these data to establish enhancer assays for a variety of different sweeteners. Primary screening identified S2423 as an enhancer of the artificial sweetener, sucralose. S2423 enhanced sucralose in both the receptor assay and in taste tests. By chemistry optimization, we discovered more potent derivatives including S2383. This compound significantly enhances sucralose in the receptor assay and enables up to a 4-fold enhancement of sucralose in taste tests. Data describing the discovery and properties of these and enhancers of other sweeteners will be reported.

Poster Session II: Wed. July 23

Neoculin, a Sweet Protein with Taste-Modifying Activity, and its Binding Site in Human T1R2-T1R3

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Neoculin is a sweet protein with taste-modifying activity to convert sourness to sweetness. It tastes sweet to humans, but mice do not detect the taste as sweet or show preference for it. The human sweet taste receptor, hT1R2-hT1R3, recognizes a wide variety of sweet ligands, and has different binding sites for different sweeteners. We found that neoculin as well is recognized by hT1R2-hT1R3, but no information is available regarding its neoculin binding site. After confirming that mouse T1R2-T1R3 (mT1R2-mT1R3) does not respond to neoculin *in vitro*, we first determined whether one or both of hT1R2 and hT1R3 are necessary for hT1R2-hT1R3 response to neoculin. We transiently expressed mismatched pairs of human and mouse T1R subunits in HEK293T cells together with chimeric Gα, Gα16-gust25, and monitored its activation by calcium imaging. The lack of response of the hT1R2-mT1R3 expressing cells to neoculin suggests that hT1R3 is required for the reception of neoculin. Next, to investigate which one of the three domains of hT1R3, an extracellular amino terminal domain (ATD), a cysteine-rich domain (CRD) or a seven-transmembrane domain (TMD), is required for the reception of neoculin, we expressed several human/mouse chimeric T1R3s along with hT1R2. These experiments revealed that the ATD of hT1R3 is required for the response to neoculin, unlike the cases of other sweet proteins such as brazzein and monellin. Our further experiments using chimeric T1R3s revealed that the site critically required for the reception of neoculin resides in the hT1R3 amino acid residues 201-300. These results support our previously proposed docking model between neoculin and hT1R2-hT1R3 (Shimizu-Ibuka et al., *J. Mol. Biol*., 2006).

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Poster Session II: Wed. July 23

Isovanillic Sweeteners Interact with a Different Site of the Sweet Taste Receptor than the Structurally Related Compound Neohesperidin Dihydrochalcone

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Isovanillic derivatives (ID) are structurally related to the natural dihydroisocoumarin *R*-(+)-phyllodulcin and to the semisynthetic sweetener neohesperidin dihydrochalcone (NHDC). The family is characterised by a large number of different active compounds, with a wide range of relative sweetness (50-20.000)[1]. These features allowed to derive structure-taste relationships by classical (Q)SARs and molecular modelling[2]. The availability of a large number of ID elected these compounds as ideal for the identification and mapping of their binding site on the sweet taste receptor (SR) heterodimer TAS1R2+TAS1R3. ID are sweet to humans, but not to rodents and indeed activate the human but not the rat SR. This observation allowed combinations and chimeras of rat and human TAS1R2+TAS1R3 to be used to identify the binding site for isovanillic derivatives on the recombinant SR. Heterologous expression of different receptor chimeras showed that these compounds interact with the heptahelical domain of the TAS1R3 subunit. Mutations that affect the responsiveness of the SR towards NHDC, cyclamate or lactisole[3] had no effect on receptor activation by ID, indicating that these substances interact with a different site within the heptahelical segments of TAS1R3. Accordingly, we found that lactisole inhibited SR activation by two isovanillic derivatives allosterically and not competitively. Taken together, our data suggest that the interactions of most ID with the sweet receptor differ from that of the structurally related NHDC. References: 1 Arnoldi A. & al. *J.Chem.Soc., Perkin Trans. 1* **1993**, 1359-1366. Arnoldi A. & al. *J. Agr. Food Chem.* **1998**, *46*, 4002-4010. 2 Bassoli A. & al. *J.Chem.Soc.,Perkin Trans. 2* **1998**, 1449-1454. Bassoli A. & al. *QSAR* **2001**, *20*, 3-16. 3 Winnig, M. & al. *BMC Struct. Biol.* **2007**, *7*:66.

Poster Session II: Wed. July 23

Structure-Function Studies on MNEI: What Makes Monellin Sweet?

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Monellin is one of a small number of proteins that are perceived as intensely sweet by humans and some old world primates. Despite extensive characterization, the basis of their sweetness, such as the molecular details of their interaction with the sweet taste receptor T1R2:T1R3, remain unresolved.

We have undertaken structure-function studies on MNEI, a single chain variant of the natural sweet protein monellin, in order to better understand what makes monellin sweet. High resolution X-ray crystallographic structures were determined of wild-type and mutant MNEI proteins with diminished or restored sweet taste. These studies have identified conformational flexibility on the surface of MNEI, including a network of side-chain conformations involving residues critical for sweetness. The role(s) played by several key residues in maintaining a functional MNEI structure or, potentially, their mediating interaction with the sweet taste receptor, T1R2:T1R3, were also identified. To correlate these structural findings with protein function (*i.e.* sweetness) we are establishing methods to quantitate the interaction between MNEI and the T1R2:T1R3 receptor proteins. The most recent results in this area will be presented. Ultimately we aim to provide a direct correlation of sweet taste, binding affinity and protein structure, to provide a complete view of what makes a sweet protein sweet.

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Poster Session II: Wed. July 23

Interaction Between Triterpene Glycoside and Sweet Taste Receptor hT1R2+hT1R3

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Gymnemic acid (GA) and Glycyrrhizin (GL) are triterpen glycosides isolated from *Gymnema sylvestre* and *Glycyrrhiza glabra*, respectively. It is known that GA selectively suppresses taste responses to various sweet compounds without affecting responses to salty, sour and bitter substances in human and chimpanzees, and that GL tastes sweet to human. In order to examine whether GA and GL directly interact with the human sweet receptor, we used the human sweet receptor hT1R2+hT1R3 assay in transiently transfected HEK293 cells. Similar to psychophysical studies in human, 0.2 mg/ml GA inhibited the $[Ca^{2+}]_i$ responses to various sweeteners completely. 0.3 mM GI elucidated $[Ca^{2+}]_i$ responses. It has also been shown that in human psychophysical study, the sweet-suppressing effect of GA is diminished by rinsing the tongue with γ-cyclodextrin (CD), and that sweetness of GI is inhibited by γ-CD. So we examined the interaction between these triterpen glycosides and (α, β and γ-) CDs in vitro. The effect of GA rapidly disappears after rinsing the cells with 1% γ-CD. The responses to1 mM GI were also inhibited by 0.1% γ-CD completely. Our present study confirmed the previous finding in human psychophysical study and demonstrated that GA and GI directly interact with hT1R2+hT1R3 on the taste cell membrane and this interaction is inhibited by forming inclusion complex between these triterpen glycosides and γ-CD.

Poster Session II: Wed. July 23

Probing the Sweet Receptor's Transmembrane Domain Ligand Binding Pocket with Lactisole Analogs

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We initially reported that lactisole inhibited human sweet taste via its interaction with the transmembrane domain (TMD) of T1R3. A model of T1R3's TMD was developed based on the solved structure of bovine rhodopsin. Now we have examined responses to several lactisole analogs of heterologously-expressed wild-type and mutant T1R2+T1R3 sweet receptors. From these studies we have identified key pharmacophores of lactisole and more finely localized the site at which lactisole binds within hT1R3's TMD binding pocket. Our data provide constraints with which to refine computational models of the ligand binding pocket within the TMD of hT1R3 and suggest a possible mechanism of inactivation of the sweet receptor.

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Poster Session II: Wed. July 23

Molecular Mechanism of Senomyx Sweet Taste Enhancers

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Sweet is one of the five basic taste modalities. Sweet taste is mediated by a heterodimer of two G Protein-Coupled Receptor subunits, T1R2 and T1R3. Recently, we have identified sweet taste enhancers for sucralose (see abstract by Servant et al.). The enhancer molecules do not activate the sweet taste receptor, but instead potentiate the activity of the receptor in the presence of a sweetener. To understand the molecular mechanism of this enhancement, we performed mapping studies and mutagenesis analysis on two sucralose enhancers, S2423 and S2383. Our results indicate that both enhancer molecules interact with the Venus Flytrap Domain (VFT) of T1R2, and several key residues required for the enhancement activity were identified. Our data suggests corporative binding of sucralose with the enhancer molecules in the binding pocket of the VFT domain. This mechanism is similar to the enhancement of glutamate by IMP for the umami taste receptor (see abstract by Li et al.), and could be applied to the enhancement of other C-type GPCRs.

Poster Session II: Wed. July 23

Candidate Sugar Receptors of *Aedes Aegypti* and their Evolution in Other Insects

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In insects, the genes responsible for discrimination of soluble molecular cues in the environment are encoded by members of a highly divergent family of receptors, the gustatory receptors (Grs). The availability of the genome sequence for *Aedes aegypti* presents an opportunity to annotate and characterize the species' olfactory receptor genes to elucidate their potential function and define their relationship to the chemoreceptor genes of other sequenced arthropods. We annotated the Gr genes of *Aedes aegypti* through iterative BLAST searches of the available raw sequence data in GenBank using annotated genes of the malaria mosquito, *Anopheles gambiae*. We then assembled hypothetical phylogenetic relationships of these genes with respect to those of *Drosophila melanogaster*, *Drosophila pseudoobscura*, *An. gambiae*, *Apis mellifera*, *Nasonia vitripennis*, *Bombyx mori*, and *Tribolium castaneum*. Of the Grs annotated in *Aedes aegypti*, we identify a distinctive subfamily of eight proteins which we designate as sugar receptors (SRs) as a consequence of their phylogenetic relationship to receptors in *Drosophila melanogaster* which appear to be required for perception of a variety of sugar ligands. Examination of the evolution of these eight proteins in the available fly, moth, beetle and hymenopteran genome sequences reveals that they appear to have originated independently from single ancestral genes in the fly and beetle lineages and from two ancestral genes in the moth and hymenopteran lineages. We also describe a wide range of patterns of gene expansion and loss, intron evolution, and an unusual exonization event is revealed in one lineage of SRs.

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Poster Session II: Wed. July 23

Follistatin Directs Patterning and Development of Sox2-Expressing Taste Bud Progenitors

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Signaling from subjacent mesenchymal tissues is known to direct the morphogenesis of many epithelium-derived organs, including hair follicles, teeth, and the ductal elements of mammary glands. Although mesenchyme-derived molecular signals that direct taste bud morphogenesis have been postulated to exist, none have yet been described. Using mouse genetics and molecular analysis of gene expression, we identified the secreted TGF-β antagonist, follistatin (Fst), as such a factor. Follistatin is expressed diffusely throughout the tongue in early development and is restricted to the mesenchyme around embryonic stage 14.5, which coincides with taste papilla induction and patterning. Tongues from mice null for *Fst* (*Fst*-/-) have morphological defects including changes in papilla spacing, dysplasia of the epithelial-mesenchymal border, and loss of barrier formation in the intermolar eminence (IE). In the anterior tongue, an absence of *Fst* results in significantly decreased *Shh* expression in fungiform papillae, whereas expression of *Sox2*, while decreased in the apex of the papillae, is expanded basally along the epithelial-mesenchymal border. Interestingly in the IE, a region normally devoid of gustatory character, loss of *Fst* results in the expansion of molecules important for patterning gustatory papillae (Sox2, β-catenin and Shh). Additionally we observed de novo localization of gustducin, and innervation of the IE in regions where *Sox2* is expanded, suggesting an expansion of functional taste buds in a non-gustatory region. Altogether, these findings demonstrate a critical role for Fst in directing morphogenesis and patterning of taste papillae, and suggest that Fst acts upstream of multiple signaling pathways involved in taste bud development.

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Poster Session II: Wed. July 23

Developmental Alterations of *BDNF*, *Ntf5* and *TRKB* Expression in the Mouse Peripheral Taste System

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Taste buds within the anterior tongue are innervated by geniculate ganglion neurons through chorda tympani nerve. BDNF and NT4 are involved in the survival and differentiation of the cells in taste bud and geniculate ganglion, and maintenance of target innervation. To determine how expression levels of *bdnf* and *ntf5* correlate with taste development, we examined the relative expression levels of *bdnf* and *ntf5* in the mouse anterior tongue and geniculate ganglion from embryonic day 12.5 (E12.5) through birth (P0), using real-time RT-PCR. In the anterior tongue epithelium, *bdnf* expression began to decrease after E16.5, when taste buds start to differentiate, probably because BDNF becomes restricted to taste cells. *Ntf5* expression in the epithelium decreased beginning at E12.5, when the axons from geniculate ganglion have reached the tongue. In the tongue mesenchyme/muscle, both *bdnf* and *ntf5* levels were reduced after E14.5, by 80% and 84%, respectively (p <0.01). Since target innervation occurs between E14 to E15, BDNF and NT4 in the tongue mesenchyme/muscle and NT4 in the epithelium may support axon growth and branching before targeting, after which they are down regulated. In the geniculate ganglion, *bdnf* expression increased after E14.5. The expression level at P0 was about 6 fold higher than that at E14.5 (p = 0.002). *Ntf5* expression decreased after E12.5, and the level is 88.5% lower at P0 than at E12.5 (p <0.001). Thus, ganglionic NT4 might have an early embryonic role in geniculate ganglion development, while BDNF may maintain geniculate neurons or taste buds or regulate central connections during late embryonic or postnatal ages. Taken together, BDNF and NT4 are expressed differently and so play different roles during the development of the peripheral taste system.

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Poster Session II: Wed. July 23

Epithelium-Derived and Neurotrophic Factors Promote Geniculate Neurite Outgrowth Postnatally

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Taste afferents must continually renew communications within taste buds because taste cells undergo turnover, a process that could entail sprouting. To determine if diffusible factors may have a role we co-cultured tongue epithelium explants with geniculate ganglion explants derived from postnatal day 7-8 rats in collagen gels. Neurite outgrowth toward tongue explants (proximal, 273 ± 10 μm, s.e.m) was significantly longer than distal outgrowth (209 ± 20 μm) when cultures were fixed before neurites contacted the epithelium ($p<0.05$, paired values t-test, n=4). When neurites contacted and grew upon/near the serosal surface of the epithelium proximal growth (282 ± 16 μm) was also significantly longer than distal outgrowth (227 ± 9 μm) ($p<0.05$, n=16). Evidently lingual epithelium promotes geniculate neurite extension despite the small fraction of epithelium occupied by gustatory papillae. Could neurotrophic factors mediate this effect? BDNF (50 ng/ml) promoted significantly longer geniculate neurite outgrowth (300 ± 18 um, n=16) than no growth factor (231 ± 13 μm, n=15) ($p<0.05$, ANOVA). When adult ganglia were used, BDNF also stimulated longer neurite growth (225 ± 9 μm, n=10) than in control cultures (139 ± 19 μm, n=9) ($p<0.05$). GDNF promoted more outgrowth (178 ± 11 μm, n=8) than in control adult cultures but the difference was not significant. Although the combination of BDNF and GDNF promoted significantly more outgrowth (193 ± 11 μm, n=10) than control conditions, the value was intermediate between those resulting from either factor alone, so GDNF may interfere with BDNF signaling. Given that both BDNF and GDNF family ligands continue to be expressed in lingual epithelium postnatally, it will be important to determine if they influence epithelium/neurite interactions in vitro and in vivo.

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Poster Session II: Wed. July 23

Taste Cell Innervation in Neurotrophin Double Knockout Mice

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We are interested in examining the roles of neurotrophins in taste bud development and innervation. Our studies of single neurotrophin knockout mice have confirmed the roles of Brain-derived neurotrophic factor (BDNF) in gustatory innervation and Neurotrophin 3 (NT-3) in somatosensory innervation of the tongue. We have generated double neurotrophin knockout mice to examine the relationship between nerve fibers and taste cells. BDNF and neurotrophin 4 (NT-4), which both recruit the same receptor components for signaling, elicit different responses from gustatory ganglia (elegantly shown

by Rochlin and co-workers) and single knockout mice of these neurotrophins exhibit variable severity in their gustatory deficits. In order to dissect their specific roles in the peripheral taste system, we generated BDNF/NT-4 double knockout mice and compared their phenotypes to those of BDNF/NT-3, BDNF and wild-type mice. While the gustatory innervation was severely reduced in BDNF/NT-4 knockout mice, all remaining anterior and posterior papillae appeared innervated, indicating NT-3 dependent somatosensory innervation was preserved and rescued taste bud innervation. Nerve fibers were present in close proximity of taste buds and entered and branched within fungiform taste buds in BDNF/NT-4 mice. There were a larger number of troma-1 positive taste cells in BDNF/NT-4 mice compared to BDNF/NT-3 mice. However, nerve fibers were only randomly associated with the few Troma-positive taste cells present in BDNF/NT-3 double knockout mice. In conclusion, double knockout mice showed not only complementary roles for BDNF, NT-3 and NT-4 in lingual innervation, distinct supplementary roles were also observed for each neurotrophin.

Poster Session II: Wed. July 23

Characterization of Brain-Derived Neurotrophic Factor in Human Saliva

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Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophic factor family, proteins necessary for the survival, maintenance, and death of many types of central and peripheral neuronal and non-neuronal cells. Recently, it was demonstrated that BDNF is present in human saliva. Very little is known, however, about the characteristics of salivary BDNF and its biological correlates. In the current study, we determined the range of salivary BDNF concentrations in healthy participants (N=29), as well as the impact of saliva collection method on these concentrations, using a sandwich ELISA technique. Furthermore, the association of salivary BDNF with several biological factors, including sex, age, BMI, presence of the common Val66Met polymorphism, and serum BDNF levels, was assessed. The median salivary BDNF concentration was 616 pg/ml, with a range of 76.5 to 2736.5 pg/ml. Nonparametric analysis indicated that collection method significantly affected salivary BDNF levels. Protein concentrations were not, however, significantly associated with sex, age, BMI, the Val66Met polymorphism, or serum BDNF levels. The lack of association with serum BDNF indicates that saliva cannot be used in lieu of blood in future studies of the protein. The role of BDNF and other growth factors in saliva is still unknown, but previous rodent studies suggest that the salivary glands may be releasing the proteins to promote survival, differentiation, and/or death of cells in the oral cavity and gastrointestinal tract. This hypothesis is supported by findings that removal of the salivary glands leads to decreased wound healing, epithelial keratosis and changes in taste cells.

Poster Session II: Wed. July 23

BDNF and NT4 Both are Essential for the Survival of Developing Gustatory Neurons but Differentially Regulate the Development of Taste Buds in the Tongue Vs the Soft Palate

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Neurons of the geniculate ganglion innervate the taste buds in the tongue and the soft palate. There is a loss of 50% of these neurons in $BDNF^{-/-}$ as well as $NT4^{-/-}$ mice. In this study we counted the number of the geniculate neurons that innervate the tongue vs the palate and the number of taste buds in each region in wild type, $BDNF^{-/-}$ and $NT4^{-/-}$ mice. DiI was placed in the peripheral target to label the neurons innervating the tongue vs palate in E18.5 mice. Taste buds were visualized for quantification using troma-1 immunohistochemistry. For wild type mice, no significant difference was observed in the number of neurons that innervate the tongue vs the palate. The tongue and palate also had equal numbers of taste buds. As compared to wild type mice, $BDNF^{-/-}$ mice showed a significant loss of geniculate neurons that innervate the tongue (51%, $p<0.001$) and the palate (28%, $p=0.038$). Similarly a taste bud loss of 59% ($p<0.001$) for the tongue and 69% ($p<0.001$) for the palate was observed in $BDNF^{-/-}$ mice. The $NT4^{-/-}$ mice showed a significant loss in the number of neurons that innervate the tongue (48%, $p<0.001$) and the palate (58%, $p=0.003$). However, only an 18% ($p=0.037$) taste bud loss was observed for the tongue and there was no significant loss of the taste buds in the palate in absence of NT4. Tongues of $BDNF^{-/-}/NT4^{-/-}$ mice are innervated by 0 to 4 gustatory neurons, but have at least a few taste buds (3 to 16) by birth. In conclusion, gustatory neurons are equally dependent on BDNF and NT4 for survival regardless of what peripheral target they innervate. However normal taste bud development depends on BDNF but not NT4, so there is no direct relationship between the number of neurons in the geniculate ganglion and the number of taste buds present in the specific peripheral target.

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Poster Session II: Wed. July 23

Plasticity of Geniculate Ganglion Cell Innervation of Taste Buds

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Taste buds on the anterior tongue of the mouse are innervated by a small number (3-5) of geniculate ganglion cells. This innervation is remarkably discrete; the ganglion cells innervate only one bud, their peripheral fibers rarely branch to nearby buds. We investigated whether this pattern is static or, since taste bud cells turn over, if it changes overtime. Single buds were injected with a fluorescent, long-lasting retrograde marker, then re-injected repeatedly with the same or a contrasting marker over a period of time (6 weeks). In these cases (n=8), the number of labeled geniculate neurons increased with time. Apparently, some ganglion cells that initially innervated the bud were joined or, since 4 ganglion cells typically innervate each bud, replaced by other neurons that grew fibers into the bud at later times.This apparent remodeling process was investigated by first labeling the ganglion cells innervating one bud, then, after periods ranging from 5 mins.-30 days, labeling surrounding buds with a contrasting marker and re-labeling the central bud with a third marker (n=55). Analysis of single-, double- and triple-labeled ganglion cells showed that over a time period of 1-3 days up to 50% of neurons innervating the central bud withdraw their fibers and deploy them to surrounding buds. Over the same time period, neurons that had innervated surrounding buds send fibers into the central bud. This rapid remodeling/re-deployment of ganglion cell peripheral fibers amongst neighboring buds could relate to the neurons' synaptic relationship with a transient population of receptor cells.

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Poster Session II: Wed. July 23

Cell Differentiation of the Taste Buds on the Soft Palate and Fungiform Papillae Reconnected to Different Gustatory Nerves

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The chorda tympani (CT), glossopharyngeal (GL) and greater superficial petrosal (GSP) nerves innervate taste buds in the fungiform papillae (FF), circumvallate papillae (CV) and soft palate (SP), respectively. Recent advances in the molecular characterization of taste cells revealed that there are regional differences in Type II cells of taste buds among loci: gustducin is dominantly co-expressed with sweet receptors in the FF, bitter receptors in the CV and both receptors in the SP. These differences in taste buds must be involved in the marked difference in the properties of nerve responses to gustatory stimulation in the individual nerves. On the other hand, denervation results in the disappearance of taste buds, and reinnervation induces the regeneration of taste buds, indicating the nerve-dependency of taste bud maintenance. However, it is unclear whether individual nerves are responsible for the regional differences in taste cell differentiation. We have recently reported gustducin was expressed in almost all (96.7%) IP3R3-expressing cells on the SP while only 42.4% in the FF. Based on these results, we are analyzing regenerated taste buds induced by cross-regeneration of the CT and GSP in rats and mice, using immunohistochemistry and *in situ* hybridization. The results at present showed no difference in the co-expression pattern of gustducin and IP3R3 between in the SP and FF after the regeneration with the different nerve supply. This suggests that the same regional difference in taste buds still remains as it was after the cross-regeneration of the nerves.

Poster Session II: Wed. July 23

Morphological Measures of Injury-Induced Persistant Alterations of Chorda Tympani Nerve Structure and Function in Adult Rats

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Unilateral chorda tympani nerve transection (CTX) produces persistent morphological changes in the peripheral and central taste systems in adult animals. Data from our lab indicates dramatic and persistent reduction of taste bud size in the periphery and chorda tympani (CT) nerve terminal field volume in the central target, the nucleus of the solitary tract following CTX. Both peripheral and central consequences of CTX persist despite functional regeneration of the injured nerve by 45 days post-CTX, suggesting transection of the taste nerve can lead to permanent alteration of CT nerve structure and function. It is our goal to elucidate the injury-induced alterations in the CT nerve in order to understand the underlying mechanisms responsible for injury induced changes in peripheral and central taste morphology. Thus, current studies are being conducted to qualitatively and quantitatively describe the effect of nerve injury on CT cell bodies and both central and peripheral axons. Techniques include whole nerve electron microscopy, light microscopy, and confocal microscopy. These techniques will allow us to detect and describe degeneration of CT cell bodies and fibers following injury. Such measures will be useful not only in understanding injury-induced changes seen in experimental animal populations, but also for understanding taste abnormalities seen in human patients after CT nerve injury. Findings from these studies may ultimately lead to effective treatment options to prevent maladaptive injury induced alteration in taste function following nerve injury.

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Poster Session II: Wed. July 23

Changes in Proliferative Activity of Taste Buds after Irradiation

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Loss of the sense of taste in humans is common after radiation therapy (Schwartz et al., 1993), although the cellular basis for this loss is not known. Throughout adult life, taste cells are replaced by proliferating progenitor cells around taste buds, while cells within buds are postmitotic (Farbman, 1980). As proliferating cells are more sensitive to irradiation than differentiated cells (Brown et al., 2002), we hypothesized that radiation may target perigemmal dividing cells, which would reduce new taste cell production and cause taste loss. To test this, we irradiated the heads of mice with a single 4Gy dose, and compared cell cycle kinetics to those of control mice at 3, 5, 7, and 9 days post-irradiation (dpi), including: 1) BrdU incorporation (S phase); 2) proliferating cell nuclear antigen immunoreactivity (PCNA-IR; entire cell cycle except early G1); and 3) phospho-histone3-IR (pH3; M phase). We found fewer BrdU-positive cells than controls at 3 dpi, a slight increase in BrdU-labeled cells at 5 days, and a return to lower levels at 7 and 9 dpi, suggesting that fewer cells enter S phase prior to 3 dpi. Consistent with this idea, we detected many fewer cells in M phase (pH3-IR) through 5 days, followed by a steady increase through 7-9 dpi. Interestingly, loss of PCNA-IR cells was not dramatic until 7-9 dpi; at days 3-5, PCNA-IR cell number was still comparable to controls. In sum, our data suggest that irradiation treatment results first in cell cycle arrest in many mitotic cells around taste buds, followed by their death. In other injury models, cell death triggers mitotic activity of progenitor cells; we observed this in taste buds at 5dpi, but not longer as expected. We are currently investigating this paradox, and attempting to better define the population of cells affected by radiation.

Poster Session II: Wed. July 23

Expression of the Basal Cell Markers of Taste Buds during Mouse Embryonic Development

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In mammals, taste buds are maintained by gustatory nerves, and the receptor cells are constantly differentiated from the basal cells throughout life. Expression of *Sonic hedgehog* (*Shh*) in the basal cells particularly depended on the nerves and decreased markedly at six hours after the denervation. In contrast to adulthood, *Shh* expression in the tongue epithelium was reported to start nerve-independently during embryonic development. Broad expression of *Shh* on the dorsal surface of the anterior tongue shifted to a focused expression corresponding to the distribution of fungiform papilla placodes. Although embryonic expression of *Shh* in the fungiform papilla placodes was reported to have a critical role in the papilla patterning, it remains unclear whether the appearance of *Shh*-expressing spots indicates the differentiation of the basal cells of taste buds. To examine the embryonic development of the basal cells, the expression of the basal cell markers of taste buds (*Shh*, *Prox1* and *Mash1*) was determined in the mouse embryo by in situ hybridization and immunohistochemistry. *Prox1* was co-expressed with *Shh* from the beginning of *Shh* expression in a punctate pattern on the anterior tongue (E12.5) and soft palatal region (E14.5), suggesting that the basal cells of taste buds and *Shh*-expressing spots in embryos share common features. *Mash1* expression lagged behind the expression of *Shh* and *Prox1* by

approximately 2 days in both regions. Nerves reached the epithelium expressing *Shh* slightly before the onset of *Mash1* expression. These results suggest that the differentiation of the basal cells in the taste bud starts nerve-independently.

Poster Session II: Wed. July 23

Gene Array Analysis to Identify Cell Cycle Target Genes of Shh Signal Disruption in Fungiform Papilla Formation

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Sonic hedgehog (Shh) is a powerful morphogen that regulates fungiform papilla development, number and pattern. When Shh signaling is disrupted in embryonic tongue, fungiform papilla numbers double on anterior tongue and form in the usually papilla-free intermolar eminence of the posterior tongue. Mechanisms that lead to development of supernumerary papillae are not known, but cell proliferation might contribute to the formation of multiple papillae. We used microarray analysis to identify cell cycle-associated genes that signal in the papilla response to Shh signal disruption. Whole tongues from gestational day 14 rat embryos were dissected and cultured for two days in standard medium (STAND) or in cyclopamine (CYCL), an alkaloid that disrupts Shh signaling at the receptor complex. Anterior tongue (AT) or intermolar eminence (IE) pieces were dissected from each culture and pooled to yield 4 groups: STAND, AT and IE; or CYCL, AT and IE. Tissue was immediately homogenized and RNA was extracted, labeled and hybridized to Affymetrix gene chips. Normalized data were analyzed with Affymetrix software. Relative to STAND AT or IE, Shh receptor and transcription factor genes were down regulated in CYCL AT or IE at 5 fold or greater change. Shh signal disruption altered a number of genes involved in cell cycle progression, including Cyclin D2, cyclin dependent kinase inhibitors, tumor suppressors, and cell cycle activators. The multiple papillae that form with Shh signal disruption are not accompanied by uniformly high activity in genes involved in cell proliferation. Rather, up and down regulation of activators and inhibitors indicates a complex involvement of cell cycle genes in the papilla response to cyclopamine.

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Poster Session II: Wed. July 23

Genome-Wide Analysis of Gene Expression in Primate Taste Buds

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Taste buds are ideal model systems to better characterize and understand sensory cell differentiation and development. We have generated a genome-wide database of gene expression in primate taste buds. We used laser capture microdissection to isolate taste buds from macaque circumvalate (CV) and fungiform (FG) papillae and compared them to lingual epithelial cells collected from sites close to but not adjacent to the taste buds. Pairs of samples (taste bud and lingual epithelium) were collected from CV papillae from four Rhesus macaque monkeys and from FG papillae from six animals. Gene expression was measured in all 20 samples following RNA extraction and cDNA amplification using Affymetrix Rhesus Macaque genome arrays representing over 47,000 transcripts. The resulting database contained over 1900 taste bud-associated genes. In addition to known taste bud-associated genes including taste receptors (T1R1 and T1R2, many T2R receptor genes and PKD2L1), taste signal transduction components (GNAT3, TRPM5 and PLCB2) and developmental genes (SHH, ASCL1 and others), mRNAs for many other interesting genes were found in the taste bud database. Two genes that encode transmembrane proteins, TMEM44 and MDSF4, were found to be expressed in cells likely to be SHH-positive (possibly progenitor) cells. Immune system-associated genes were expressed at high levels including those encoding several cytokines and chemokines genes suggesting that the taste bud is a site of active immune protection. Genes associated with neuronal and sensory cell development were also prominently expressed, including SOX1, SOX21 and SIX1. We conclude that our database of gene expression in primate taste buds is a powerful tool for better understanding the biology of taste. Peter Hevezi and Bryan D. Moyer are co-first authors.

Poster Session II: Wed. July 23

Immune Cell Populations in Healthy Human Fungiform Papillae

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Our aim was to characterize immune cells in healthy human fungiform papillae taken from the anterior tongue that did and did not contain taste buds. We examined dendritic cells, macrophages and lymphocytes via immunohistochemistry in 3-4 fungiform papillae from each of six healthy subjects. Among the innate immune cells, CD11c+ dendritic cells were more prevalent than CD64+ macrophages, while the density of CD83+ mature and CD209+ immature dendritic cells were similar. Dendritic cells were mostly localized in the lamina propria and subepithelial region, but intraepithelial dendritic cells were also detected. T lymphocytes were present in epithelium and underlying connective tissue with CD4+ T cells more common than CD8+ T cells. In contrast to T cells, only very few CD19+ B lymphocytes were detected, making T cells more prevalent across the fungiform papillae. Another notable finding is that none (or very few) of the detected immune cells were found within taste buds, which were embedded within the outer layers of the epithelium. This finding suggests that there may be an immunologic barrier around taste buds, that prevents immune cells from entering and disrupting normal bud signal processing. We hypothesize that since taste bud cells undergo rapid programmed cell death (~every 9 days), immune cells entering the taste bud would flood this region and interfere with normal taste function. To our knowledge, this is the first report on immune cells in human taste organs. These immune cells in fungiform papillae represent key players in our oral mucosal immune system. These results will help us to understand the impact of inflammation on gustatory activity and the interaction between the immune and gustatory systems.

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Poster Session II: Wed. July 23

Structure-Function Analysis of a Subclass of Mosquito Odorant Receptors

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Genes encoding two odorant receptors (OR2 and OR10) display a remarkable level of sequence conservation between and within two disease vector mosquitoes, *Anopheles gambiae* and *Aedes aegypti*. Microsyntenic relationships, gene structure, and expression pattern in olfactory tissues suggest

these genes are the products of a gene duplication event pre-dating the separation of the Anopheline and Culicine lineages. We have utilized a *Xenopus laevis* oocyte heterologous expression system coupled with two-electrode voltage-clamp recording to functionally characterize OR2 and OR10 from *An. gambiae* (AgOR2/AgOR10) and *Ae. aegypti* (AaOR2/AaOR10). Here we report that a cognate odorant of *An. gambiae* and *Ae. aegypti* evokes responses in all OR2/OR10 proteins from both species. Based on our sensitivity criterion (EC50), OR2 and OR10 represent two distinct functional groups. The OR2 gene lineage is approximately 10-fold more sensitive than the OR10 gene lineage. Homologous gene lineages retain similar function, whereas paralogous genes, OR2 versus OR10, display diverging function. *Ae aegypti* possesses an additional paralog in AaOR9, which based on genomic structure and sequence similarity would be predicted to be functionally closer to AaOR10 than to AaOR2. We see a direct correlation between gene phylogeny and sensitivity to odorant. Based on these results, we are proceeding with low-resolution mutational analyses to determine which regions are responsible for odorant-binding within this clade and provide new insight into the functionality of this protein family.

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Poster Session II: Wed. July 23

Analysis of Insect Olfactory Receptors Reveals a GPCR-Atypical 7tm Topology

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Insect Odorant Receptors (ORs) have been identified from genomic sequences based on the presence of multiple transmembrane domains and were hypothesised to be G Protein-Coupled Receptors (GPCRs). However, recent evidence has suggested that insect ORs possess an orientation atypical of GPCRs, with an intracellular N-terminus. This has been confirmed for the non-canonical chaperone receptor Or83 using glycosylation site mapping. In order to generalise this model to the ligand-binding ORs of insects, we have pursued an epitope-tagging approach. C-Myc or FLAG epitopes were inserted onto the N- and C-termini, as well as into the predicted hydrophilic regions of the ester receptor Or22a from the fly, *Drosophila melanogaster*. We also assessed the orientation of an odorant-sensitive OR from the moth *Epiphyas postvittana*, EposOR1, by inserting c-myc epitopes onto the N- and C-termini of this receptor. The accessibility of the epitope to its cognate antibody was determined in S2 cells transiently expressing the receptor in the presence and absence of detergent. From these data we could infer the location of the epitope as either intra- or extracellular. Each construct was tested for functionality using heterologous expression in Sf9 cells and calcium imaging. Using this system, we have shown that Or22a possesses an intracellular N-terminus, an extracellular C-terminus and at least seven transmembrane domains, and that this GPCR-atypical orientation of ORs extends to the Lepidoptera.

Poster Session II: Wed. July 23

Insect Olfactory Receptors are Heteromeric Ligand-Gated Ion Channels

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Insect olfactory organ possesses one of the most sophisticated chemosensory systems. Recent progresses in molecular- and neuro-biology revealed that insect olfactory receptor neurons (ORNs) express a novel class of olfactory receptors (ORs), which lack homology to G-protein-coupled receptor family and possess a distinct membrane topology with the intracellular N-terminus. The functional insect OR consists of a heteromeric complex together with the Or83b family co-receptor. Here, we provide evidence that insect OR complex comprise a ligand-activated cation channels. We overcame the difficulty in intracellular recording of insect ORN activities by expressing ORs in a heterologous expression system, and analyzed the function of the OR complex. HeLa and HEK293T cells expressing fruit fly, silkmoth, and mosquito OR complex exhibited extracellular calcium influx and nonselective cation conductance upon odorant stimulation. Inhibitors for known G-protein-coupled second messenger pathways had no effect on the odorant response. The odorant response kinetics was completely different from that in vertebrate ORNs. Ion permeability and the degree of response inhibition by a calcium channel blocker were dependent on the OR subunit composition. These results suggest that OR complexes directly elicit G-protein independent responses. The final evidence for the current production by ORs was obtained from the outside-out single-channel recording of OR complex-expressing cell membranes. The channels were spontaneously active, and the open probability increased upon stimulation with the cognate ligand. Conductance of fruit fly ORs was larger than that of mosquito ORs at −60 mV. We conclude that insect OR complexes are spontaneously-active odor-gated ion channels that likely regulate the ORN receptor potential.

Poster Session II: Wed. July 23

Effect of Ligand Conformation on the Activity of the Olfactory Receptor OR-I7

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Octanal is a potent ligand of the rat olfactory receptor OR-I7. Although the lowest energy conformer of octanal is fully extended, other conformations are only slightly higher in energy. To investigate which carbon-carbon bonds must be able to rotate freely in order to elicit activity at OR-I7, we have synthesized a series of eight carbon compounds in which an increasing number of carbons are constrained via cyclization. This panel thus mimics various non-extended conformations of octanal. Using calcium imaging, these compounds were screened in rat olfactory neurons transiently expressing OR-I7. We find a dramatic reduction in activity between 4-cyclobutylbutanal (with three freely rotating carbons between the aldehyde and point of conformational restriction) and 3-cyclopentylpropanal (with two free carbons). However, while the analogs with two or fewer free carbons were unable to activate OR-I7, they served as moderate antagonists of octanal. This panel may thus help efforts at modeling the weak intermolecular forces underlying ligand binding versus activation at an olfactory receptor.

Poster Session II: Wed. July 23

Receptor Ligand Interactions in Olfaction: the OR1D2 Receptor as an Example

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The exact functional interaction between odorant receptors and odor molecules is currently unknown. In general, most receptor-ligand models are based on a 'lock and key' concept, but there is some uncertainty if this concept also holds true for odorant receptors. To obtain a more detailed understanding of the structure-function relationship between receptors and their cognate ligands, we characterized a recombinant human odorant receptor,

OR1D2, which was previously identified in both olfactory sensory neurons and spermatozoa. The most potent agonist identified so far is bourgeonal, which has a muguet (lily of the valley) odor character. Based on this result, one could assume that all agonists must have a muguet character. Using recombinant receptor expression in a heterologues cell system (HEK293), receptive field analysis reveals a broad array of active and inactive odorants. Adding to our previously published data, we here, selected a range of 20 additional agonist candidates based on their odor character and screened for receptor acrivity. Our results show that activation of OR1D2 is not a guarantee that a substance will possess a muguet odor. Combining these molecular results with studies on specific anosmia, we show that there is no simple correlation between activation of a single type of receptor and the ultimate odor percept. Taken together, our results show that simplistic 'lock and key' models of olfaction provide limited information for odor quality perception. Thus, structure/function correlations on the receptor level should only be considered as activity determinants in the periphery of the olfactory pathway.

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Poster Session II: Wed. July 23

Differential Expression of Ret Receptor Isoforms in the Olfactory System

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The olfactory system has a unique topographical organization but the mechanisms controlling this remain largely unknown. Previously, we showed two members of the glial cell line-derived neurotrophic factor (GDNF) family were expressed in the olfactory neuroepithelium (ON) (Maroldt et al., 2005), GDNF broadly, whereas neurturin (NTN) was concentrated in zone 3. Subsequently, GDNF was shown to have a migrational effect on olfactory ensheathing cells (OECs) *in vitro* (Cao et al., 2006). NTN and GDNF signal via the receptor Ret which has two predominant isoforms, Ret9 and Ret51. In this study, we aimed to examine the roles of Ret9 and Ret51 by determining their cellular expression in the rat olfactory system. Adult animals were cardiac-perfused and neonates immersion fixed with 4% PFA. Olfactory tissue was paraffin embedded and examined by immunofluorescent histochemistry. 1^0 antibodies included pAbs specific for Ret9 and Ret51 (Santa Cruz), markers of immature and mature neurons and markers of OECs. In neonatal and adult ON, Ret9 was expressed by immature and mature ORNs. Ret51 was expressed by a rare subpopulation of ORNs restricted to zone 3 and in the adult colocalized with GAP43 +ve axonal fibers passing thru the basal lamina. Ret51 +ve ORNs were also Ret9 +ve. In the olfactory nerve layer (ONL), both isoforms were expressed by OECs as evidenced by double-labeling with s100 and NPY. These data suggest Ret9 is the main functional isoform in immature and mature ORNs, while Ret51 plays a role in a restricted odorant receptor zone of the ON. One possible explanation is that Ret51 may have a selective relationship with NTN which is also expressed in this zone. Expression of both receptors on OECs, a unique type of axonal growth-promoting glia, is consistent with GDNF playing a key role in their function.

Poster Session II: Wed. July 23

Cellular Differentiation in the OE of MASH1 Knockout Mice

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Notch signaling drives cell fate choice in the MeBr lesioned-recovering adult rodent olfactory epithelium (OE), as shown by increased expression of the downstream Notch effector Hes1 in globose basal cells (GBCs) that differentiate into sustentacular (Sus) cells and by the effects of manipulating Notch signaling. Hes1 is known to block the expression of Mash1 and other proneural genes. Accordingly, the OE of Mash1 knockout mice (KO) expresses no Hes1 mRNA at E12.5 (Cau et al., 1997), while 3′ UTR of mutated Mash1 is expressed in presumptive Sus cells at E14.5 and E17.5 (Murray et al., 2003). The prior findings prompt exploration of Sus cell differentiation and Hes1 expression in Mash1 KO mice where neuronal differentiation need not be repressed. We have examined the OE in perinatal knockout and control mice, with particular attention to the expression of cell type-specific markers. At this stage, cells at the apex of the mutant OE retain an olfactory phenotype as shown by the absence of respiratory epithelial markers. An increasing percentage of keratin (+) cells at the apex of the OE of Mash1 KO mice express Hes1 over this period, while other cells deeper in the OE are also Hes1 (+); heterozygous littermates restrict expression mainly to Sus nuclei at the apex of the OE. In addition, the widespread inability of the mutant epithelium to execute neuronal commitment and differentiation produces an increase in cells expressing Sox2 and c-kit – proteins expressed by GBCs that correlate with multipotency in the OE. Thus, Hes1 is eventually expressed in Sus cells, for unknown reasons, but is not required for their initial differentiation.

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Poster Session II: Wed. July 23

Expression of *Splash*, a Proneural Gene, in the Olfactory Organ of Adult Spiny Lobsters

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Adult neurogenesis occurs in the olfactory pathway of many animals including the spiny lobster *Panulirus argus*. Proliferation of olfactory receptor neurons (ORNs) and other associated neurons is located in the antennular lateral flagellum (LF), the olfactory organ of spiny lobsters. ORNs are added in the proliferation zone, become functional in the mature zone, and are shed in the senescence zone. The proliferation rate of ORNs is highest in premolt compared to post- or intermolt animals. Using a candidate gene approach to elucidate the molecular mechanisms associated with adult neurogenesis in the LF, we identified and characterized proneural gene *splash* (**s**piny **l**obster **a**chaete-**s**cute **h**omolog), a homolog of *achaete-scute*. RT- PCR shows *splash* expression is significantly higher in premolts, where the highest rate of proliferation occurs, compared to post- or intermolts. We used *in situ* hybridization (ISH) to identify the cellular expression of *splash* in the LF. We tested whether molt stage or developmental zone affects *splash* expression. To identify proliferating neurons, we used *in vivo* incorporation of bromodeoxyuridine (BrdU). Using digoxigenin-labeled RNA probes, we show that *splash* is expressed in the epithelium, in the soma of proliferating and mature ORNs, in auxiliary cells, and in other, large bipolar sensory neurons, whose identity is currently unknown. *splash* is not expressed in haemocytes or axons and dendrites of ORNs. *splash* expression is variable in the epithelium and ORNs but not in the large sensory neurons across the developmental zones. Our data suggest that *splash* is associated with but not restricted to the proliferation of ORNs in adult spiny lobsters.

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Poster Session II: Wed. July 23

Neurod1-Derived Cells in Olfactory Epithelium

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The transcription factor NeuroD1 is expressed by globose basal cells (GBCs) in the mouse embryonic olfactory epithelium (OE) as well as during lesion-induced regeneration in rat, but no phenotype has been described for the OE of NeuroD1-knockout animals. We have used a BAC transgenic, NeuroD1-driven Cre recombinase to determine whether all olfactory sensory neurons (OSNs) are derived from a common NeuroD1-expressing progenitor by crossing them with Rosa26-LacZ reporter mice. Tissue sections of adult OE reveal universal expression of β-gal by all or nearly all neurons within the OE, as shown by co-localization of β-gal with neuronal markers, including PGP9.5. In addition, isolated β-gal (+) cells are observed in whole mounts amidst the respiratory epithelium and may correspond to isolated chemoreceptors. Co-localization of β-gal with Pax6 demonstrates that necklace glomeruli-targeting neurons also derive from NeuroD1-expressing progenitors. In addition, we investigated the effect of NeuroD1 knockout on OSNs. As compared with adult wild type (WT) and heterozygous (het), the OE from homozygous (null) mice mouse OE reveals only a subtle neuronal abnormality. In some portions of ventral mucosa of null mice, the OE is devoid of mature, OMP (+) OSNs, although retaining an expanded population of immature, PGP9.5 (+) OSNs. Our results support the idea that the vast majority of (if not all) OSNs derive from a NeuroD1-expressing progenitor. Moreover, absence of NeuroD1, although not critical for initial OSN differentiation, may compromise neuronal survival in some fashion.

Poster Session II: Wed. July 23

The Effect of Blocking Retinoic Acid Signaling on the Regeneration of Olfactory Sensory Neurons in the Adult Mouse Olfactory Epithelium

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In many systems, retinoic acid (RA) is essential for patterning complex fields of neurons, first for inducing neural differentiation and then for specifying neuronal subtypes within the field. It may exert a similar influence in the olfactory epithelium, whose olfactory sensory neurons (OSNs) are highly organized across the epithelial plane. We have previously shown that the three main synthetic enzymes for RA are expressed in a differential manner across the epithelium suggesting that concentration of RA may influence neuronal subtype specification with regard to odorant receptor (OR) expression. RA, however, may be required before OR choice is made and terminal differentiation occurs. We examined the effect of disrupting RA signaling, therefore, early in neuronal differentiation on the fate of OSNs. We cloned a dominant-negative form of the RA receptor (dnRARalpha403) into the pLIA-IRES.GFP retroviral vector and infected progenitor cells with it at one-day post MeBr-lesioning. Animals were allowed to recover for 21 days at which time they were sacrificed and colonies arising from virally-infected progenitors were analyzed. Clones arising from pLIA-dnRARalpha403-IRES.GFP infection had a significantly lower percentage of mature OSNs when compared to control clones (3% and 37%, respectively, $p<0.001$ Chi Square Analysis) although the pLIA-IRES.dnRARalpha403 clones had a significantly higher percentage of OSNs overall (74% vs. 59% $p = 0.01$ Chi Square Analysis). Whether OSNs are failing to become mature or if they are reaching maturity and then dying is unclear. However, we can conclude that RA plays a role in differentiating OSNs coincident with the timing of OR choice leaving open the possibility that affected OSNs fail to express a proper OR, which may, in turn, lead to their early death.

Poster Session II: Wed. July 23

Sequential Expression of Pre-Synaptic Molecules Duringolfactory Sensory Neurons Maturation

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The mammalian Olfactory Epithelium (OE) possesses the rare capacity of continuous neurogenesis during adulthood, which provides an opportunity to study the intrinsic and extrinsic factors critical for establishing the identity of a sensory neuron. It has been postulated that the formation of a synapse between the axons of the sensory neurons and the dendrites of second order neurons in the olfactory bulbis a critical step in the process of sensory neuron maturation. However, it is not yet clear whether OSNs follow an intrinsic maturation program or whether they require synapse formation in order to fully mature. Furthermore, it is unknown when in the life of the sensory neuron pre-synaptic differentiation starts to take place. Identifying synapse-associated molecules expressed by sensory neurons and their expression patterns within the epithelium is a first step toward understanding the relation between synapse formation and sensory neuron maturation. By using a panel of specific in situ hybridization probes, we assessed in the epithelium the expression patterns of messengers for proteins involved in the pre-synaptic vesicle release machinery. Our results indicate a sequential onset of expressions for the pre-synaptic molecules as sensory neurons mature. Interestingly, subsets of pre-synaptic molecules display similar expression patterns, suggesting common regulatory mechanisms. Also, the expressions of all pre-synaptic molecules so far tested are restored after recovery from bulbectomy, suggesting that OSNs would mature as pre-synaptic cells independently of their target. Our data set the stage for understanding the molecular events underlying the differentiation and the maturation of pre-synaptic sensory neurons.

Poster Session II: Wed. July 23

Establishment of Transgenic Rats in which Olfactory Receptor Cells Overexpress BDNF

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We have demonstrated that brain-derived neurotrophic factor (BDNF) was expressed by both olfactory receptor cells (ORCs) and sustentacular cells in rat olfactory epithelium. To clarify specific roles of BDNF secreted from ORCs, we generated transgenic (Tg) rats in which BDNF was overexpressed by olfactory marker protein (OMP)-expressing ORCs. In brief, a rat BAC clone (pTARBAC2.1) that contains promoter, coding, and enhancer regions for rat OMP was chosen as a backbone of ''expressing cassette''. The coding region for OMP was replaced by human BDNFcDNA and cDNA of c-Myc (an antigenic peptide located near the carboxyl terminus of human myc protein) using Red/ET technology. This recombinant clone, named OMP-BDNF-myc RecBAC (size: about 200kb), was inserted as the expressing cassette into fertilized eggs of wister rats by ''semi-knock-in'' technology''. These eggs were implanted into rat uteli, and founders in which the OMP-BDNF-myc RecBAC was inserted into their genomes were determined using southern hybridization technique. Then, the F1 rats of the founders were analyzed immunohistochemically using antibodies to c-Myc and OMP. In transgenic (Tg) F1 rats in which the OMP-BDNF-

myc RecBAC was present in their tail cells, OMP-immunoreactive ORCs contained c-Myc immunoreativity in the supranuclear region of ORC somata. By contrast, c-Myc immunoreacitivity was not detected in nasal respiratory epithelium of Tg rats. Thus, we conclude that Tg rats in which human BDNF in addition to rat BDNF was expressed by ORCs were established. Analyses for determining biological effects of overexpressing BDNF by ORCs are underway in our laboratory.

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Poster Session II: Wed. July 23

Identification of a Ciliary Enrichment Element Sufficient for Adenylyl Cyclase Compartmentalization in Primary Cilia

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Normal olfactory function requires the compartmentalization of the sensory transduction cascade in the highly specialized cilia of the sensory neurons. Genetic loss of signaling pathway components leads to olfactory deficiencies in mice and human diseases, disrupts cilia function, and results in profound deficiencies in odor detection. A conserved sequence element has been identified in several proteins that localize to cilia. This motif, RVXP, is found in the CNGB1b subunit of the olfactory channel and appears to contribute to cilia localization in olfactory tissue. The olfactory-specific adenylyl cyclase, AC3, lacks the motif but is highly enriched in cilia layer of olfactory neurons. We have initiated efforts to identify essential sequences in AC3 for cilia enrichment and determine the mechanism underlying this compartmentalization. We employed the IMCD3 cell line, a polarized kidney cell line that develops primary cilia in cell culture, to study the targeting of AC3 to the cilia membrane. Heterologous expression of AC3 protein results in significant enrichment in the cilium of these cells while another family member, AC8, showed no enrichment in cilia. To identify domains that regulate cilia targeting, we performed structure/function studies using a targeted chimeragenesis strategy creating hybrid adenylyl cyclases. These studies identified a short sequence in AC3 that is necessary for its enrichment in primary cilia and sufficient to enrich another adenylyl cyclase (AC8) not normally concentrated in the cilia compartment. Biochemical analysis using these chimeras should allow us to identify AC3 interactors involved in cilia sorting. Additionally, we are investigating the relevance of this putative cilia enrichment sequence *in vivo* through viral infection of olfactory sensory neurons in mice.

Poster Session II: Wed. July 23

Regional Variation in Survival of the Olfactory Sensory Neurons Expressing Mutant CNGA2 in Heterozygous Mice

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The olfactory system detects a wide range of airborne odorants. The main olfactory epithelium is composed of olfactory sensory neurons (OSN). A majority of OSN express the cyclic nucleotide-gated channel subunit A2 (CNGA2), a critical channel of the canonical odor transduction pathway. Previous studies have shown that in heterozygous females, OSN with a dysfunctional CNGA2 are gradually eliminated due to the activity dependent competition with OSN that express functional CNGA2. We examined the pattern of elimination of OSN carrying the mutant CNGA2 in the main olfactory epithelium using mice in which the CNGA2 coding region is replaced with the green fluorescence protein. OSN expressing the mutant CNGA2 are GFP positive. We found in sexually experienced adult females that the remaining GFP positive OSN were largely located in the lateral and ventral regions of the olfactory epithelia, with few GFP positive OSN in the dorsal medial regions. Further, we found the axons from the GFP positive OSN projected to a heterogeneous population of glomeruli in the olfactory bulb, including some microglomeruli that stained with *Ulex europaeus* lectin (UEA-I). Our results demonstrate regional variation in the survival of the OSN that expressed the CNGA2 mutant. Further experiments are needed to determine whether the life span of these OSN vary in different locations of the olfactory epithelium.

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Poster Session II: Wed. July 23

ATP-Induced Proliferation is Mediated Via Neuropeptide Y (NPY) in Adult Mouse Olfactory Epithelium

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Extracellular ATP exerts multiple neurotrophic actions such as proliferation in the CNS. However, the neurotrophic role of ATP on regeneration of the adult olfactory epithelium (OE) has not been investigated. We tested the hypothesis that ATP induces proliferation in the OE of adult Swiss Webster mice via a NPY-mediated mechanism. We found that intranasal instillation of ATP (200μM) significantly increased $BrdU^+$ cells in the OE by 124% of control ($p<0.001$; n=3). Pre-intranasal treatment with purinergic (P2) receptor antagonists PPADS (25μM) and suramin (100μM) did not alter the number of $BrdU^+$ cells in the OE (n=3), but significantly reduced the ATP-induced increase in $BrdU^+$ cells to 42% ($p<0.01$; n=3). These results indicate that ATP activation of P2 receptors induces proliferation. We observed that NPY expression in sustentacular cells was increased 20 hours after intranasal instillation of ATP (200μM) compared to control animals (n=3). Pre-intranasal treatment with PPADS (100μM) blocked ATP-induced NPY expression, indicating that ATP activation of P2 receptors induces NPY expression in sustentacular cells. Intranasal instillation of NPY Y1 receptor antagonist BIBP3226 (10μM) following intranasal ATP treatment did not alter the number of $BrdU^+$ cells (n=3), but significantly blocked the ATP-induced increase of $BrdU^+$ cells to 38% of control ($p<0.01$; n=3). These results indicate that blockade of NPY Y1 receptors significantly reduces ATP-induced proliferation in adult mouse OE. Thus, we demonstrate that ATP activation of P2 receptors upregulates the expression of NPY and increases proliferation in the adult mouse OE via activation of NPY Y1 receptors. This suggests that ATP, acting in synergy with NPY, may have a role in neuroregeneration of the adult OE.

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Poster Session II: Wed. July 23

Immunohistochemical Studies on the Olfactory Organ of Lake Sturgeon

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As a first step in understanding the role of olfaction in mediating lake sturgeon (*Acipenser fulvescens*) behavior, we conducted immunohistochemistry studies on yearling lake sturgeon olfactory organ. We found that the

olfactory epithelium was composed of morphologically distinct regions, the sensory epithelium and nonsensory epithelium. Elongated sensory neurons and supporting cells were packed together in the thick sensory epithelium while cuboidal glandular-like cells were the major components in the nonsensory epithelium. Clusters of these cells were also found between the sensory epithelium, dividing the sensory epithelium into regions. Sensory neurons stained positive for a monoclonal antibody SV2, a synaptic vesicle protein. Some of these neurons are immunoreactive to $G_{\alpha o}$ or $G_{\alpha olf/s}$. They were intermingled with cells that were immunoreactive to GFAP (Glial fibrillary acidic proteins). The GFAP positive signals were mostly limited to upper half layers of the sensory epithelium and a thin layer at the apical of nonsensory epithelium. Double immunolabeling suggests that immunoreactivity of GFAP did not overlap with that of $G_{\alpha o}$ or $G_{\alpha olf/s}$. We did not find S100 positive cells in the sensory epithelium in lake sturgeon. This is the first report on anatomical and cellular structures of the lake sturgeon olfactory organ.

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Poster Session II: Wed. July 23

Development of Crypt Cells in *A. Naccarii* Sturgeon Embryos

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All olfactory receptor neurons (ORNs) in fish form part of a single sensorial epithelium: the olfactory epithelium (OE). ORNs are bipolar neurons with cilia and microvilli (ciliated ORNs) or with microvilli alone (microvillous ORNs). In 1996, Morita and Finger reported another type of ORN in teleosts, characterized by a crypt-like apical invagination (crypt cells: CC). In 2000, Hansen and Finger described CC as a common characteristic in the OE of actinopterygians, and they have subsequently been observed in adult chondrichthyes and ray embryos (Ferrando et al., 2006, 2007). In acipenserids, CC have only been observed in juvenile specimens, and it has not been clarified whether they differentiate along with the rest of the ORNs during the lecitotrophic stage or during later development stages. Furthermore, a detailed optical (OM) or electronic (EM) microscopy study on the development of CC has not been published to date. In the present study, we used OM and EM to follow the development of CC in *A. naccarii* from hatching to the establishment of exogenous feeding. Based on these observations, we can affirm that CC are present from the first few post-hatching (PH) days. The CC appear with their nucleus very close to the basal lamina of the epithelium and enveloped by support cells In addition, from the beginning of day 2 PH, we observed cells with very similar characteristics to CC (absence of knob, abundant mitochondria on apical cytoplasm, numerous microtubules, enveloping support cells) but with cilia still remaining on their non-invaginated apical surface. We conclude that these cells may correspond to immature CC in which the crypt, the final feature of their morphological differentiation, has not yet formed.

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Poster Session II: Wed. July 23

An Integrated Immunohistochemical Analysis of Human Whole-Mount and Cryosectioned Olfactory Tissue

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To understand olfactory dysfunction in humans a more complete analysis of the normal olfactory mucosal (OM) histology is needed. The characterization of olfactory epithelium (OE) through the use of immunohistochemical (IHC) markers has expanded in lower vertebrates but has not translated to the same degree in humans. Our understanding of the human OM has been limited to epithelial biopsies and sections of autopsy material. In depth analysis of OM biopsies has provided little information on the mucosal condition as a whole. In addition, there is a dearth of knowledge in humans regarding the status of axonal projections onto the olfactory bulbs (OB) in relation to the condition of the mucosa. We obtained autopsy specimens of intact formalin-fixed OM and OB through the National Disease Research Interchange. Whole-mount (WM) IHC with anti-PGP9.5 was performed on sheets of OM to delimit that part of the olfactory area of the nasal lining that is neuroepithelium. Additional IHC was performed on sections obtained from a smaller region of the WM tissue and performed on the OB using anti-OMP, TuJ-1, and anti-PGP9.5. The analysis revealed a surprising degree of preservation of neuroepithelium even in tissue obtained from elderly patients with a history of dementia. Tissue-section analysis confirmed the presence of immature neurons in abundance admixed with a smaller population of OMP-(+) mature neurons. In the OB olfactory axons often projected deep to and beyond the glomeruli. Our findings suggest that neurogenesis persists in the OE even into pronounced old age. The finding of abundant immature neurons (a sign of disconnection) and aberrant axonal projections within the OB suggests that age-related olfactory loss may be due to an inability of olfactory neurons to form first order synapses.

Poster Session II: Wed. July 23

Transsynaptic Viral Tracing from Defined Neuron Populations in the Olfactory System

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In previous work, we demonstrated distributed columnar connectivity in the olfactory bulb by transsynaptic tracing using the pseudorabies virus (PRV). One disadvantage of this and all transsynaptic tracing techniques is that the population of neurons which were initially labeled can only be inferred by proximity to the injection site. In some cases it is difficult to determine if labeling arises from axons or dendrites innervating the injection site or if the labeling represents synaptic connection from neurons with cell bodies near the injection site. To address this issue, we have developed a technique which combines adult *in vivo* electroporation of DNA bearing a red fluorescent Cre recombinase with a PRV strain that will only fluoresce green and replicate in the presence of the recombinase. Source neurons are therefore labeled red and green, while synaptically connected neurons fluoresce only in the green. Preliminary results are discussed.

Poster Session II: Wed. July 23

Modified Transsynaptic Tracing Viruses Suggest an Extensive Axon Collateral Network in the Rat Olfactory Bulb

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Previous studies using a retrograde specific pseudorabies virus (PRV) strain showed distributed granule cell columns labeled through the lateral

dendrites of mitral and tufted cells. In further studies, we have engineered transsynaptic viral tracing tools which are capable of both anterograde and retrograde spread. GFP or mRFP1 under a CMV promoter was inserted into the gG locus of a (PRV) strain that bears a truncated gE to reduce virulence (Tirabassi and Enquist, J Virol 1999). The resulting strains, JD-1 and RD-1 respectively, were injected into the glomerular layer of the rat olfactory bulb. In addition to previously observed columnar patterns, widespread labeling of large cell types in the granule cell layer consistent with Blanes cells was evident two days after injection. These cell types are not widely labeled following injection with retrograde specific PRV strains (Bartha), suggesting that the labeling arises from anterograde transfer through mitral and tufted cell axon collaterals. These results are consistent with a previous physiological study (Pressler and Strowbridge, Neuron 2006) and suggest that Blanes cells may play a more prominent role in olfactory information processing than was previously thought.

Poster Session II: Wed. July 23

Visualizing Mitral Cell Axon Projection in Transgenic Zebrafish

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In the zebrafish, different classes of odorants such as bile acids and amino acids preferentially activate glomeruli within spatially distinct regions, developing an odor map in the olfactory bulb (OB). Using *OMP:RFP* and *TRPC2:Venus* double transgenic fish, we previously demonstrated that olfactory sensory neurons (OSNs) expressing OR-type and V2R-type olfactory receptors project axons to anteromedial and ventrolateral regions of the OB, respectively. In contrast, the dorsomedial region was innervated by neither RFP- nor Venus-positive axons, indicating the presence of at least three classes of glomeruli with distinctive OSN innervations. However, it remains unknown how the odor map in the OB is transferred via mitral cells to the higher olfactory centers. Here, we fluorescently labeled mitral cells and visualized their axonal projections using transgenesis with three different gene promoters. We found that the mitral cells are classified into three heterogeneous subsets in a spatially segregated manner in the three transgenic fish lines. In one line, the labeled mitral cells preferentially innervate glomeruli in the dorsomedial region of the OB and project axons into the telencephalon and further to the habenula directly. Single-cell labeling of these mitral cells revealed a relatively stereotyped projection pattern: the axon extends through the medial olfactory tract, makes branches to innervate both ipsi- and contra-lateral telencephalon, and further extends posterodorsally to reach the right habenula asymmetrically. We are currently carrying out the single-cell labeling of different subsets of mitral cells that innervate glomeruli in the ventral and lateral regions of the OB for comprehensive understanding of the neuroanatomical basis of the secondary olfactory pathway.

Poster Session II: Wed. July 23

Differential Projection Patterns of Mitral/Tufted Cells to Olfactory Cortex Versus Tubercle

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Advances in molecular biology of odor receptors have revealed a strikingly exquisite organization in the sensory projection from the nose to the olfactory bulb (OB), which provides critical insights into the initial coding and processing of odorants. Another fundamental question that remains is how olfactory signals are further relayed beyond the bulb into higher olfactory centers. Here we address this issue by electroporation dye labeling of different populations of projection neurons in the mouse OB and tracing their axonal trajectories along the lateral olfactory tract (LOT) into the piriform cortex (PC) and olfactory tubercle (OT). Our published work has established that the effective dye-loading area by local electroporation is small and confined within a diameter of a few tens of microns (Nagayama et al., Neuron, 2007). This allowed us to load neural tracers in a relatively specific manner into either mitral or tufted cells, via targeting a pipette to different depths in the external plexiform layer. The axons of tufted cells in the dorsal OB were found to send their terminals primarily to OT (red), while the mitral cell axons tended to branch out at a 90° angle mostly into both the anterior and posterior PC (green). Our data thus reveal a clear segregation between the central projections of mitral and tufted cells. Considering our previous work on the M/T cell functions (Nagayama et al., J Neurophysiol, 2004), these two types of projection neurons may process different aspects of odor information.

Poster Session II: Wed. July 23

Anatomical and Functional Organization of Kenyon Cells in the Mushroom Bodies of Male *Bombyx Mori*

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In insects, a variety of odors in the environment are detected by an array of antennal olfactory receptor neurons. Olfactory information is transmitted by axons of the olfactory receptor neurons to glomeruli in the first olfactory center called antennal lobe. Olfactory information is conveyed by projection neurons (PNs) to higher olfactory centers, the mushroom bodies (MBs) and the lateral protocerebrum. In the male silkmoths, the antennal lobe is divided into two subsystems: a macroglomerular complex, which is dedicated to pheromone processing, and a group of ordinary glomeruli, which is devoted to general odor processing. The macroglomerular complex consists of three subdivisions called toroid, cumulus, and horseshoe. PNs innervating the toroid respond to the major pheromone component, and PNs innervating the cumulus respond to the minor pheromone component. Their branching patterns of those neurons in the calyx of the MB are different. However, the organization of intrinsic neurons of the mushroom body (Kenyon cells) that receive inputs from the PNs is not well understood. Here, we describe anatomical organization of the MB. Whole mount immunolabeling with antibodies against *Drosophila* DC0, a catalytic subunit of protein kinase A preferentially expressed in the MBs, and FMRFamide revealed four subdivisions in the MB. Morphological characterization of single neurons labeled by intracellular staining showed that there were four morphological types of Kenyon cells, each of which corresponded to one of the four subdivisions. In addition, we isolated a gene that is homologous to *Drosophila* DC0 gene from the silkmoth brain. Our results indicate that the Kenyon cells are functionally diverse and the PKA cascade is involved in pheromone processing in the silkmoth MBs.

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Poster Session II: Wed. July 23

Sparse Odor Representation in the Mushroom Body and Associative Learning

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Sensory systems create neural representations of environmental stimuli; these representations can be associated with other stimuli through learning. Are patterns of spikes the neural representations that get directly associated with reinforcement during conditioning? In the moth *Manduca sexta*, using intracellular and extracellular recordings we found that long odor presentations (4 s), which are commonly used for olfactory conditioning, elicit only one or two spikes upon the odor's onset (and sometimes offset) in each of a small fraction of Kenyon cells (KCs). Varying the timing of sucrose reinforcement relative to odor-elicited spiking in KCs in a proboscis extension conditioning paradigm, we found the intervals between odor pulses and reinforcement that produced associative conditioning included no temporal overlap between spiking in KCs and sucrose presentation. Further, increasing the temporal overlap between spiking in the KCs and sucrose reinforcement actually reduced the efficacy of conditioning. Thus, spikes in KCs do not constitute the representation of odor that coincides with reinforcement, and spike-timing-dependent plasticity alone cannot underlie this learning.

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Poster Session II: Wed. July 23

A Standard Brain Atlas in the Search for Neural Networks Involved in Chemosensory Coding and Learning in the Moth *Heliothis Virescens*

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We are using heliothine moths as model organisms for studying mechanisms of chemosensory coding and learning. The goal is to uncover the neuronal network involved by providing morphological and physiological characterisations of neurons. Challenged by the need to integrate the data from different brain preparation a common framework was required. Therefore, we have made a standard brain atlas of the moth using the Interative Shape Procedure (ISP), developed and used in the honeybee standard brain (Rohlfing et al., 2001 and Brandt et al., 2005). This procedure is based on the average of many brain preparations. By using a proportional scaling system to reference a given preparation into the atlas brain, it is compensated for individual variations like brain shapes and sizes. Here, we present a three dimensional model of the moth standard brain atlas and show how it serves as a common framework into which neurons from several brain preparations are transformed. The following brain compartments are included in the model: Deutocerebrum with the antennal lobes (primary olfactory centres), mushroom body calyces, peduncles and lobes (secondary chemosensory centres, important for learning and memory), suboesophageal ganglion and tritocerebrum (primary gustatory centres), protocerebrum, central complex, protocerebral bridge, noduli, the optical ganglia (medulla, lobulla, lobulla plate) and the anterior optic tubercles. The integrated gustatory and olfactory neurons have been physiologically characterised according to responses to biologically relevant stimuli.

Poster Session II: Wed. July 23

Integration of Characterized Olfactory Interneurons into the Standard Brain Atlas of the Moth *Heliothis Virescens*

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We are studying chemosensory coding using heliothine moths, a serious pest insect, as model organisms. The goal in the present study is to understand how the primary olfactory center, the antennal lobe (AL), process plant odor information and how the information is further mediated to higher olfactory centers. The neural network of the AL is formed by synaptic connections in the glomeruli between the receptor neuron terminals, local interneurons and projection neurons. Also terminals of modulatory neurons innervate the glomeruli. By intracellular recordings we characterize AL interneurons physiologically by testing for sensitivity to antennal stimulation with biologically relevant plant odorants. Receptor neurons, classified by gas chromatography linked to single cell recordings, are narrowly tuned to one primary odorant and respond weaker to a few structurally related odorants (Røstelien et al., 2005). The minimal overlap of the molecular receptive ranges indicates a labeled line input to the AL. The test protocol includes primary odorants and mixtures as well as pheromone components. The AL neurons are stained with fluorescent dyes for visualization in confocal laser scanning microscope followed by 3-dimensional reconstruction. The neurons are morphologically classified according to their innervations of glomeruli (uni- or multi-glomerular) and through which of the four antenno-cerebral tracts the axon projects to the mushroom bodies (involved in olfactory learning) and the premotoric area in lateral protocerebrum (Rø et al., 2007). The neurons identified in individual brains are integrated into the standard *H. virescens* brain atlas (Kvello et al., abstract in this meeting). We here present physiologically and morphologically classified antennal lobe neurons integrated in the standard brain atlas.

Poster Session II: Wed. July 23

The Serotonin Immunoreactive Neuron Retrieved in the Antennal Lobe of the Male Oriental Moth *Helicoverpa Assulta*? Physiological and Morphological Characteristics of a Presumed Centrifugal Neuron

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Moths are widely used for studying neural pathways mediating olfactory signal information. In particular, the chemotopic organization of the male-specific macroglomerular complex (MGC) has been thoroughly described in several heliothine moths, one of them being *Helicoverpa assulta* (Berg et al., 2005, J Comp Neurol). In this Asian species the numerous pheromone receptor neurons converge onto 3 male specific antennal lobe glomeruli whereas the plant odor neurons target 62 ordinary units (Berg et al., 2002, J Comp Neurol). Like in other insects, two main categories of central interneurons arborize in the antennal lobe, i.e. projection neurons and local interneurons. Here we present data about a small number of interneurons making up a third category, centrifugal neurons. By intracellular recordings combined with iontophoretic staining and confocal microscopy reconstructions we have identified a neuron which is morphologically similar to the serotonin-immunoreactive antennal lobe neuron initially described in the sphinx moth *Manduca sexta* (Kent et al., 1987, J Neurobiol). The neuron presented here has a large soma in one antennal lobe and projects via the protocerebrum to the contralateral lobe where it innervates each glomerulus including the MGC units and the ordinary glomeruli. In the protocerebrum

fine processes arborize in several bilateral areas. As concerns physiological characteristics, the neuron responded to mechanical stimulation of the antennae. This corresponds with previous findings in *Bombyx mori* (Hill et al., 2002, Chem Senses). Interestingly, the neuron reported here exhibited two distinctly different spike amplitudes. The presence of serotonin will be investigated by use of immunocytochemical techniques.

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Poster Session II: Wed. July 23

Effect of Culture and Familiarization on Odor Categorization

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This study aims at evaluating the effect of culture and familiarization on odor categorization. Two very different groups of subjects were involved in the study, Dutch and Swiss subjects. Both groups were composed of trained panelists with many years experience in descriptive analysis and difference tests. An important difference between the 2 groups lies in the type of training they received regarding descriptive tests. The Dutch and Swiss subjects were trained to characterize the flavor profile of commercial products according to two different flavor languages (a flavor language being a collection of sensory descriptors, each descriptor being defined by a reference flavor). More specifically, two families of flavor references sharing similarities between the Swiss and the Dutch panels were studied: ''Fatty'' and ''Fruit berries'' flavors. The Dutch and Swiss subjects were instructed to perform a Hierarchical Sorting Task (Egoroff, 2005) on 35 Fatty flavors (26 coming from the usual flavor references of the Swiss and 9 from the Dutch) and on 38 Fruit flavors (30 Swiss and 8 Dutch flavor references). For Fatty flavors, 22 Swiss and 7 Dutch participated, and for Fruits 24 Swiss and 6 Dutch participated. The data were analyzed according to Distatis (Abdi et al., 2007) and to additive tree representation (Barthélémy and Guénoche, 1991).The results clearly show different organization patterns between Dutch and Swiss subjects. The Swiss had a tendency to make large flavor clusters, whereas the Dutch split those clusters or allocated the flavors to other flavor clusters. It seems the Swiss were more straightforward than the Dutch and categorized the flavors according to preconceived categories, because the flavor set comprised more flavors familiar to the Swiss that to the Dutch.

Poster Session II: Wed. July 23

Effects of Grapefruit Scent on Enhancing Cognitive Performance

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Certain scents have been found to influence mood and mental functioning. However, the effectiveness of citrus scents has been minimally studied. In the present study, the effect of grapefruit scent on cognition was examined. Ps completed two conditions: scent/cognitive evaluation and no-scent/cognitive evaluation. Ps then completed the NASA-Task Load Index (NASA-TLX, a self reported assessment of workload) and the Profile of Mood States (POMS, a self reported assessment of mood). Ps also completed a neurological cognitive assessment (Impact Applications, Inc.). Physical demand was significantly lower during the grapefruit scent and cognition condition. There was also a main effect found for the composite visual scores on the Impact test…scores were found to be significantly higher during the grapefruit condition than during the control condition. A main effect was found for the composite reaction time on the Impact test…scores were significantly lower during the grapefruit condition. Ps in the grapefruit condition perceived the cognitive test to be less physically demanding than did the Ps in the control condition. There was also significance found within two of the sections of the cognition test. The composite visual score on the Impact test was higher during the grapefruit condition; therefore, participants were better able to discriminate between the visual stimuli. The reaction time during the cognitive Impact test was found to be significantly faster during the grapefruit condition than during the control condition. Thus, not only were participants better able to discriminate between the visual stimuli, but they also responded more quickly to the appropriate stimuli. Grapefruit scent may have many implications, being used as a means to improve stimulus discrimination and reaction time.

Poster Session II: Wed. July 23

Effects of Congruent Vs. Incongruent Scent During a Scent Dependent and Information Dependent Learning Task

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A connection between scent and memory has long been recognized. Scent dependent learning exists when the same scent is present in both the learning and assessment phase, which leads to greater performance. The present study assessed scent dependent learning interactions between scent congruent vs. incongruent information. Prior to participation, Ps completed the Profile of Mood States (POMS). They then watched a 50 min. video on coffee history under one of three ambient scent conditions (none, coffee, cherry). Following the video, a questionnaire related to the video information was completed under one of three ambient scent conditions (none, coffee, cherry). Following the questionnaire, Ps again completed the POMS, in addition to the NASA-TLX to determine perceived workload and task performance. Between-subjects ANOVAs were conducted controlling for coffee preference and consumption. Scent dependent learning was validated, such that performance was better when the same scent was in both the learning and recall situations. Recall was greater than control when the scent in both the learning and recall situations matched the information presented (i.e. coffee). Recall was greater than control when coffee scent was present in the recall situation, regardless of whether it was presented in the learning condition. Thus, scent dependent learning interacts with the type of information being presented, and can provide greatest performance with congruent testing information, even in the absence of that scent being presented in the learning condition.

Poster Session II: Wed. July 23

Effects of Jasmine Scent on Sleep Quality and Cognitive Performance

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Previous research has indicated that jasmine scent can improve the overall quality of sleep. The present study evaluated the effectiveness of jasmine scent on sleep quality, mood, and cognitive functioning. Ps underwent two conditions. In condition 1, Ps placed a jasmine air freshener in their bedroom for one week and rated their quality of sleep, cognition, mood, performance, and workload. In condition 2, Ps rated their quality of sleep, cognition, mood, performances, and workload for one a week in the absence of the jasmine air freshener. There was a week break between the two conditions. The results showed that the directions of the jasmine scent condition were positive, leading to greater sleep quality, cognitive function, mood, and

performance. The implications are particularly salient for finding a natural sleep aid that increases cognitive performance following sleep.

Poster Session II: Wed. July 23

Chemosensory Stimulation During Sleep

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The interaction of sensory physiology and sleep has been studied for various sensory systems. Nevertheless, chemosensory (especially olfactory) stimulation during sleep has hardly been investigated to date. As the central processing of olfactory information shows fundamental differences compared to other sensory systems, significant differences have to be expected, especially with regard to arousal reactions. Five young healthy, normosmic volunteers were included in this prospective controlled trial and 23 nights of testing were performed. Intranasal chemosensory stimulation during sleep was based on air-dilution olfactometry. For olfactory stimulation H_2S was used in 4 concentrations (1, 2, 4, and 8 ppm) while for trigeminal stimulation CO_2 was also administered in 4 concentrations (10%, 20%, 40%, and 60% v/v) while odorless stimuli were used for control. Arousal reactions were assessed during overnight polysomnography 30 seconds after every stimulus. For olfactory testing, an average number of 703 olfactory stimuli and 157 odorless controls were used for analysis per subject. Even the highest stimulus concentration did not produce an increase in arousal frequency. For trigeminal testing, an average number of 405 stimuli and 79 controls were used for analysis per subject, and an increase in arousal frequency was observed following the increase of stimulus concentration. With the present results we were able to demonstrate for the first time that, in contrast to trigeminal stimulation, the presentation of a strong but selective olfactory stimulus does not lead to arousals during sleep in humans. These results demonstrate that sleep in humans may be influenced with olfactory stimulation, a concept which is currently investigated with regard to the impact of olfactory stimulation on dreams.

Poster Session II: Wed. July 23

Effects of Peppermint Scent on Enhancing Weight Lifting, Strength, and Endurance

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It is well known that scent can be used to alter mood, sleep quality and physical performance. Experiments have shown peppermint raises arousal levels, which indicates an excited effect, can distract participants from or relax them during burdensome tasks, and is an alternative to pharmacological means to be used as an accessory to athletic training. The purpose of the present study was to examine the effects of peppermint scent administration on weightlifting, specifically to determine the effectiveness of increasing strength of the participant. Participants underwent 2 conditions. In condition 1, the participants inhaled peppermint scent every 15 minutes during the course of their regular weightlifting workout over a period of 2 weeks. In condition 2, participants were asked to perform their regular weightlifting workout for 2 weeks with the absence of the peppermint. Before and after both conditions, participants were tested on how much maximum weight they could lift on chest press, lateral pull down, leg extension, and leg curl machines. There was a week separation period between condition 1 and condition 2 to allow for recovery. Peppermint scent inhalation was associated with increased number of repetitions performed and increased muscle endurance. The direction of the perceived workload in the areas of mental, effort, and frustration were less and performance was greater in the peppermint condition. The implications are particularly salient in relation to finding an all-natural, alternative supplement to increase strength without harmful side effects.

Poster Session II: Wed. July 23

Retronasal and Orthonasal Time-Intensitypatterns in Relation to Judged Pleasantness, Familiarity, and Food-Relatedness

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Perceived odorant intensity is phasic-tonic,with increases within the 1st 20 seconds preceding decreases. Retronasal reaction times are longer than orthonasal reaction times, and decreased retronasal intensities occur earlier than orthonasal. Retronasal maximum and final intensities are less than orthonasal, but initial intensities do not differ. We wondered if there would be a relationship between judgments of pleasantness, familiarity, and food-relatedness of 5 natural extract odorants and changes in their perceived intensity over time.

Methods: Twenty participants (14 females, median age = 20) 1st selected isointense concentrations of 5 vapor-phase odorants (anise, orange, peppermint, strawberry, and vinegar) orthonasally and then retronasally; 2nd, using Likert-scales, rated pleasantness, familiarity, and food-relatedness of each odorant; 3rd, judged intensity during natural breathing over 60 sec trials orthonasally and retronasally. Intensity judgments were made on a computer by adjusting the vertical position of the display to correspond to changes in perceived intensity while the horizontal position (time) advanced at a constant rate under program control. Real-time visual feedbackwas provided on the computer display.

Results: Reaction times, times to maximum, and intensities over time varied between odorants. Maximum intensities interacted with odorant familiarity, pleasantness, and mode of odorant presentation. Times to maximum and intensities over time interacted with mode of presentation and food-relatedness of odorants.

Conclusions: Changes in odorant intensity over 60 sec interact with odorant pleasantness, familiarity, and food-relatedness.

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Poster Session II: Wed. July 23

Chemosensory Function in Firefighters: A Longitudinal and Cross-Sectional Analysis

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Firefighters are regularly exposed to a wide range of chemical gases and fumes. These exposures are potentially lethal and firefighters rarely have the benefit of *a priori* knowledge of the chemical makeup of the fire. Despite the use of respiratory protection, even brief contact with these irritants can cause airway inflammation and lead to chronic respiratory problems. Given these conditions the loss of olfactory function is highly probable. For firefighters this not only impacts quality of life, but more importantly represents an occupational hazard, as reductions in the ability to localize the source of smoke or burning electrical wires poses a significant handicap. In spite of this there are no published longitudinal studies of olfactory function among this group. In an attempt to document these effects we are conducting a

longitudinal study of new recruits coupled with an initial cross-sectional evaluation of Philadelphia firefighters having varying years of job experience. Nasal inflammation was evaluated using cytokine profiles and inflammatory cell counts as markers and mucociliary clearance function was tested using saccharine transit time. Olfactory function was evaluated using a standard test battery including an odor identification task, odor detection thresholds for PEA and butanol, as well as lateralization thresholds for butanol. Results from the cross-sectional study indicate olfactory dysfunction and inflammatory changes are associated with increased years of employment. Although there have been significant improvements in the personal protective equipment available to firefighters, the results illustrate both the need to ensure compliance in the use of such equipment and the continued surveillance of the chemosensory health of this population.

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Poster Session II: Wed. July 23

Molecular Mechanisms for Enhancement of Umami Taste Receptor by IMP

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The umami taste receptor recognizes L-glutamate and mediates the savory taste of monosodium glutamate (MSG). The receptor is a heteromer consisting of T1R1 and T1R3, both of which belong to the C family of G Protein-coupled Receptors. The ribonucleotide, inosine-5'-monophosphate (IMP), is a natural enhancer of the umami receptor and umami taste. Using sweet-umami chimeric T1R receptors, we show that both L-glutamate and IMP interact with the N-terminal extracellular domain of T1R1. Molecular modeling proposes a novel mechanism for the synergy between L-glutamate and IMP. L-glutamate interacts with the ''hinge'' region of the Venus flytrap domain (VFD) and induces closure of the flytrap, whereas IMP coordinates the positively charged residues at the opening of the flytrap, further stabilizing the closed conformation. This model was confirmed by a mutagenesis approach. Four residues at the hinge region are crucial for the L-glutamate response. Four other residues at the opening of the flytrap are required for the enhancement effect of IMP. Furthermore, as predicted by the model, changing some of the residues on one side of the flytrap from amino acids having positively charged to negatively charged side chains stabilized the closed confirmation of the VFD and resulted in a more sensitive receptor than the wild type. Taken together, our results demonstrate coordinate binding of L-glutamate and IMP to the T1R1 VFD pocket. This represents a novel binding mechanism unique for the umami taste receptor.

Poster Session II: Wed. July 23

Effect of Chorda Tympani Nerve Transection on IMP-Enhanced Preference to MPG and Arginine in Mice

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An umami substance, monosodium L-glutamate (MSG) represent considerably different taste quality from monopotassium L-glutamate (MPG). However, the taste quality of MPG with inosine 5'-monophosphate (IMP) seemsto be close to that of MSG because we found using a conditioned taste aversion (CTA) paradigm that C57BL/6 mice, whose umami sensitivity is relatively similar to that of human, discriminated MSG from MPG, but dose-dependently failed to discriminate MSG from MPG with IMP (MPG+IMP). The expression pattern of MPG-stimulated Fos-like immunoreactivity (FLI) in the parabrachial nucleus (PBN), a secondary center of gustatory sense, was altered by addition of IMP to be similar pattern of MSG-stimulated FLI: MSG-stimulated FLI tended to distribute in anteromedial part of PBN, but MPG-induced FLI, which dispersedly located in the posterior part of PBN, shifted to anteromedial part of PBN with addition of IMP. However, only the alteration of pathways toward antero-posterior axis was observed in the nucleus of solitary tract (NTS). Similar results were observed between arginine and arginine+IMP, where taste quality of arginine seemed to shift from bitter dominant taste to sweet dominant taste in mice. On the other hand, such an IMP- induced alteration was excluded by the bilateral transection of chorda tympani nerve (CTn). These results suggest that T1R1/R3 receptors in the area innervated by CTn mediate IMP-induced taste quality changes of MPG and arginine accompanied by the alteration of pathways within PBN and NTS. Furthermore, pathway of taste signal for IMP induce an enhancement at the anteromedial part of PBN, but an inhibition at the posterolateral of PBN.

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Poster Session II: Wed. July 23

Recovery of Umami Taste Responses After Crush of the Mouse Chorda Tympani Nerve

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Recent molecular studies proposed that various receptors, such as a truncated type 4 metabotropic glutamate receptor (taste mGluR4), heterodimers of T1R1/T1R3, taste mGluR1, and brain-type mGluR4, might underlie umami taste. To date, however, the roles in umami taste of each of these receptors and their downstream signaling molecules have not been made clear. In the present study, we examined recovery of responses to umami compounds in the mouse chorda tympani (CT) nerve after crushing the nerve. At about 2 weeks after the nerve crush, no significant responses to taste stimuli were observed in the CT. At about 3 weeks after the crush, taste responses reappeared and response to 0.1M monopotassium glutamate (MPG) was significantly suppressed by AIDA and CPPG, mGluR1 and mGluR4 antagonists respectively. At about 4 weeks after the crush, although responses to MSG + 0.5mM inosine monophosphate (IMP), 0.1M MPG + IMP and 0.1M L-Ala + IMP recovered to their control levels, synergism between 10mM quisqualic acid (mGluR1 agonist) and IMP and/or that between10mM L-AP4 (mGluR4 agonist) and IMP were not significantly detected. After more than a month, the CT showed recovered responses to all stimuli tested including10mM quisqualic acid + IMP and 10mM L-AP4 + IMP to similar levels to those shown by intact animals. These results suggest the differential restoration of T1R1/T1R3, mGluRs and transduction pathways, providing additional evidence for existence of multiple receptors and transduction pathways underlining umami taste in mice.

Poster Session II: Wed. July 23

Effect of Inosine Monophosphate (IMP) on Behavioral Response to Lysine and Arginine in Mice

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Recent *in vitro* heterologous expression studies showed that most L-amino acids, for example L-methionine (Met), activate the mouse T1R1+T1R3 receptor. However, L-lysine (Lys) and L-arginine (Arg) evoke only negligible activation of the mouse T1R1+T1R3 receptor, but activation of this receptor increases considerably when Lys and Arg are mixed with IMP

(Nelson *et. al.*, 2002). This suggests that addition of IMP changes the taste quality of Lys and Arg. We tested this hypothesis using a conditioned taste aversion (CTA) technique. Separate groups of C57BL/6J mice were exposed to 50 mM Lys or Arg with or without 2.5 mM IMP, or to water (control) and injected with LiCl to form CTA. Conditioned mice were presented with five basic taste solutions, Met, Lys and Arg, and their lick responses were recorded. An aversion to Lys generalized only to Lys mixed with IMP (Lys+IMP). An aversion to Lys+IMP generalize not only to Lys but also to a mixture of 50 mM monosodium glutamate (MSG) and 30 μM amiloride (Ami; added to block sodium taste) with and without 2.5 mM IMP (i.e., MSG+IMP+Ami and MSG+Ami). An aversion to Arg generalized to quinine and Arg mixed with IMP (Arg+IMP). An aversion to Arg+IMP generalized to MSG+IMP+Ami and MSG+Ami but not quinine. This suggests that, as predicted by the *in vitro* study, addition of IMP changes the taste quality of Lys and Arg *in vivo*.

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Poster Session II: Wed. July 23

Suppression of Obesity by Spontaneous Drinking of Monosodium L-glutamate (MSG) Solution in Rats

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Monosodium L-glutamate (MSG), an umami-taste substance, may be a key molecule coupled to a food signal, possibly mediated through a specific L-glutamate (GLU)-sensing mechanism in the gastrointestinal tract. Here we investigated the spontaneous ingestion of 1% MSG solution with water on food intake and body weight in male adult Sprague-Dawley rats that fed high fat diet. Fat mass and lean mass in the abdomen, blood pressure, and several blood metabolic markers were also measured. Rats with free access to water but not MSG acted as controls. Rats had high preference (95%) for MSG solution. Rats ingesting MSG had a significantly smaller weight gain, reduced abdominal fat mass, and lower plasma leptin levels, compared to rats ingesting water alone. Naso-anal length, abdominal lean mass, food and energy intakes, blood pressure, blood glucose, and plasma levels of insulin, triglyceride, total cholesterol, albumin, and GLU were not influences by access to MSG. Together, these results suggest that MSG ingestion reduces weight gain, body fat mass, and plasma leptin levels. Moreover, these changes are likely to be mediated by increased energy expenditure, not reduced energy intake or delayed development. Conceivably, these effects of MSG might be mediated via gut GLU receptors functionally linked to afferent branches of the vagus nerve in the gut, or the afferent sensory nerves in the oral cavity.

Poster Session II: Wed. July 23

New Assessment of Gustatory Disorders Using Umami Taste Sensation

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Patients with gustatory disorders often complain of the persistent impaired taste of umami, a synonymy with savory or broth-like, although the other four basic taste sensations (sweet, salty, sour, bitter) have improved in the recovery process. However, there is no clinical test for umami, although the other four basic tastes have been widely used in quantitative gustometry. The purpose of this study was to develop a new method for clinical assessment for the umami taste sensitivity. First, we investigated appropriate concentration range of the test solution in 80 healthy volunteers (age: 18 to 88). Next, we applied the method to 8 patients with gustatory disorders (age: 38 to 78). Recognition thresholds for umami taste were measured using aqueous solutions of monosodium glutamate (MSG) and inosine 5'-monophosphate (IMP) (1 to 200 mM). A filter-paper disc, 5 mm in diametor, saturated with each solution was placed on the tip and on the posterior third of the tongue (the areas innervated by the chorda tympani nerve and the glossopharyngeal nerve, respectively), and on the soft palate (the area innervated by the greater superficial petrosal nerve). Mean recognition thresholds in the healthy volunteers showed less than 50 mM for MSG and 10 mM for IMP on the posterior third of the tongue and on the soft palate. The threshold at the tongue tip was higher than in the other areas. Many patients showed higher thresholds than the volunteers, but the thresholds showed improvement after medical treatment. Changes in recognition thresholds for MSG and IMP were consistent with increase in subjective umami taste intensities. These results indicate that this method is available for the assessment of gustatory disorders in umami taste.

Poster Session II: Wed. July 23

Has Norwich's Entropy Theory of Perception Derived Stevens' Law for Taste?

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Norwich's Entropy Theory of Perception (1975) reveals a startling conclusion: Stevens' Law with an Index of 1 arises for taste purely from theory. Norwich's theorizing starts with some extraordinary hypotheses. First, "multiple, parallel receptor-neuron units" without collaterals "carry essentially the same message to the brain", i.e. the rate-level curves are identical (Percept Psychophys 1981; Sensory science theory & applications in foods, Marcel Dekker Inc, NY, 1991). Second, sensation is proportional to firing rate (ibid.). Third, firing rate is proportional to the taste receptor's "resolvable uncertainty" (see Chem Senses 2001). Fourth, the "resolvable uncertainty" is obtained from Shannon's Information Theory (ibid.). Finally, "resolvable uncertainty" also depends upon the microscopic thermodynamic density fluctuation of the tasted solute (Percept Psychophys 1984). Norwich (ibid.) proves that density fluctuation is density variance which is proportional to solute concentration, all based on the theory of fluctuations in fluid composition in Tolman's The Principles of Statistical Mechanics (1962). Altogether, taste sensation is theoretically proportional to solute concentration. Norwich calls this Stevens' Law for taste with Stevens' Index = 1. Now, a universal rule for taste that is regardless of solute identity, physiological differences, and psychophysical task is well-deserving of scrutiny. Norwich's crucial step, the derivation of density variance, was meticulously reconstructed. It transpires that the appropriate fluctuation is Tolman's mean-square fractional density fluctuation, not the density variance. The error is uncorrectable. Thus, Norwich's Entropy Theory of Perception has not derived Stevens' Law for taste.

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Poster Session II: Wed. July 23

The Influence of Color and Label Information on Perceptions of Chocolate

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Several studies have independently shown that manipulations of color or label information can influence perceptions of food or beverage flavor. The present study examined how the *simultaneous* manipulation of these

two cues modulates flavor perception. Thirty participants rated 12 identical (except in color) chocolate M&Ms on scales of *chocolateyness* and *likeability*. The "m" logos were hidden from sight, and chocolates were said to be part of a "new line of chocolate products." In order to indicate what was meant by *chocolatey*, blindfolded participants tasted three samples of chocolate (white, milk, and dark) prior to testing, which were said to span the *chocolatey* scale in ascending order. In the color-only condition, sighted participants were given 2 green and 2 brown M&Ms. In the label-only condition, blindfolded participants were given 2 M&Ms with a "milk chocolate category" label and 2 with a "dark chocolate category" label. In the color-label condition, sighted participants were given an M&M of each possible color-label combination (green-milk, green-dark, brown-milk, & brown-dark). We found a significant effect on chocolatey ratings of color in the color-only condition ($p = .040$), label in the label-only condition ($p = .012$), and of both color and label in the combined condition ($p = .022$ and $p = .018$ respectively, interaction n.s.). There was no significant effect on likeability. Brown M&Ms were rated as more *chocolatey* than green ones, and "dark chocolate" labeled M&Ms were rated as more *chocolatey* than "milk chocolate" labeled ones. These influences held true when both color and label cues were simultaneously available to the participant. These data reinforce the idea that flavor is a percept that originates not only from chemosensory information, but also from color and label information.

Poster Session II: Wed. July 23

Labeled Hedonic Scale for Assessing Liking/Disliking of Oral Sensation

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Oral sensation has two components: one is discriminative (i.e., quality and intensity) and the other is affective (i.e., pleasantness/unpleasantness). The general Labeled Magnitude Scale (gLMS) has been widely used to obtain ratio-level data on the perceived intensity of oral sensation. Although a few scales for measuring affective experience have been patterned after the gLMS, none were developed and validated using an equivalent psychophysical procedure. The current study therefore was aimed to develop a semantically-labeled hedonic scale that would yield ratio-level data on the magnitude of liking and/or disliking of oral sensation. The 'Labeled Hedonic Scale' (LHS) was constructed by having Ss (N=54) who were practiced in magnitude estimation rate the semantic magnitude of 11 terms commonly used to express degree of liking and disliking (e.g. slightly, very much) within the context of a broad range of imagined sensations. The resulting bipolar scale is bounded at its ends by the "most liked/disliked sensations imaginable" and is nearly symmetry around neutral, i.e., the locations of the positive and negative descriptors are not significantly different. Experiments are continuing to compare data obtained using the LHS, the traditional 9-point hedonic scale, and magnitude estimation. Test stimuli used in the experiments include food item names on cards that cover a wide range of liked and disliked oral sensations, and various chemical and food stimuli to test whether the LHS can be used to assess liking/disliking for both simple and complex flavor systems. The results will be discussed in terms of data distributions, sensitivity, inter-scale correlations, and other statistical considerations in addition to the characteristics of the scale.

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Poster Session II: Wed. July 23

Impact of Sweeteners on Ortho-and Retronasal Aroma Perception

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In our daily lives, diet products have become more and more present in recent years. Much attention has been paid to the substitution of "simple" sugars in beverages, slightly neglecting aroma perception, a key to flavor experience. Aim of the present study was to investigate the influence of aroma perception on different sweeteners. Flavor perception was assessed in 34 normosmic subjects who reported a habit of regular soda consumption. There were two different aroma conditions (lime and cola aroma) presented either ortho- or retronasally by means of a flow controlled olfactometer. On the other hand a variety/selection of four sweeteners (two artificial sweeteners: sucralose, aspartame; and two natural sweeteners: sucrose, HFCS) complemented by a non-sweetened solution were tested. Subjects were asked to sip the taste solutions while aromas were presented, and then to rate the hedonics, overall flavor intensity, and sweetness of the stimulus. Among all sweeteners sucrose was most liked. Retronasal presentation of aroma produced much higher hedonic ratings and higher ratings for sweetness, when compared with orthonasal presentation, thus overall intensity ratings were very strongly correlated with overall sweetness. Furthermore, cola aroma boosted overall intensity of all solutions except for aspartame, while this was not found for lime. Sweetness exhibited negative correlations with hedonic ratings. Cola aroma produced much higher sweetness ratings than lime aroma. These findings clearly show the intimate relationship between olfactory and gustatory components of beverages. Aromas and different sweeteners contribute differently to overall flavor experience.

Poster Session II: Wed. July 23

Effects of Context on Perceived Intensity of Flavor Mixtures near Threshold

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Last year, we reported results of a study asking how stimulus context affects the perceived intensity of flavor mixtures (Marks et al., AChemS, 2007). In that study, subjects rated, on a labeled magnitude scale, the perceived intensity of 16 stimuli constructed by combining 4 possible concentrations (including zero) of the gustatory flavorant sucrose with 4 concentrations of the retronasal olfactory flavorant citral. In one contextual condition, concentrations of sucrose were relatively high and those of citral low; in the other, sucrose concentrations were lower and citral concentrations higher. There were two main findings: First, ratings obtained in both conditions approximated linear (additive) sums of the perceived components presented alone, consistent with several earlier findings (e.g., Murphy & Cain, Physiol. Behav., 1980; McBride, Chem. Sens.,1993). Second, stimulus context produced flavorant-specific contrast effects: The perceived intensity of a given concentration of a flavorant was reduced when other concentrations of that flavorant were high rather than low. That is, high concentrations led to'contextual adaptation.' Our two new studies followed the same design as last year's study, but used near-threshold concentrations of citral and sucrose. Again, each set of ratings showed approximate linear additivity, but now contextual adaptation was small in magnitude in sucrose and absent in citral. Because the stimulus sets included water, it was also possible to analyze the data using methods derived from signal detection theory. Results of this analysis suggest a possible dissociation between effects of stimulus context on detectability and effects on perceived intensity of gustatory-olfactory flavor mixtures near threshold.

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Poster Session II: Wed. July 23

Selective Adaptation Exposes Component Odors and Tastes in Mixtures

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In mixtures of as few as two odor or taste stimuli, identification of stimulus-components by humans is impeded (Laing et al., 2002), but characteristic component odors emerge after brief selective adaptation (Goyert et al., 2007). To study variables that affect identification in binary mixtures, 7-14 subjects sampled mixtures after exposure to one component. Odor adapt-test pairs were sniffed from squeeze bottles; taste adapt-test pairs were applied to the tongue tip as mists via atomizing spray caps. Average correct identification of 1 or 5 mM vanillin as *vanilla* odor and 1 or 5 mM phenethyl alcohol as *rose* odor in mixtures was 66% without selective adaptation. Regardless of intensity, an adapted odor mixture component was identified less frequently, 30% ($p = 0.0001$), and an unadapted component more frequently, 82% ($p = 0.002$), following selective adaptation. Average correct identification of 50 or 100 mM NaCl as *salt* taste and 150 or 300 mM sucrose as *sugar* taste in binary mixtures was 86% without selective adaptation. An adapted taste mixture component was identified less frequently, 40% ($p = 0.001$), but an unadapted mixture component was identified as frequently as a single component, 96%, after selective adaptation. Increasing adapting sniffs from 1 to 5 did not affect emergence of characteristic *vanilla* and *rose* odors from binary mixtures. With 1 or 5 sniffs, an adapted stimulus was identified less frequently, 40% ($p < 0.001$), and an unadapted stimulus more frequently, 90% ($p = 0.04$), than the 70% identification without selective adaptation. Regardless of intensity or sniffing time, characteristic odors and tastes emerge from mixtures following brief selective adaptation, allowing identification of recently introduced compounds in a dynamic chemical environment.

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Poster Session II: Wed. July 23

Perception of an Odor from Common Taste Mixtures

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It was previously reported that some taste stimuli emit detectable odors in aqueous solution (Mojet et al., 2005). Here we report evidence that odors can also arise from certain taste mixtures in aqueous solution. During preliminary testing for a different study, Ss noticed that some taste mixtures appeared to have a weak, nondescript smell. We therefore designed a study in which 23 Ss sniffed and tasted 0.56 M sucrose, 0.32 M NaCl, 10 mM citric acid, and 0.18 mM QSO4 as well as all possible 2-stimulus (n=6), 3-stimulus (n=4) and 4-stimulus (n=1) mixtures. Solutions were kept in a 37°C water bath and deionized water was included as a control. In separate sessions the Ss either sniffed or tasted 10-ml samples poured into plastic medicine cups. On sniffing trials the gLMS was used to rate odor intensity and Ss were invited to describe or identify, if possible, any odors they perceived. In two separate tasting blocks, Ss rated either overall taste intensity or the intensity of sweetness, sourness, saltiness, bitterness, and 'other', with nose open or nose closed. The results from the sniffing task confirmed the presence of an odor in a subset of the stimuli. A repeated-measures ANOVA revealed a significant effect of stimulus: the mixture of sucrose+NaCl as well as all mixtures that contained sucrose+citric acid were rated significantly more odorous than water (Tukey HSD, $p<0.05$). However, a comparison of taste ratings with nose open vs. nose closed showed no specific effect of retronasal odor on taste quality or intensity. Experiments are continuing in an effort to identify the source of the odor (e.g., headspace analysis) and to determine if its presence may affect the perceived pleasantness of certain aqueous taste mixtures independent of perceived intensity.

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Poster Session II: Wed. July 23

Differential Effects of Body Mass Index and Eating Style on Neural Response to Milkshake

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Previous studies have shown differential responses in obese subjects to food-related stimuli. It is unclear whether these differences reflect the altered metabolic state or, the presence of metabolism-independent behavioral traits that contribute to obesity. We used fMRI to determine how eating style (ES) traits - such as self-reported ratings of cue responsiveness, disinhibition, compulsive eating and binging - contribute to obese-specific brain responses independently of actual body fatness (assessed by body mass indexes, BMI). Lean (LE) and overweight/obese (OV) subjects (Ss) passively consumed milkshake (mlk) and tasteless (t) solutions. Comparison of mlk-t in OV - LE, matched for ES, showed increased dorsal midbrain response and decreased caudate response. In contrast, comparing high-ES – low-ES, when matched for BMI, showed increased amygdala (Amg) and anterior hippocampus (Hi) responses, and decreased ventral medial prefrontal cortex (vmPFC) response. This indicates that BMI and ES have distinct effects on the neurophysiology of food reward. We also found that these distinct effects were differentially accounted for by measures of anticipatory and consummatory food reward. While in OV subjects caudate responses directly reflected the subjects ratings of mlk pleasantness (a measure of consummatory reward), in high-ES subjects these ratings correlated with activations in Amg/Hi. Conversely, salivary response (a measure of anticipatory reward) was associated with greater midbrain responses in OV and lower vmPFC responses in high-ES. Our data indicate that obese-specific responses to food consumption result from an interaction between BMI and ES, which differentially affect the anticipatory and consummatory phases of food reward.

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Poster Session II: Wed. July 23

Investigating the Existence of Differences for Innovative Expectations and Olfactive Preferences Across Age: The Case of Mature People

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Objectives: The objective of this study is to investigate the existence of different olfactive perceptions and preferences across age and their consequences on acceptance and consumption of innovative novel food products.

Materials and methods: 19 molecular mixes have been selected to cover a large variety of fruit flavours. Some of the flavours were considered mainstream ones, others were potentially innovative ones and others were chosen for their nostalgic evocation potential. Flavours were presented on a Mini-Vas®, device allowing a rapid and easy delivery and evaluation of a large number of smells. 327 people were recruited according to four age targets: [7-12], [30-45], [50-65] and [66-80]. Subjects were asked to smell each flavour, to imagine a fruited beverage flavoured with it, and to give a hedonic score

on a seven point scale from "I dislike extremely" to "I like extremely". Following this, consumption habits and food choice criteria questions were asked. In particular, reluctance or willingness to taste the evaluated flavours in a beverage were subsequently measured on a four point hostility scale.

Results: Large differences in both preferences and innovative potentials were observed. However, regarding preferences, only three flavours (smell only blind evaluation) showed a clear age effect. On the contrary, the age effect had important consequences on the flavours expectations and their innovative potentials. Discussion and conclusions This study shows that age might affect preferences towards flavours and can justify a product development adapted to the mature segment. It also clearly shows that the smell evaluation should be linked to the context of evaluation and the information given to the panelists.

Poster Session II: Wed. July 23

The Mouth Feel, Taste, and Biophysical Properties of Feeds Given to Patients with Dysphagia

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Introduction: Dysphagia is a common disorder that is diagnosed with a modified barium swallow. It is likely that the surface properties, such as sessile **c**ontact **a**ngle (CA), **s**urface **t**ension (ST), and dynamic **v**isco**e**lasticity (VE), can affect mouth feel and palatability of diagnostic and nutritive liquids.

Methods and Subjects: A questionnaire to assess taste preferences, mouth feel, and taste of 8 test substances in 30 healthy volunteers was administered twice on different days and in different order to validate the reliability, reproducibility, and discriminating ability of the items. CA, ST, and VE of all test substances were measured using validated techniques developed in the PI's lab. Data were analyzed by ANOVA and simple linear regression.

Results: There was a significant relationship between the overall rating of the product and the overall flavor ($R = 0.964$, $p<0.0001$) as well as the overall texture ($R = 0.763$, $p<0.0002$). There was a significant relationship between the overall flavor and the overall texture ($R = 0.917$, $p=0.001$), but not to oiliness, CA, ease of swallowing, saltiness, sweetness, or bitterness. ST correlated with the overall rating ($R=0.91$), overall texture ($R=0.84$), overall flavor ($R=0.96$), salty feel ($R=0.97$), sweet feel ($R=0.97$), and bitter feel ($R=0.95$; $p<0.01$ for each). There was a strong correlation between oiliness and CA ($R = 0.837$, $p<0.01$). Participants had an overwhelming preference for the Varibar® line of products over the E-Z-HDTM Barium Sulfate for Suspension.

Conclusion: The product's flavor and texture nearly equally influence the palatability of the product. ST correlated with how well the subjects liked a product. This is potentially useful, as physical properties can be altered to improve palatability.

Poster Session II: Wed. July 23

Smell and Taste Function in Children with Chronic Renal Failure

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A major problem for renal clinicians is the loss of appetite and unwillingness to eat that is exhibited by patients with chronic renal failure (CRF). Commonly, this results in malnutrition and anorexia, compromising treatment and recovery. However, in children, the poor nutrition can have severe lifelong disabling consequences, namely, growth failure and failure to experience puberty and reproductive activities. Since impaired olfaction and/or gustation may be a cause of the unwillingness of CRF patients to eat sufficient food to maintain normal nutrition, the present study investigated smell and taste function in children with CRF. Sixty children, aged 5-16 years, participated: 20 had CRF, 20 were clinical controls, 20 were healthy controls. All were matched for age and gender. Olfactory function was assessed using a 16- odour identification test developed for children aged > 4 years. Each child used a squeeze bottle to sniff one odorant at a time and chose from 3 photos which one best described the odour. Gustatory function was measured using a test in which children identified 5 concentrations of sweet, salty, sour and bitter solutions and water, using sets of 3 photos. The results indicated there were no differences between the odour identification levels of the 3 groups ($p > .05$), however, the CRF group was significantly poorer at identifying the tastants than the other 2 groups ($p <0.001$). In addition, there was a positive correlation between kidney function and total taste identification score ($r = .43$, $p <.01$). Children with CRF, therefore, have reduced taste function and their smell function is normal. Accordingly, impaired taste function may be one factor that affects the willingness of CRF children to eat a diet that is sufficient to maintain their nutrition.

Poster Session II: Wed. July 23

Oral Phantasies: the Perceptual World of Thermal Tasters

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The study of individual variation in oral sensation has long focused on differences between 6-n-propylthiouracil (PROP) taster groups (PTS). Recently, 'thermal taste' was described, the phenomenon whereby some individuals perceive 'phantom' taste sensations as a result of thermally stimulating small areas of the tongue (Cruz & Green, 2000). As with PROP sensitivity, thermal taster status (TTS) has been proposed as a proxy for general sensitivity to oral stimuli. This study examined the influence of TTS on the intensity of sweet, sour, salty, bitter, PROP, astringent and metallic stimuli, and the perception of temperature on heating or cooling the tongue. PTS was determined after Porubcan & Vickers (2005). Lingual thermal stimulation (via Peltier device with thermocouple feedback) and TTS categorization followed Green & George (2004). 24 thermal tasters (TTs) and 49 thermal non-tasters (TnTs) rated oral sensation intensities on the gLMS. Fungiform papillae (FP) density and salivary flow rate (SFR) were also determined. One-way repeated measures ANOVA examined main effects of TTS on intensity ratings. Two-way repeated measures ANOVA examined effects of gender, ethnicity, smoking, PTS, and their interactions with TTS. TTS was not associated with either SFR or FP density. All logged oral stimuli and temperature ratings, except sourness and PROP intensities, were higher for TTs than TnTs. A TTS*PTS interaction was not found for any oral stimuli. We conclude that TTs possess greater sensitivity across a range of taste and trigeminal stimuli and concentrations, independent of PTS and FP density. Research recently completed in our lab demonstrates the significance of the TTs' acuity 'advantage' in both food and beverage behavior and in health status.

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Poster Session II: Wed. July 23

Modeling of Nasal Airflow and Odorant Transport in Patients with Chronic Rhinosinusitis

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Our 5-year multi-center study seeks to quantitatively characterize the conductive mechanisms contributing to olfactory loss in chronic rhinosinusitis (CRS) patients and in patients with other inflammatory disorders. As yet, the functional impact of the nasal obstruction experienced by CRS patients and the treatment outcomes in these patients have not been successfully indexed using existing tools such as acoustic rhinometry (AR), rhinomanometry (RM) or computed tomography (CT), the measurements of which correlate poorly with subjective symptoms. In this study, computational fluid dynamics (CFD) techniques are utilized to simulate nasal airflow and predict odorant delivery rates to the olfactory epithelium for each patient based on their pre & post-treatment CT. In an earlier report, we preliminarily supported the hypothesis that the calculated olfactory delivery rate is a better predictor of olfactory sensitivity among CRS patients than are conventional methods. In this updated report, 16 additional CRS patients (total n= 37) have been evaluated using AR, RM and CT, and their olfactory function characterized using measures of unilateral threshold sensitivity to *l*-carvone, *d*-limonene and phenethyl alcohol. Patient symptoms and pathology varied considerably, as did their olfactory abilities. Correlations between measured olfactory sensitivity and CFD, AR and RM predictions were examined. In the future, we envision that CFD modeling techniques may provide predictive models of treatment for CRS and an important pre-treatment guide to optimize airflow and odorant delivery in human nose.

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Poster Session II: Wed. July 23

Spatial and Temporal Odorant Transport Patterns in Rat Nose: A Computational Study

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Past research indicates that inhaled odorants may differentially deposit onto nasal mucosa in spatial patterns predetermined by nasal aerodynamics, odorant physiochemical properties and flow directions. Their implications on odor perception are not well-understood. Using computational fluid dynamics, we attempted to further quantify the transport patterns in rat to include the temporal effects of breathing cycles, transition, frequency and odor plume fluctuations and to compare the results with published EOG measurements. 3 sets of simulations were performed based on a published rat nasal model: 1) Steady state ortho- & retro nasal airflow 2) Time-dependent simulations of continuous breathing cycles at 2 frequencies (1.5Hz 2.55 ml/s and 8 Hz 10 ml/s) 3)Fluctuation of odor source at same or different frequencies than sniffing frequency *(data not completed yet)*. 4) Finally, the above simulations were repeated in a straight tube of similar volume. Preliminary results indicate that 1) the calculated deposition rate at the recording sites can be predictive of the measured EOG responses to odorants of various solubility (Scott et al., 2007); 2) the temporal profile of deposition rate at high sniffing frequency significantly differs from that at low frequency or constant flow, with elevated rates, damped fluctuations and phase shift, however the effects were only prominent in the peripheral zone; 4) many of the spatial or temporal features can not be replicated in the straight tube, implying that they are unique to the structure and aerodynamics in rat nose. In conclusion, the spatial and temporal feature of the "imposed pattern" combined with the motor regulation of the sniffing behavior employed by rodent may lead to a structure-functional optimization between nasal airflow and olfactory function.

Poster Session II: Wed. July 23

Identification of a Lysyl Residue Defining the Binding Specificity of a Human Odorant-Binding Protein

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Vertebrate Odorant-binding proteins (OBPs) are small abundant soluble proteins belonging to the lipocalin superfamily. They reversibly bind odorants with dissociation constants in the micromolar range and are good candidates for carrying airborne odorants, which are commonly hydrophobic molecules, through the aqueous nasal mucus towards olfactory receptors. In contrast with other vertebrate OBPs studied so far, human variant hOBP-2A binds numerous odorants of different chemical classes with a higher affinity for aldehydes and large fatty acids. A computed three-dimensional model of hOBP-2A revealed that three lysyl residues of the binding pocket (K62, K82 and K112) may interact with odorant aldehyde function, stabilizing odorant docking. In order to identify the lysyl residue involved in the higher affinity of hOBP-2A for aldehydes, we independently substituted these residues for alanine using site-directed mutagenesis, generating K62A, K82A and K112A mutants. By measuring the displacement of fluorescent probes by odorants, we showed that only the mutation K112A led to a dramatic reduction of binding affinity for aldehydes and small aliphatic acids (from 9- to 12-carbons), whereas binding of larger fatty acid (14- and 16-carbon length) were not affected by any mutation. Furthermore, we comforted these data by molecular docking of undecanal inside hOBP-2A binding pocket.

Poster Session II: Wed. July 23

Xenopus Laevis and *Xenopus Tropicalis* have Different Number of OBP Genes: Xlaeobp and Xtroobp

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Olfactory Binding Proteins (OBP), commonly associated with aerial olfaction, are currently found in mammals olfactory mucus, but have never been identified in fish. It is not clear yet if OBP is an adaptation of the olfactory system to an aerial environment. Adult olfactory system Xenopus is organized into two olfactory chambers which are thought to be devoted respectively to aquatic and aerial olfaction. This specificity provides us the opportunity to test this alternative hypothesis. We have identified for the first time Olfactory Binding Protein in *Xenopus laevis* and *tropicalis*. A reverse transcription and 3' RACE strategy has been applied and yielded two products, which were cloned and sequenced. These cloned sequences were used to analyse the expression pattern of the gene in the olfactory system of two Xenopus species: *X. laevis* and *X. tropicalis.* Using *in situ* experiments we showed that in both *Xenopus laevis* and *Xenopus tropicalis,* XOBP (xenopus Olfactory Binding Proteins) transcripts are only present in the aerial chamber supporting the idea that OBPs are an adaptation to aerial

olfaction. Moreover, from an EST (expressed sequence tag) library we also demonstrated that *X. laevis* has 2 different OBP genes while *X. tropicalis* has only one gene.

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Poster Session II: Wed. July 23

Associations of SNPS in Odorant Binding Protein Genes with Olfactory Behavior in *Drosophila Melanogaster*

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Odorant binding proteins (Obps) are the first components of the insect olfactory system to encounter odorants. Their functions, however, remain poorly characterized. We designed a population genetics strategy to uncover historical patterns of natural selection acting on *Obp* genes while at the same time providing information about their binding specificities. We sequenced all 4 *Obp* genes of the *Obp99* cluster in ~300 lines from a wild-derived population of *Drosophila melanogaster*, which were inbred for 20 generations to minimize genetic variation within lines while preserving genetic diversity among lines. Population genetic analyses revealed different patterns of historical recombination with a strong signature of balancing selection for *Obp99d*. *Obp99d* is exceptionally polymorphic. We measured variation in olfactory behavior to benzaldehyde, acetophenone, which is structurally similar to benzaldehyde, and hexanol, an unrelated odorant. Four SNPs exceeded the permutation threshold for association with variation in the response to benzaldehyde, 8 SNPs were associated with variation in the response to acetophenone, and 2 SNPs were associated with variation in response to hexanol. These SNPs were distinct for each odorant and included SNPs in coding regions and regulatory regions, including a SNP associated with variation in response to acetophenone that changes a cysteine into a tyrosine. This SNP is in strong linkage disequilibrium with 4 additional SNPs, two of which are nonsynonymous substitutions. These results show that at least some Obps are broadly tuned and, like odorant receptors, recognize odorants in a combinatorial manner. Furthermore, our observations illustrate how SNPs that arise during evolution can alter odorant binding properties and generate individual variation in Obp specificities.

Poster Session II: Wed. July 23

Xenobiotic Metabolizing Enzyme and Pheromonal Perception in *Drosophila Melanogaster*

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Xenobiotic Metabolizing Enzymes (XME: P450, UGT…) take in charge exogenous molecules and eliminate them from the organism. There are several evidences of specific XME activity in the chemosensory organs but still very few experimental evidence to support their functional role. Our goal is to investigate the functional involvement of XME in the discrimination of sex pheromones (hydrocarbons secreted by the fly cuticle) in *Drosophila melanogaster*. Using a Drosophila mutant strain containing a P element inserted in a putative UDP-Glycosyl transferase (UGT) gene, we observed several biological consequences. First, males of this strain cannot discriminate the sexes and show a decreased courtship of target females. This behavioral defect is specific since the mutation does not alter other behavioral responses. Second, the level of expression of the UGT, probed by qPCR, is significantly increased in several tissues including the sensory appendages. Using UAS/Gal4 system, we targeted specifically the sensory appendages with a transgene carrying the RNA interference (RNAi) of this UGT to affect its expression. The RNAi transgene was validated with both qPCR and behavior. We found that the level of expression of the UGT is logically reduced but surprisingly, transgenic males showed a higher sexual discrimination, especially because they decreased their courtship of target males. In conclusion, our data indicate that the overexpression of the *UGT* gene induces a loss of pheromonal discrimination whereas its underexpression leads to a higher discrimination. This suggests that the modulation of expression of a putative *UGT* tends to modulate pheromone perception and/or discrimination in Drosophila. We are currently investigating the involvement of the UGT activity in the metabolism of Drosophila pheromones.

Poster Session II: Wed. July 23

Combinatorial Co-Expression of Major Urinary Protein (MUP) Genes During Ontogenesis is Essential for Olfactory Coding and Social Recognition in Mice

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The major urinary proteins (MUPs) are widely assumed to be a key component of individual recognition in *Mus musculus L.* (Hurst et al., 2001; Sharrow et al., 2002; Beynon, Hurst, 2003; Armstrong et al., 2005; Cheetham et al., 2007). MUPs can bind volatile pheromone ligands and convey essential olfactory information about genotype, sex, social status and individuality of donors (Novikov, 2007). We investigated the ontogenetic profile of MUPs content in urine from male and female mice of two common strains CBA/Lac and C57BL/6J using SDS-PAGE electrophoresis followed by detailed densitometry study. Correlation analysis between rank orders of particular MUP bands at different stages of ontogenesis revealed positive correlation between juvenile and adult animals of both sexes. Obtained data indicate that specific "adult proportion" profile of different MUP fractions emerges very soon after weaning and resembles a "bar code". Our results can reflect the functional significance of co-expression of the *Mup* multigene family and give evidence for the important role of MUPs' combinatorial pattern in formation of the genotype- and gender-specific pheromone signature. In the light of recent findings on direct activation of the vomeronasal neurons by MUPs (Chamero et al., 2007) and on the combinatorial expression of pheromone receptors, V2Rs (Silvotti et al., 2007) the presented data provide valuable insight into fine molecular mechanisms of olfactory coding, discrimination and social recognition in laboratory mice.

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Poster Session II: Wed. July 23

Functional Imaging after Occlusion of the Vomeronasal Organ

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The vomeronasal organ (VNO) is an intranasal chemosensory system that exists in many mammals. It is not clear whether or not the VNO has a function in humans. In this study we aimed to investigate its functionality in the perception of the odor of androstadienone (AND), a putative human pheromone. Twelve healthy young women underwent PET scans while they were stimulated with AND or a perceptually similar, nonendogenous control odor, polysantol (CON). Presence of a VNO was ascertained by endoscopy. In half of the scans subjects' VNO was occluded with a latex patch, which was moved to a sham location on the nasal mucosa in the other half of the scans. Six subjects were tested on the left nostril, and six subjects were tested on the right; the respective other nostril was blocked with a tape. Brain activations during the presentation of AND and CON were contrasted against water. When compared to the water baseline, both odors activated the piriform cortex; CON additionally activated the orbitofrontal cortex. Further, neither odor activated the hypothalamus, a region previously implicated in the processing of AND, according to global and regions of interest analyses. Covering of the VNO did not render any significant change in brain activation patterns (contrast: VNO covered – sham location covered). Thus, functional blocking of the VNO appears not to change brain activations following stimulation with either a putative human pheromone or a nonendogenous odorant. This lends further support to the notion of the VNO's lack of function in humans.

Poster Session II: Wed. July 23

An Intact Whole-Organ Preparation of the Mouse Vomeronasal Organ: Confocal Life-Cell Imaging in the Microvillous Layer of the Sensory Epithelium

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In most mammals, olfactory stimuli are detected by at least two different sensory systems, the main olfactory epithelium (MOE) and the vomeronasal organ (VNO). The VNO, a blind-ended tube at the ventral part of the nasal septum is specialized for detection of social information important for reproduction, mate selection, gender identification and social status. So far, the majority of physiological studies investigating the vomeronasal signal transduction machinery used either freshly dissociated vomeronasal sensory neurons (VSNs) or acute coronal sections of the VNO. While these approaches provide a wealth of important insights into the molecular machinery of vomeronasal signal transduction, such preparations are inherently limited by mechanical perturbation of the natural cellular environment. Here, we report a novel highly intact mouse VNO whole-organ preparation suitable for physiological recordings of pheromone-induced Ca^{2+} signals from the dendritic surface of the sensory epithelium. In this preparation, the sensory epithelium is essentially undamaged and axonal projections to the accessory olfactory bulb are in sound condition. Combining life-cell confocal fluorescence microscopy of Ca^{2+}-sensitive reporter dyes with specific pharmacological approaches, we are able to investigate the role of various signaling enzymes and ion channels. Our current findings confirm a critical role of phospholipase C as well as members of the TRP ion channel family in vomeronasal signal transduction. Employing this novel whole-mount preparation method in future studies, we envisage to obtain significant new insights into social signaling in the accessory olfactory system.

Poster Session II: Wed. July 23

Male-Specific Exocrine Peptide ESP1 Activates a Selective Neural Pathway Via V2Rp5 Receptor in the Mouse Vomeronasal System

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The mammalian vomeronasal organ (VNO) comprises two functionally different populations of vomeronasal sensory neurons (VSNs): the apical-layer VSNs express V1R-type receptors and Gαi, while the basal-layer VSNs express V2R-type receptors and Gαo. Although it has been suggested that V1R- and V2R-expressing neurons detect small volatile chemicals and nonvolatile peptides, respectively, only a few ligand-receptor pairs have been identified. We previously discovered the male-specific exocrine gland-secreting peptide 1 (ESP1) as a strong candidate for V2R-ligand. In this study, we identified a functional receptor for ESP1 designated as V2Rp5 by a combinatorial approach detecting ligand-induced c-Fos expression and *in situ* hybridization for V2R mRNAs in VSNs. To obtain further evidence for ESP1-V2Rp5 interaction, we generated a BAC transgenic mouse line (V2Rp5-Red) in which V2Rp5-expressing VSNs were labeled with fluorescent protein DsRed. When V2Rp5-Red mice were exposed to ESP1, c-Fos expression was faithfully observed in DsRed-expressing VSNs, demonstrating the *in vivo* evidence for ESP1 stimulation of V2Rp5-expressing VSNs. In addition, we found that DsRed-expressing VSNs projected their axons onto 4-9 glomeruli lining along the medio-lateral axis in the accessory olfactory bulb (AOB). Furthermore, ESP1 stimulation induced c-Fos expression in a small population of mitral cells and granule cells that were distributed beneath the DsRed-positive glomeruli. These results demonstrate for the first time a link between a specific ligand-receptor interaction in VSNs and a selective vomeronasal neural pathway, paving the way to the better understanding of mechanisms for peptide pheromone perception in mammals at the molecular and neural circuit levels.

Poster Session II: Wed. July 23

Immunocytochemical Evidence for Steroid Metabolism and Modification in the Primary Vomeronasal System in Rodents

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Rodent vomeronasal receptor cells (VRCs), which detect pheromonal chemosignals, contain well-developed smooth endoplasmic reticulum (SER) in their somata, suggesting that they are involved in steroid metabolism that takes place in SER of gonadal and adrenal endocrine cells. Also, the physiological activity of the rodent vomeronasal system is markedly modified by sex steroids. To obtain immunocytochemical basis for understanding metabolism and modification of steroids in VRCs, progesterone, testosterone, beta-steroid dehydrogenases (bHSDs), and estrogen receptor alpha (ERa) were identified and localized in adult Sprague-Dawly rats of both sexes. For light microscopic study, rats were transcardially perfused with Zamboni's fixative, nasal regions containing VNOs were dissected out, decalcified, and processed for cryostat sectioning. For electron microsopic study, a mixture of formaldehyde and glutaraldehyde solution was used as a fixative, VNOs were dissected out, and processed for LR-White resin embedding. Immunoreactivity for progesterone was identified in VRCs, sustentacular cells, and vomeronasal nerves, whereas strong immunoreactivity for testosterone was localized in the apical surface of the vomeronasal sensory epithelium. Using an antibody that recognizes several types of bHSDs, such

as 3b-, and several types of 17b- HSD, it was demonstrated that VRCs contain its immunoreactivity. Post-embedding immunogold electron microscopy using an antibody to bHSDs demonstrated that immunoreactivity was localized in SER of VRCs. The immunoreactivity for ERa was prominently present in the apical dendrites and dendritic endings of VRCs. The above results demonstrate that metabolism of steroids take place in rat VRCs, and suggest that the function of the VRCs is to be modified by estradiol.

Poster Session II: Wed. July 23

Mechanism of Pheromone Adaptation in Vomeronasal Neurons: Calmodulin Feedback Regulates Diacylglycerol-Operated TRP Channels

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The mouse vomeronasal organ (VNO) has emerged as a valuable model for investigating the regulation of social behaviors by chemical cues such as pheromones. Understanding VNO signaling requires knowledge of the dynamic processes that regulate the sensitivity of pheromone detection in vomeronasal sensory neurons (VSNs). Here we show that pheromone-induced Ca^{2+} entry plays a crucial role as a negative feedback modulator of VSN sensitivity. VSN responses undergo effective sensory adaptation that requires the influx of Ca^{2+} and is mediated by calmodulin (CaM). Removal of the Ca^{2+}-CaM feedback eliminates pheromone adaptation. A key component of this feedback module is the pheromone-sensitive, diacylglycerol-operated cation channel of VSNs, as its activation is strongly inhibited by Ca^{2+}-CaM. Our experiments reveal a previously unrecognized feedback mechanism that is essential for adjusting the sensitivity of pheromone detection in the VNO. These findings are not consistent with a model in which the entire chemotransduction process in VSNs fails to adapt.

Poster Session II: Wed. July 23

The Involvement of Calcium Activated Chloride Channels in Urine Responses of Mouse Vomeronasal Sensory Neurons

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In most mammals, the vomeronasal organ (VNO) plays a role in mediating animal behavior by detecting pheromones and general odors through vomeronasal sensory neurons (VSNs). These neurons transduce chemical signals from the environment into electrical signals by G-protein coupled receptors and activation of the phospholipase C pathway (PLC-pathway). This activation leads to an increase in diacylglycerol (DAG) and arachidonic acid (AA) and an influx in Na^+ and Ca^{2+} ions. Whether or not there is any downstream signal amplification of the initial cation influx is unclear. We decided to investigate this possibility using gramicidin-perforated patch clamp recordings with two chloride channel blockers, niflumic acid (300 mM) and 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid (DIDS) (300 mM). We found that up to 80% of the urine-induced inward current (V_{hold} = -80 mV) in isolated mouse VSNs was carried by chloride. Further, the urine-induced responses decreased significantly (70%) when external calcium was replaced by barium, which suggests that these Cl channels are calcium dependent. In addition, reverse transcription polymerase chain reaction (RT-PCR) and immunocytochemistry studies revealed the presence of a calcium-activated chloride channel, Bestrophin-2, in mouse VSNs. Taken together, these studies suggest that Cl channel activation amplifies odor responses in VSNs similar to that observed in olfactory sensory neurons. This work was supported by grants NIH-DC006939, NIH-P20RR16435 and NSF-EPS0236976.

Poster Session II: Wed. July 23

The Canonical Transient Receptor Potential Channel 2 (TRPC2) Forms Protein-Protein Interactions with Homer and RTP in the Rat Vomeronasal Organ

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The protein machinery transducing chemosignal cues in the vomeronasal organ (VNO) has been individually well-characterized, but little attention has been paid to the role of protein-protein interactions amongst these molecules or to the mechanisms that might regulate surface expression. Previously we found that TRPC2 and the type 3 IP_3 receptor co-localize in VNO microvilli and a peptide designed to prevent co-immunoprecipitation of the two channels inhibited chemosignal-induced currents. We now present evidence for two additional protein partners that couple with the channel in native VNO. Purified membrane preparations of adult VNO were separated by SDS-PAGE and probed with antisera directed against members of the Homer family of scaffolding proteins. Homer 1b/c and 3 were expressed in both the VNO and the olfactory bulb whereas Homer 2 was only expressed in the latter. RT-PCR supported VNO expression of two chaperone proteins originally identified in olfactory receptor neurons, namely receptor transporting protein 1 (RTP1) and receptor expression enhancing protein 1 (REEP1). Adult VNO cryosections were processed with an aviden peroxidase chromagen method that revealed RTP1 antiserum labeled the VNO sensory epithelium, goblet cells, and the soft palate, but not respiratory cilia. Both RTP1 and Homer 1b/c formed protein-protein interactions with TRPC2 in native reciprocal co-immunoprecipitation assays. Utilizing a transient lipofectamide-based transfection protocol in HEK293 cells, RTP1 increased surface expression of TRPC2 *in vitro* as demonstrated by surface biotinylation of the channel. We conclude that TRPC2 activity could thereby be regulated by both chaperones and scaffolding-associated proteins to modulate pheromone information.

Poster Session II: Wed. July 23

A Conserved Vomeronasal Signaling Pathway in a Non-Mammalian Vertebrate

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In terrestrial lungless salamanders, Plethodon Receptivity Factor (PRF) is a male pheromone protein that affects female receptivity. A male delivers this pheromone to the female's nares and then the pheromone is shunted to the vomeronasal organ (VNO). In mammals, the V2R signaling pathway has been shown to respond to amino acids and proteins. This V2R pathway generally has been shown to utilize the $G\alpha_o$ subunit, PLC, DAG and the TRP2C channel. We hypothesized that this V2R pathway also may function in the VNO of female *P. shermani* salamanders. First, we used PCR on cDNA from VNO tissue to investigate the presence and diversity of V2R receptors in salamanders. Our initial sequence analyses of these V2Rs showed that the V2R family appears to be as diverse in *Plethodon* as in other vertebrates. To determine the histological distribution of V2R receptor expression, we conducted an *in situ* hybridization study utilizing probes

designed from V2R sequences obtained from *P. shermani* VNO. Probes demonstrated the presence of V2R RNA in VNO epithelium, but not in main olfactory epithelium. In addition, we have evidence for the expression of other members of the cellular cascade. We amplified a 1200 bp fragment of the TRP2C from VNO cDNA. We also verified that *P. shermani* VNO neurons express the $G\alpha_o$ subunit by conducting an immunocytochemical study using antibodies against $G\alpha_o$. Vomeronasal epithelium demonstrated intense labeling in the region of the dendritic knobs and microvilli. Thus, our preliminary work indicates that the salamander VNO contains elements of a conserved signaling pathway that potentially transduces sex pheromones.

Poster Session II: Wed. July 23

Intrinsic Plasticity in the Mouse Vomeronasal Organ: The Role of the Ether-à-Gogo Related Gene Ion Channel

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Conspecific social and sexual behavior is regulated by complex chemical communication strategies. Social cues - pheromones - are detected by sensory neurons of both the main olfactory epithelium and the vomeronasal organ (VNO). Despite the fundamental significance of social chemosignaling, the principle mechanisms underlying pheromone detection and processing remain poorly understood. Here, we report expression of an ether-à-gogo related gene (ERG) ion channel in basal vomeronasal sensory neurons (VSNs) of C57BL/6 mice. Activity-dependent mRNA expression profiling and semi-quantitative immunoblotting reveal increased vomeronasal expression of ERG1 channel subunits after long-term exposure to social stimuli. Patch clamp recordings from basal VSNs in acute VNO slices show that ERG-mediated currents are activated during action potential discharge. Pharmacological block of ERG channels strongly diminishes tonic firing in response to depolarizing current injections. Thus, our data indicate an important role of ERG channels in extending the dynamic response range of basal VSNs, revealing a previously unknown form of intrinsic plasticity in the VNO.

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Poster Session II: Wed. July 23

Hyperpolarization Activated Cyclic Nucleotide Gated Channels in Mouse Vomeronasal Sensory Neurons

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Pheromones are chemicals released from animals that can cause changes in physiology and behavior in members of the same species. Pheromones are mainly detected by vomeronasal sensory neurons located in the vomeronasal organ (VNO), or Jacobson's organ. Some of the biophysical properties of vomeronasal sensory neurons are still not completely characterized. We measured the properties of hyperpolarization-activated currents (I_h) from acute slices of the mouse VNO. In voltage-clamp studies, I_h was identified by the: characteristic kinetics of activation, voltage-dependence, and blockage by Cs^+ or ZD-7288, two blockers of the I_h, but not by Ba^{2+}. Forskolin, an activator of adenylyl cyclase, shifted the activation curve for I_h to less negative potentials. A comparison of the measured I_h properties in VNO neurons with those of heterologously expressed hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, together with RT-PCR experiments in the VNO, indicate that I_h is due to HCN2 and/or HCN4 subunits. In current-clamp recordings, blocking I_h with ZD-7288 induced a hyperpolarization of 5.1 mV and an increase in input resistance from 4.1 to 5.1 GΩ but did not modify the frequency of action potentials elicited by a 5 pA current step of 3-sec duration. It has been shown that in VNO neurons some pheromones induce a decrease in cAMP concentration, but the physiological role of cAMP is unknown. After application of blockers of adenylyl cyclase we measured a hyperpolarization of 5.8 mV in six of eight neurons, suggesting that basal levels of cAMP could modulate the resting potential. In conclusion, these results demonstrate that mouse VNO neurons express HCN2 and/or HCN4 subunits and that I_h contributes to setting the resting membrane potential.

Poster Session II: Wed. July 23

A Putative Endoplasmic Reticulum Chaperone, Calreticulin 4, is Expressed in Mouse Vomeronasal Organ

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Intraspecific communication in animals is often mediated by pheromones and partly detected by the accessory olfactory organ, Vomeronasal Organ (VNO) in mammals. Previous studies have uncovered molecules that are specifically expressed in the VNO, including two independent groups of putative pheromone receptors, the V1Rs and the V2Rs. The VNO however appears to be vestigialized in humans and the vast majority of the V1Rs and all of the V2Rs as well as the VNO-specific ion channel, Trpc2, are pseudogenes in the human genome. We hypothesized that genes that have specific functions in the VNO are pseudogenized in humans. We used a published list of human pseudogenes [(1)] to identify intact orthologues in mouse and asked if any of them might be specifically expressed in the VNO. We performed RT-PCR and *in situ* hybridization to assay transcription of these genes in different mouse tissues and found calreticulin 4, a homologue of calreticulin, with highly enriched expression in the mouse VNO. Since calreticulin is a ubiquitously expressed endoplasmic reticulum resident chaperone with essential roles in quality control of glycoproteins, calreticulin 4 could have specific roles in the VNO in biogenesis of the VNO-expressing transmembrane and secreted molecules. (1) International Human Genome Sequencing Consortium. Finishing the euchromatic sequence of the human genome *Nature* (2004) 431 (7011): 931-45.

Poster Session II: Wed. July 23

A Novel Role for JNK Signaling in Olfactory Sensory Neuronal Death

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Olfactory sensory neurons (OSNs) represent a unique population of neurons in which death and regeneration are ongoing throughout adulthood, a feature that makes them an attractive model cell type for the investigation of neuronal death. However, the mechanism by which OSNs die remains elusive. Therefore, we developed a culture system for studying pathways

involved in OSN death. Here, we show that inhibition of transcription or translation, by actinomycin D or cycloheximide, respectively, suppresses pathways leading to death, prolonging the survival of OSNs in culture. We discovered that caspase activity and jun N-terminal kinase (JNK) signaling both play a role in OSN death, and inhibition of JNK activity suppresses effector caspase (caspase-3) activation. Results from studies in culture were confirmed in vivo, in a mouse bulbectomy-induced OSN death model. These findings provide new insights into the nature of OSN death and a means of studying OSNs in vitro.

Poster Session II: Wed. July 23

Degenerative Changes in Olfactory Sensory Neurons Deprived of Neuronal Targets

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Synaptic contacts with bulb target neurons are thought to regulate neuronal survival in the olfactory epithelium. We characterized degenerative changes in olfactory sensory neurons (OSNs) at both the light and electron microscopic level following disruption of these contacts. Chemical ablation of bulb neurons with NMDA was employed to rapidly and permanently remove the normal synaptic targets of OSNs without damaging their bulbar projections. Adult rat OSNs proved remarkably resilient to acute synaptic disconnection and maintained normal expression of olfactory marker protein (OMP) and growth-associated protein-43 (GAP-43) out to two weeks post-lesion. However at this time, increases in TUNEL labeling indicated delayed, retrograde apoptosis in the target-deprived epithelium, and decreased cell survival was verified by post-lesion administration of bromodeoxyuridine. At the light microscopic level, sensory axons making contact with the neuron-depleted bulb were mostly restricted to the olfactory nerve layer. Electron microscopic examination of the bulb remnant three weeks after NMDA lesion showed ensheathing glia-enclosed axon bundles in the nerve layer, and numerous individual axon terminals in the process of dissolution that appeared swollen, and more electron lucent than normal, with some undergoing filamentous degeneration. Sensory axon synaptic contacts were lacking, with the exception of those made on rare surviving dendritic processes in outer portions of the remnant. These appeared heavily innervated by sensory terminals. Our results indicate that preservation of an undamaged axon projection pathway allows sensory axons to project to the neuron-depleted bulb, but their inability to maintain or establish stable synaptic contacts is correlated with OSN degeneration.

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Poster Session II: Wed. July 23

Loss of *Notch2* in Sustentacular Cells of the Main Olfactory Epithelium Leads to Neurodegeneration

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Defects in olfaction are common in patients with Alzheimer's disease, and olfactory sensory neurons (OSNs) of Alzheimer's patients show signs of oxidative stress. However, why OSNs of Alzheimer's patients are susceptible to oxidative damage is not well-understood. Sustentacular cells in the olfactory epithelium are thought to protect OSNs by detoxifying environmental stimuli so as to reduce oxidative stress. However, a direct role for neuroprotection by sustentacular cells has yet to be shown. Employing mouse genetics we provide the first direct evidence confirming this hypothesis. We show that the Notch pathway is critical for maintaining sustentacular cell function. Loss of *Notch2*, a cell-surface receptor, results in the decreased expression and activity of key enzymes responsible for the neuroprotective function of sustentacular cells. Interestingly, this results in OSN neurodegeneration confirming the neuroprotective role of sustentacular cells. These studies show for the first time that sustentacular cells are important for OSN survival.

Poster Session II: Wed. July 23

Pacap Reduces Cytokine-Induced Apoptosis in Olfactory Neuronal Cells Via Both AC and PlC Transduction Pathways

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PACAP protects neurons in the olfactory epithelium (OE) against axotomy-induced apoptosis. TNF-α is a cytokine intrinsic to the OE. We examined the neuroprotective role of PACAP against TNF in the olfactory placodal cell lines, OP6 and OP27. Cells were treated with TNF ± PACAP, and labeled with propidium iodide (PI) to mark dying cells. Treatment with TNF significantly increased the number of PI-labeled cells vs. control in both cell lines. Data is presented as percent of PI-labeled cells in the TNF treatment. We found that PACAP reduced PI-labeled OP6 cells to 45 ± 4% of that seen with TNF alone. In OP27 cells, PI-labeling was reduced to 62 ± 5%. The effect of PACAP against TNF was mimicked by the PAC1 receptor agonist, maxadilan. Addition of PAC1 receptor antagonists PACAP6-38 or M65 abolished PACAP's effect, implying that PACAP mediates neuroprotection in the OE by activating the PAC1 receptor. We then asked if PACAP functions via the phospholipase C (PLC) or the adenylate cyclase (AC) signal transduction pathways. Addition of the PLC blocker U73122 reduced the protective effect of PACAP: PI-labeling increased from 45% to 71 ± 17% (OP6), and from 62% to 79 ± 9% (OP27). Co-incubation of TNF and the PLC activator PMA reduced PI labeling to 28 ± 10% (OP6) or 33 ± 6% (OP27). Similarly, co-incubation of TNF and the AC activator forskolin mimicked the effect of PACAP by reducing PI-labeled cells to 53 ± 14% (OP6) and 48 ± 2% (OP27). Addition of the AC blocker SQ22536 reduced the anti-apoptotic effect of PACAP: PI-labeling increased from 45% to 111 ± 10% (OP6) and from 62% to 79 ± 8% (OP27). We therefore show that both the PLC and AC pathways can be involved in PACAP-mediated inhibition of TNF-induced apoptosis in olfactory neuronal precursor cell lines.

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Poster Session II: Wed. July 23

Pacap is Required for Maintenance of Olfactory Epithelial Integrity in Adult Mice but not in Neonates

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The adult olfactory epithelium (OE) is capable of continuous regeneration and expresses pituitary adenylate cyclase-activating polypeptide (PACAP) and its receptor (PAC1). PACAP is a pleiotropic peptide important for many stages of neuronal development such as proliferation, differentiation, maturation, and survival. We investigated the in vivo role of PACAP on OE development and maturation using neonatal and adult PACAP knockout (KO) and wild-type mice. Mice were injected with BrdU, sacrificed at 2 hrs, fixed, and cryo-sectioned. OE tissue sections were either processed for BrdU labeling (to measure cell proliferation) or TUNEL labeling (to measure apoptosis), or were trypan blue stained (to measure OE thickness). Six comparable regions were measured in each tissue section, and all measurements were conducted blind to the genotype. Surprisingly, in P3 neonates

there were no significant differences in OE thickness or TUNEL labeling between PACAP-KO and wild-type mice. However, BrdU labeling was significantly decreased by 35% in neonatal PACAP-KO compared to wild-type, indicating that PACAP plays a role in cell proliferation in the neonate OE. In contrast, the OE from adult PACAP-KO mice was severely compromised, with a 32% decrease in OE thickness and a 265% increase in TUNEL labeling compared to wild type. Interestingly, BrdU labeling was increased by 318% in adult PACAP-KO mice suggesting that PACAP is not required for adult basal cell proliferation. We conclude that in adult mice, PACAP is required for preventing apoptosis in the OE, while in development, PACAP either plays a lesser role or perhaps other factors actively compensate for lack of PACAP.

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Poster Session II: Wed. July 23

Expression of Survivin in Rat Olfactory Epithelium during Postnatal Development

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Olfactory epithelium is known for neuronal turnover throughout life by a tight regulation of proliferation and apoptosis. However, proliferation density decreases postnatally (Weiler & Farbman, J Neurosci 1997, 17, 3610-22) and thus apoptosis should be inhibited to retain the olfactory sheet. Therefore we asked, whether apoptosis inhibitors are expressed in olfactory tissues, especially in older animals. Using RT-PCR and duplex-RT-PCR we investigated the expression of the apoptosis inhibitor survivin, also known as Birc5, in olfactory mucosa of rats at different postnatal ages (P10-900). We describe here, that survivin is expressed in olfactory mucosa at all postnatal ages and furthermore, at much higher levels in young animals compared to older ones; survivin expression decreases postnatally as does proliferation density. The question arises, what function does survivin fulfill in the olfactory system, why is the expression much higher in young animals, where there is a backup by high proliferation? In young animals, where proliferation is high, many olfactory sensory neurons compete for the target cells in the olfactory bulb, and we know that synaptic input is essential for neurons to survive. So it seems reasonable for a neuron to express an apoptosis inhibitor. On the other hand, when proliferation density is decreased in older animals, the turnover pressure is low and apoptosis inhibitors are not necessary as much. Thus we conclude, that the apoptosis inhibitor survivin is expressed to help neurons to survive during their competition for target contacts until they adjust enough stable synapses, which then take over the survival function.

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Poster Session II: Wed. July 23

Triton X-100 Treatment in Zebrafish Alters Olfactory Epithelium Morphology

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Our goal was to study the effect of sensory loss on central structures by chemically ablating olfactory sensory neurons while limiting damage to adjacent structures. Chemical ablation offers a way to study turnover in the olfactory epithelium of zebrafish, about which little is known. A 0.7% solution of the detergent Triton X-100 was applied to the right olfactory organs, leaving left sides untreated. Fish were sacrificed 1 to 5 days later and whole heads were fixed, decalcified, embedded in paraffin, and sectioned at 10μm. Sections were stained with hematoxylin & eosin to examine and measure olfactory structures and labeled with anti-acetylated-tubulin to distinguish olfactory epithelium from respiratory epithelium. We measured a significant decrease in epithelial thickness of the treated sides compared to controls 1 day post Triton X-100 treatment (treated = 10.9 ± 0.554, control = 17.0 ± 0.563, p = 0.04). Thickness of epithelium recovered over time, with no significant difference by 5 days (treated = 17.6 ± 0.312, control = 18.2 ± 0.105, p=0.8). We observed that anti-acetylated-tubulin labeling was low in treated olfactory organs at short survivals but comparable to controls by 5 days. Our data suggest that the most significant reduction in the olfactory epithelium following a Triton X-100 treatment corresponded to the region of supporting cells and mature olfactory sensory neurons while not severely affecting the basal cell layer, allowing for swift regeneration of both olfactory and respiratory cell types. Thus, chemical ablation causes temporary deafferentation of the olfactory bulb with regeneration of the epithelium occurring within a week; therefore Triton X-100 can be a useful tool for olfactory regeneration and reinnervation studies in the zebrafish.

Poster Session II: Wed. July 23

Effect of Ginko Biloba and Dexamethasone in the Treatment of 3-Methylindole- Induced Anosmia Mouse Model

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Objective: Treatment of olfactory loss is challenging. Although glucocorticoids are widely used, it was reported that it potentiated neural damage in the early period of treatment. This study is aimed to identify the effect of ginko biloba in the treatment of olfactory injury aggravated by dexamethasone.

Materials and Methods: Anosmia mouse model was developed by intraperitoneal injection of 3-methylindole (3-MI). Twenty-five mice were divided into one normal control and four anosmia groups according to the treatment of dexamethasone or ginko biloba. The effects of treatment were evaluated by Western blot and immunohistochemistry two weeks after 3-MI injection.

Results: Induction of anosmia was confirmed by behavioral tests. The thickness and cell number of olfactory neuroepithelium more significantly decreased in anosmic mice treated with dexamethasone alone than in mice treated with combination of dexamethasone and ginko biloba. The expression of olfactory marker protein (OMP) in olfactory epithelium was also lower in dexamethasone treatment group than in combination treatment group. The expression of OMP significantly decreased in the olfactory bulbs of anomia groups but there were no differences between experimental groups.

Conclusions: Dexamethasone deteriorated olfactory loss induced by 3-MI and olfaction was restored by treatment of dexamethasone and ginko biloba. The anti-oxidant effect of ginko biloba might be playing a role in these findings and effective only in condition that oxidative stress is maximized by dexamethasone. Clinically, it might be suggested that combination treatment might be safer than single-agent glucocorticoid therapy in patients with olfactory deficit.

Poster Session II: Wed. July 23

Probing Olfactory Coding Directly at the Human Cortical Surface

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The rules underlying cortical coding of olfaction remain unknown. Moreover, whether the identity of odors is encoded within the temporal patterns of neural activity, the spatial patterns of neural activity or a combination of both, is unresolved. Here we set out to address this by recording electrical activity directly at the cortical surface of patients with implanted electrodes before resection surgery for intractable epilepsy. To date, we recorded from one patient. We used a computer-controlled olfactometer to deliver one of 5 different odorants or clean air in an event-related design (Flow = 6 lpm, ISI = 30s, Stimulus duration = 3s, Number of events per condition = ~15). The odorants were selected based on their spanning a validated perceptual odor space. Intracranial EEG (256Hz) was recorded from 44 sub-dural electrodes (Ad-Tech), 20 in a grid overlaying the right fronto-pariet-temporal junction, and 24 in three 8-electrode strips laid roughly in frontal, occipital and sub-temporal regions. Initial analysis in the time domain revealed a clear sniff-related response that played out within ~4 seconds, and that was evident in 24 electrodes. Within this response, in 16 electrodes there was a pronounced difference in absolute peak response between sniffs with and without an odorant (all $p<0.001$). Critically, an inline high-sensitivity pneomatotachograph revealed that there were no differences in sniff airflow across these conditions, suggesting the response reflected a genuine response to odor. That said, no EEG parameter within the time domain dissociated the different odors. Although additional analysis in the frequency domain, as well as additional patients are necessary, these results nevertheless suggest that probing olfactory coding directly from the human cortical surface is feasible.

Poster Session II: Wed. July 23

Cerebral Responses after Olfactory Stimulation with Phenyl Ethyl Alcohol – How much Stimulation is Necessary? - A Functional MRI Study on Olfaction

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Neurodegenerative diseases are often associated with olfactory dysfunction. A novel tool for investigation of the human olfactory system is functional magnetic resonance tomography (fMRI) after odorant application. Necessary requirements for this technique are the artefact-free application of olfactory stimuli in the scanner environment and the establishment of a useful MRI sequence. First aim of this study was to develop a robust fMRI design for investigations on cerebral olfactory processing. Since the length of the fMRI examination is a limiting factor in patient care a short as possible paradigm should be found in order to reduce the overall examination time. A MRI-compliant constant flow olfactometer was developed. 9 healthy normosmic subjects were measured in a 1.5 Tesla scanner. Odorant was phenyl ethyl alcohol (PEA). 300 whole brain EPI volumes were collected over 11:26 min while 16 PEA stimuli were given. The statistical evaluation of the data was performed by the SPM5 software package. The group analysis showed bilateral cerebral activations within insula and adjacent operculum, cingulum, amygdala and cerebellum. This activation pattern is in accordance with results described in the literature. All mentioned areas could already be identified after the application of 8 (out of 16) olfactory stimuli. Bilateral insular activations and activations in the left amygdala were even shown after 4 stimuli. Therefore the study design as well as the developed olfactometer were appropriate to show reliable neuronal activations during odour perception. The paradigm can be used in studies on patients with neurodegenerative diseases and other olfactory disorders. A further reduction of the examination time at least by the half seems to be possible.

Poster Session II: Wed. July 23

Olfactory Perceptual Decision-Making in the Human Olfactory Brain

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Little is known about how percepts of odor quality develop in the context of olfactory decision-making. Using functional magnetic resonance imaging (fMRI) and psychophysical testing we investigated the evolution of odor percepts in the human brain and the role of perceptual decision-making in the disambiguation of odor mixtures. A binary odor-mixture set of citral (lemon) and eugenol (clove) was assembled, systematically varying between 100% citral/0% eugenol and 0% citral/100% eugenol (total of nine discrete mixtures). In initial pilot experiments, six participants were asked to make as many sniffs as needed to confidently identify which of the two odors was more prevalent in a given odor mixture trial. Odor mixtures yielded a ratio of perceptual decisions that reflected the ratio of the pure odorants in a sigmoidal fashion, and reaction times increased with the ambiguity of the odor. At this time, we have completed fMRI data analysis from one subject (with analysis of further subjects to be completed soon). These preliminary imaging findings show that odor-evoked activation in the piriform cortex conformed to a "U-shaped" curve, such that the two pure odorants at the extremes of the scale (least ambiguous) had the largest activation, while those at the middle of the scale (most ambiguous) had reduced activation. Conversely, activation in the orbitofrontal cortex (OFC) conformed to an "inverted U-shaped" curve, with increasing neural responses for the more ambiguous odor mixtures. These results suggest that piriform cortex and OFC play different roles in evaluating the content of odor mixtures. Further analysis will specifically explore the neural interactions and dynamics of these brain regions in olfactory perceptual decision-making.

Poster Session II: Wed. July 23

What's on your Mind when you Smell the Roses?

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We used activation likelihood estimation (ALE) meta-analysis of 34 olfactory functional neuroimaging studies to reveal brain activation probabilities during different olfactory tasks: passive smelling, simple odor detection, and tasks demanding higher order olfactory processing. Only 'odor' versus 'odorless baseline' scans were included. During all three tasks, significant ALE peaks were found in the piriform cortex (PIR) bilaterally. While the likelihood of activating the orbitofrontal cortex during passive smelling was minor (left anterior OFC), the probability of the bilateral OFC activation during odor detection was high (both peaks in the region surrounding the transverse orbital sulcus). A direct contrast [passive smelling vs. odor detection] confirmed a stronger likelihood of bilateral OFC activation in association with odor detection, and also revealed a stronger likelihood of bilateral PIR activation in association with passive smelling. Likelihood of activating PIR during higher order olfactory processing was lower than during both passive smelling and odor detection. However, widespread activation in frontal and parietal cortex bilaterally was associated with increased cognitive processing while smelling. In addition, we addressed the question of whether warning signals announcing the delivery of olfactory stimuli affect the pattern of brain activation probabilities. A warning signal increased the likelihood of bilateral PIR activation and produced an asymmetry in the OFC: whereas warning signals produced larger values in the left OFC, unannounced odors rendered higher values in the right OFC. Taken together, these findings demonstrate the importance of the task performed

during olfactory functional neuroimaging and confirm an important mediating role of cognition in olfactory perception.

Poster Session II: Wed. July 23

Odor Deprivation in the Human Brain

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What are the effects of olfactory sensory deprivation on odor perception and its neural correlates in the human brain? We used functional magnetic resonance imaging (fMRI) and psychophysics techniques to measure odor-evoked brain activity from healthy subjects, both before and after a 7-day deprivation period. Subjects were admitted as inpatients for 7 days to Northwestern Memorial Hospital, where they were given scent-free hygiene products and a low-flavor diet. During waking hours, subjects' nostrils were occluded using Microfoam surgical tape and Merocel nasal foam. Dressing materials were changed every 4 hours. Behavioral results from one pilot subject showed transient improvements in odor detection thresholds (11 to 12.25 on the "Sniffin' Sticks Test") and odor identification (34 to 36 on the "UPSIT") following deprivation. In contrast, perceptual variance in ratings of odor quality similarity was greater from pre to post deprivation. Concurrently, odor-evoked fMRI activity increased in piriform cortex, but decreased in right olfactory orbitofrontal cortex (OFC), immediately after deprivation. Notably, these behavioral and neural effects returned to baseline levels after a 7-day recovery period. These preliminary results partially concord with visual deprivation studies in humans showing enhanced perceptual thresholds and cortical excitability as a result of visual deprivation (Boroojerdi et al., Cereb Cortex 2000), though the reductions of odor quality discrimination and OFC activity seen here suggest that a lack of olfactory sensory experience may disrupt higher-level odor processing at perceptual and neural levels.

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Poster Session II: Wed. July 23

The Localization of Human Olfactory Cortex: An Ale Meta-Analysis of Functional Neuroimaging Studies

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Functional neuroimaging has been used extensively during the last two decades to explore the substrates of olfactory neuronal processing. We reviewed all published olfactory imaging studies to date (PET and fMRI; n = 81) using the activation likelihood estimation (ALE) meta-analysis technique. ALE is an objective statistical method that searches for concordance in data by modeling each reported foci as the center of a 3D Gaussian probability distribution by permutation testing, then creating statistical parametric maps. We determined areas commonly activated during olfactory processing as well as differences in activations between PET and fMRI. A total of 34 studies (53 contrasts, 399 foci) met our 10 criteria, one of which was the inclusion of Odor vs. Baseline only. Significant ALE peaks were observed in areas commonly referred to as primary and secondary olfactory cortex - piriform (PIR) and posterior orbitofrontal cortex (OFC). In addition, high ALE scores were observed in insular, medial frontal, and superior frontal cortex. Differences between PET and fMRI were observed in both PIR and OFC. PET demonstrated higher ALE scores in the frontal part of PIR and the right caudal OFC whereas fMRI demonstrated higher ALE scores in the temporal part of PIR and bilaterally in the posterior OFC. These results map the olfactory brain with a high degree of statistical certainty. We demonstrate that areas outside the traditional olfactory cortices are commonly activated by olfactory stimuli. In addition, clear differences exist between imaging methods in their ability to map neuronal activation within olfactory regions. Implications for future imaging studies and potential remedies will be discussed.

Poster Session II: Wed. July 23

The Privileged Status of First Olfactory Associations: An FMRI Study

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Psychophysical data suggests a privileged status for first olfactory associations (Lawless, 1977). We set out to use fMRI in order to probe for neural correlates of this privileged link. Olfactory brain areas are activated differently at retrieval of visual objects that were previously associated with either positive or negative olfactory contexts (Gottfried, 2004). Here we consecutively associated visual objects with 2 differently valanced odorants and set out to ask whether/where the object-induced brain activity maintained a potential trace of the initial association. Four subjects participated in a 2-session fMRI experiment. At study1 they learned to associate 66 objects with pleasant, unpleasant or no odor. In fMRI test1 subjects viewed the objects, and indicated with which type of odor they were previously associated. At study2 the same objects were associated with an opposite valanced odor, and subjects were tested again in fMRI test2. We defined odor context brain regions by contrasting objects associated with unpleasant odor vs. objects associated with pleasant odor for the first association. This contrast revealed activity in right orbitofrontal cortex, left parahippocampus and posterior cingulate. We tested activation for the second association in these regions. Right orbitofrontal cortex was activated both for the first and second association (valance main effect $p < 0.028$, valance by association interaction $p = 0.18$). In other words, it maintained a candidate trace of the initial association. The other areas were activated only for the second association. Altogether our results suggest that it is possible to differentiate between brain activity for the first and second association. Conclusions, however, depend on a non-olfactory control to be reported.

Poster Session II: Wed. July 23

The Role of the Amygdala in Perception of Graded Pleasantness

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The role of the amygdala in the chemical senses still remains a source of debate. While some researchers have claimed that the amygdala is preferentially tuned to intensity rather than to valence[1], others have reported that it is preferentially activated to high intensity pleasant and unpleasant stimuli but not to neutral or low intensity stimuli[2]. We used a set of binary odor mixtures to establish whether the amygdala responds to odor valence regardless of odor intensity. Twelve subjects underwent PET, and scanned under 8 conditions: pyridine (unpleasant), citral (pleasant), five mixtures of citral and pyridine in varying physical proportions (from 10/90 to 90/10), and an odorless baseline. All stimuli were perceived as being isointense and moderately strong. A linear increase in perceived pleasantness was observed as one progressed from pyridine to the 50/50 mixture (neutral) and to citral. Using volume of interest analyses we extracted mean regional cerebral blood flow (rCBF) in left and right amygdala for all eight conditions. For both VOIs in the amygdala we found a U-shaped function: maximum rCBF in response to the pleasant and unpleasant mixtures (10/90 and 90/10 proportions of

citral and pyridine) and the smallest rCBF response for the neutral midpoint (50/50). In conclusion our results are consistent with previous findings which suggest that the amygdala responds to odor valence in both directions, i.e. to pleasant and unpleasant stimuli but not to neutral stimuli. Also, as our stimuli were not high in intensity[3] these results suggest that the amygdala also responds to pleasant and unpleasant stimuli of medium intensity.

Supported by grant MOP 57846 from the CIHR to MJG. [1]Anderson et al., Nat Neurosci, 2003. **6**(2): p. 196-202. [2]Winston et al., J Neurosci, 2005. **25**(39): p.8903-7.

Poster Session II: Wed. July 23

Preferential Response to Food Compared to Nonfood Odors in the Insula and Operculum

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Odors may have taste-like characteristics, and odors sensed in solution with a taste may come to smell like that taste (Stevenson et al., 1995, 1998). Here we performed two fMRI studies to test the prediction that food odors would preferentially activate insular gustatory cortex compared to equally pleasant and intense nonfood odors. In both studies, odors were delivered as vapors orthonasally and retronasally via tubes ending either at the external nares or nasophyarynx. In the first study (N = 11) one food odor (chocolate) was compared to 3 nonfood odors (lavender, farnesol and butanol). In the second study (N = 11), 4 food odors (chocolate, pineapple, peach, tomato) were compared to 2 nonfood odors (rose, lilac). In both studies we identified multiple regions of insula and overlapping operculum that responded preferentially to the food compared to the nonfood odors, irrespective of the route of odorant delivery (orthonasal vs. retronasal). This effect occurred despite the fact that subjects rated food and nonfood odors as similarly intense. Pleasantness ratings were equated in study 1 and in the pilot of study 2. However, during scanning subjects rated the food odors as more pleasant than the nonfood odors. To determine if this influenced the insular effect, we calculated the difference in rated pleasantness between food and nonfood odors and regressed these difference scores against the differential insular response. No relationship was observed. These results support our prediction that food odors, which have been previously experienced in the mouth with taste, result in greater response in insular taste cortex. We speculate that this response reflects re-activation of taste neurons that were involved in the initial encoding of the flavor.

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Poster Session II: Wed. July 23

Investigation of Mental Representation of VOC-Exposure Risks in Asthmatics

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Exacerbation of asthmatic symptoms is commonly attributed to exposure to various odors and irritants. However, the mechanisms by which chemical exposure elicits adverse health symptoms in this sensitive subpopulation are still unclear and may involve both physiological and psychological processes. For example, cognitive factors such as individuals' mental model or expectations regarding disease triggers may also be powerful in inducing adverse airway responses. Indeed, in the field of chemosensory perception recent research has shown that expectancies related to a chemical or an exposure situation can modulate subjective responses to airborne chemicals among healthy individuals. The aim of this study is to investigate how asthmatics' expectancies of sensory and health effects from VOC exposure covary with factors intrinsic to the exposure scenario (e.g., proximity, source of the chemical…) and factors associated with the individual (e.g., personality, disease severity…). Groups of asthmatics (mild and moderate) and healthy controls are presented with pictorials of a chemical exposure scenario and an unfamiliar odor. Participants are instructed to imagine themselves in a specific situation in which the odor stimulus is described as the emission odor from the environment depicted in the pictures. Subjects are asked to rate how intensely they would expect to experience a variety of health symptoms as a consequence of the odor/pictorial combination. Ratings of the odor stimulus quality are obtained before and after the pictorials presentation. Preliminary data show variations of health symptoms ratings in relation with the situation characteristics as well as differences in the sensory properties of the perceived odorant between asthmatics and control subjects.

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Poster Session II: Wed. July 23

Inter-Individual Variability in Olfactory Event-Related Potentials is Related to Reaction Time

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Olfactory event related potentials (OERPs) are mainly studied by means of grand average: means of ERPs of a large number of subjects. The inter-individual variability is less known. We recorded event-related potentials (OERPs) induced by 200 ms monorhinal stimulations with amyl acetate, under constant humidified air flow (6 L/min; 31°C) without pressure change in the air flow. OERPs were identified by averaging 65 trials (with random inter-stimulus interval of 7.0 s ±25%) and comparing with 65 control trials without odorant presentation. The procedure allows the subject to indicate the perception of the stimulus by pressing a button 2.5 s after the stimulus presentation. The reaction time was measured in another session by using the same stimulatory procedure. 20 normosmic subjects participated in the experiment. OERPs were hard to define in some subjects but easily identified in others. In the latter case, significant differences in the time course and, sometimes, in the polarity of the signal were observed among subjects. To try to understand this discrepancy, we examined and found a certain relationship with inter-individual differences in the reaction time to the odorant stimulation among subjects. We are also examining a possible relationship with the detectability index (d') measured by the signal detection theory (SDT). Finally, current analyses are performed, using wavelet analysis, to examine the changes in the frequency-time pattern in the main band-pass of the EEG (alpha, theta, delta waves, for example).

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Poster Session II: Wed. July 23

Olfactory Nerve Scintigram with Nasal Administration of Thallium-201 *in vivo*

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Although olfactory nerve damage is a contributing factor in the diagnosis of posttraumatic olfactory loss, at present there are no methods to directly assess injury to these nerves. Radioactive thallium-201 (^{201}Tl) has been widely used by systemic administration in isotope imaging for clinical diagnosis. The transport of ^{201}Tl in the olfactory nerve is decreased following transection of the olfactory nerve fibers (Kinoshita et al., 2007). A correlation has been shown between odor detection ability (ODA) and the rate of transport of ^{201}Tl in the olfactory nerve in mice (Shiga et al., 29th AChemS). In this study, we assessed the transport of ^{201}Tl from nasal cavity to olfactory bulb with gamma camera, and then with combined a single photon emission computed tomography (SPECT) and CT scanner (SPECT/CT) systems *in vivo*. The rats (Wistar rat, female, 8w) were exposed with both the right and left olfactory bulbs and administered with steel wire fragments in the left olfactory bulb. Those rats were administered with ^{201}Tl into the right nasal cavity. The transport of ^{201}Tl from nasal cavity to olfactory bulb assessed using gamma camera and plain X-ray was significantly increased eight hours after ^{201}Tl nasal administration. The normal rats were administered with ^{201}Tl into the right nasal cavity, and assessed with SPECT/CT *in vivo*.The transport of ^{201}Tl from nasal cavity to olfactory bulb could be precisely detected with SPECT/CT. Our results warrant ^{201}Tl olfactory nerve scintigram by a simple nasal administration of ^{201}Tl for patients with hyposmia due to head injury, it may be possible to diagnose injuries to the olfactory nerves.

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Poster Session III: Thurs. July 24

Enkephalinergic Signaling in Limbic Forebrain Circuits Mediates Palatable Food Intake

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Opioid signaling promotes palatable food intake, acting at multiple brain regions in the gustatory pathway as well as in forebrain reward circuits. The endogenous signaling mediating this effect is poorly defined. In particular, electrophysiological encoding underlying opioids effects is not well understood, particularly in forebrain regions. We used pharmacological, immunocytochemical, and in vivo electrophysiology approaches in wild-type and preproenkephalin knockout (PPENK) mice to study the effects of enkephalin on palatable food intake. Administration of the nonspecific opioid antagonist naltrexone (NTX) in moderate doses decreased intake of an array of palatable foods in WT mice; in PPENK KO mice, however, NTX administration elicited a slight trend toward increased consumption. This effect was not dependent upon caloric content, as it was observed for saccharin intake. Using cfos immunochemistry, we found that systemic NTX, while increasing cfos expression in the central nucleus of the amygdala (CeA) in WT mice, had no effect on cfos expression in the CeA of PPENK mice. To elucidate opioid effects on neural firing in the amygdala, we recorded from this brain region during intraoral infusion of appetitive (sucrose) or aversive (quinine) tastants in WT and KO mice. Systemic NTX administration modulated baseline and taste-evoked amygdalar firing in a population of neurons, including a subset that responded differentially to sucrose versus quinine. Consistent with a previous report (Hayward et al., 2006), our behavioral results suggest that enkephalin underlies endogenous opioid signaling promoting palatable food intake. Moreover, our results demonstrate that tonic enkephalinergic signaling modulates taste-sensitive neural responses in the amygdala.

Poster Session III: Thurs. July 24

Convergent, not Serial, Striatal and Pallidal Circuits Regulate Opioid-Induced Palatable Food Intake

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Mu opioid receptor (MOR) signaling in the nucleus accumbens (NAcc) elicits marked increases in the consumption of palatable tastants. Multiple downstream target regions have been implicated in mediating this effect but the role of the ventral pallidum (VP), a primary target of NAcc efferents, has not been well defined. Using lick microstructure analysis, we first identified behavioral changes in licking patterns following NAcc MOR stimulation. Secondly, we used a combination of pharmacological inactivation and lesions to define the role of the VP in hyperphagia following infusion of the MOR-specific agonist DAMGO in the NAcc. In agreement with previous studies, results from lick-microstructure studies suggest that NAcc MOR stimulation augments intake through a palatability-driven mechanism. In addition, our results confirm an important role for the VP in normal feeding behavior: pharmacological inactivation of the VP suppressed baseline and DAMGO-induced consumption of a high fat chow. NAcc projections to the VP are primarily ipsilateral (Nauta et al., 1978). To further investigate a role for the VP in NAcc DAMGO-induced hyperphagia, we unilaterally lesioned the VP. We then tested the effects of unilateral infusion of NAcc DAMGO contra- and ipsilateral to the lesion. Surprisingly, contra- and ipsilateral infusion sites potentiated high fat chow consumption equally. Thus, direct projections from the NAcc to the VP appear not to be necessary for NAcc DAMGO to elevate palatable food consumption. Our results suggest NAcc and VP circuits converge on a common downstream target regulating palatable food intake, rather than forming a serial circuit through which DAMGO-mediated hyperphagia is effected.

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Poster Session III: Thurs. July 24

Role of Central Opioids in Benzodiazepine Modulation of Gustatory Behavior

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Benzodiazepine receptor agonists induce hyperphagia through selective increases in the hedonic gustatory evaluation of foodstuffs. Recently, systemic administration of a subthreshold dose of the opioid antagonist naltrexone (NTX) was shown to block the benzodiazepine-induced increases

in ingestive oromotor taste reactivity responses to a quinine-sucrose mixture (Richardson et al., 2005). We sought to determine whether a central brain region was important for this interaction, using a behavioral licking microstructure analysis. Rats were fitted with cannulas aimed to either the lateral ventricle (LV), to provide brain stimulation over a broad region, or to the hindbrain fourth ventricle (4V), to favor maximal dose effects in the brainstem region. Dose-response analyses indicated that NTX doses delivered to the LV that were greater than 10 ug (15, 25, and 50 ug) significantly suppressed 0.3M sucrose intake in a 90-min intake test ($p < 0.05$). Interestingly, intake suppression was not mediated through a reduction of microstructure measures of taste evaluation (mean burst size and initial lick rate). Analysis of responses after 4V injection produce similar results, suggesting that one site of NTX action may be in the brainstem, as there was no shift in the dose response curves across injection sites. Next, we evaluated whether a subthreshold dose of NTX (10 ug/2 ul) suppressed hyperphagia induced by the benzodiazepine agonist, chlordiazepoxide (CDP; 10 mg/kg). Preliminary results suggest that NTX injections to either LV or 4V reduced but did not completely abolish the CDP-induced hyperphagia for 0.1M sucrose. No similar result was observed for 4mM saccharin, although CDP alone more than tripled saccharin intake. CDP alone also enhanced measures of gustatory evaluation (burst size and initial lick rate) for both taste solutions. Additional groups are being tested to confirm these results and to determine whether or not the NTX effects are mediated through influences on measures of gustatory evaluation. Overall, the results obtained thus far suggest that opioid receptor (particularly mu receptor) systems in multiple brain regions may indirectly contribute to benzodiazepine induced hyperphagia, possibly through behavioral processes that are not directly related to gustatory evaluation.

Poster Session III: Thurs. July 24

MU Opiates Infused into the NST Alter Fluid-Induced Licking and Gaping

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Administration of mu opiates into the ventral pallidum and nucleus accumbens increase food intake and differentially enhance or suppress affective oromotor responses to palatable and unpalatable stimuli. A role for opiate modulation of taste reactivity and feeding via effects on the nucleus of the solitary tract (NST) also seems likely. The NST contains mu opiate receptors, and agonist injections into NST modify the firing of taste neurons and promote feeding. However, assessment of how NST mu opiate modulation influences affective oromotor responses has not been explored. We hypothesized that mu opiates infused into NST would increase licking to sucrose and suppress aversive reactions (i.e. gaping) to quinine, parallel to their forebrain influences. To test this, rats ($n = 6$) were implanted with intraoral cannulae, EMG electrodes, and brain cannulae aimed at the gustatory NST. Oromotor responses elicited with intraoral infusions of 0.5 M sucrose, 0.001 M QHCl and water, before and after bilateral NST infusions with 0.9% saline or the mu opiate agonist DAMGO (20 pm/40 nl) were videotaped and EMG activity of the anterior digastric muscle recorded. Preliminary analysis revealed that DAMGO lengthened or maintained licking to sucrose, but decreased licking to quinine and water ($p = 0.02$). Quinine-evoked gaping remained intact. Interestingly, after DAMGO, the neutral stimulus, water, elicited gapes ($p = 0.03$). Finally, DAMGO slowed the rhythm of both licking and gaping ($p < 0.01$). Thus, mu agonists in NST produce only some of the effects seen in the forebrain and are more complex. Some of this complexity likely arises from spread to the adjacent reticular formation, a region known to orchestrate oromotor responses. Further experiments will be necessary to pinpoint the origin of each of these effects.

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Poster Session III: Thurs. July 24

The Stimulation of μ-Opioid Receptors in the Ventral Pallidum Attenuates Learned Taste Aversion in Rats

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When animals experience nausea (unconditioned stimulus, US) after intake of a taste stimulus (conditioned stimulus, CS), they acquire an aversion to the CS (conditioned taste aversion, CTA). It is considered that acquisition of CTA induces the change in taste hedonics of the CS from ingestive to aversive. Previous studies have suggested that μ-opioid receptors in the ventral pallidum (VP) play a role in positive taste hedonics. To elucidate the possible involvement of the opioidergic system in the VP in the hedonic shift after CTA, we examined the effects of microinjections of μ-opioid receptors agonist D-Ala^2-N-Me-Phe^4-Glycol^5-enkephalin (DAMGO) into the VP on taste responses to the CS after acquisition of CTA. Rats received a paired presentation of 5 mM sodium saccharin (CS) with 0.15 M lithium chloride (US). Two days after the conditioning, simultaneous bilateral microinjections of DAMGO (10 or 100 g/0.25 μl) or vehicle (Ringer solution) were made in the rats just before the re-exposure of the CS. We counted orofacial hedonic responses (taste reactivity test) and measured the intake of the CS (single-bottle test). In the taste reactivity test, the microinjections of DAMGO into the VP significantly decreased the occurrence of aversive responses (e.g. chin rubbing) and tended to increase ingestive responses (e.g. lateral tongue protrusion). In the single-bottle test, the DAMGO-injected group showed significantly higher consumption of the CS than the vehicle-injected group. These results suggest that the application of DAMGO into the VP attenuated the aversion to the CS after acquisition of CTA, resulting in the higher intake of the CS. We conclude that the stimulation of μ-opioid receptors in the VP may reduce the expression of aversive behavior after establishment of CTA.

Poster Session III: Thurs. July 24

Taste is Modulated by Exogenous Opiates in Opiate Dependent Patients

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Heroin addicts consume an increased amount of refined sugars. We hypothesized that 1) chronic opiate users have altered perception of sweet taste that is acutely enhanced by opiate administration, and that 2) this altered sweet perception can be reversed by acute opioid receptor antagonism. This study investigated the effect of opiate use and antagonism on sweet taste in chronic opiate users (Users) and detoxified former chronic opiate users (Detox). Sucrose taste recognition thresholds were determined from psychophysical taste functions before and 4 hours after 1) a single dose of methadone (Users, $n = 6$) or 2) naltrexone (Detox, $n = 6$). Control subject data were taken from a cohort of healthy volunteers ($n = 41$). Taste intensity and pleasantness of a supra-threshold sucrose (1 M) solution were measured using labeled magnitude scales. Sweet taste thresholds were significantly increased in Users and in Detox subjects, compared to non-opiate-using control subjects (33 ± 4 mM control; 128 ± 26 mM Users; 169 ± 38 mM Detox, $p < 0.05$). Acute methadone tended to further increase sweet thresholds (220 ± 31 mM). Increased sweet thresholds in Users were associated with increased sweet intensity and pleasantness ($p < 0.05$) compared to controls, whereas sucrose intensity and pleasantness were not significantly different in Detox subjects. Increased sweet thresholds in Detox subjects were reversed by acute opioid antagonism, returning thresholds towards control levels (169 ± 38 mM before, 64 ± 9 mM after naltrexone, $p < 0.001$). These results suggest that opiate use alters sweet perception. This altered sweet perception does

not immediately reverse on detoxification, but can be reversed by opioid antagonism. Changes in sweet taste perception may underlie altered consumption of refined sugars in opiate users.

Poster Session III: Thurs. July 24

Intracellular Signaling Mechanisms of Activation of Postsynaptic δ_1-Opioid Receptors that Mediate Opioid-Induced Reduction of Solitary Tract-Evoked Epscs of the Parabrachial Nuclei Projection Rostral NST Cells

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Our previous work showed that opiates reduced solitary tract (ST)-evoked EPSCs of the gustatory parabrachial nuclei (PbN)-projection rostral NST cells, and their effect was mediated by postsynaptic δ_1-opioid receptors (D_1OR). For instance, SNC80, a selective DOR agonist, reduced ST-evoked EPSCs. The SNC80 effect was eliminated by 7-benzylidenenaltrexone (BNTX), a selective D_1OR antagonist but not by neltriben mesylate, a selective D_2OR antagonist. In the present study, we investigated the intracellular signaling pathway of the activation of DOR using a combination of whole cell recording, western blotting and single-cell RT-PCR techniques. Intracellular administration of 15 μM G-protein antagonist peptide eliminated the reduction of ST-evoked EPSCs induced by SNC80. In addition, intracellular administration of 1 μM U73122, a phospholipase C (PLC) inhibitor, eliminated SNC80-induced reduction of ST-evoked EPSCs but 2 μM U73343, the negative control of U73122, did not. The effect of SNC80 was not abolished by intracellular administration of 15 μM BAPTA, a selective chelator of intracellular Ca^{2+}, and/or 4 μM ryanodine, a potent inhibitor of Ca^{2+} release from intracellular calcium stores. Western immunoblots showed the presence of D_1OR, PLCγ_1, and ryanodine receptor proteins in the rostral NST tissue. We also detected D_1OR, PLCγ_1, and ryanodine receptor mRNA within a single PbN-projection rostral NST cells whose ST-evoked EPSCs were reduced by SNC80, and were eliminated by BNTX. These results indicate that D_1OR are G-protein-PLC coupled receptors, and that G-protein-PLC second messenger system is involved in SNC80-induced reduction of ST-evoked EPSCs.

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Poster Session III: Thurs. July 24

Greater Connectivity between Anteroventral Insula and Hypothalamus during Sensation of Nutritive vs. Harmful Oral Stimuli

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We used functional magnetic resonance imaging to investigate the effective connectivity between regions encoding four oral stimuli in humans. Sixteen healthy right-handed subjects were scanned on a 3T magnet while receiving two potentially nutritive solutions (sweet and salty) and two potentially non-nutritive or harmful solutions (bitter and capsaicin), as well as a tasteless control. The anteroventral insula responded equally to all four stimuli. Responses in this region to potentially nutritive and harmful stimuli were extracted to serve as ''seed'' responses for a psychophysiological interactions analysis, which enables identification of the degree of connectivity between a seed region and other brain areas under one condition (i.e. nutritive stimulation) compared to another condition (i.e. harmful stimulation). We found that processing in the anteroventral insula influences responding in the hypothalamus and bilateral ventral striatum more in response to nutritive than harmful tastes. No areas were influenced more by the insula in response to harmful vs. nutritive tastes. These findings indicate that the anteroventral insula represents gustatory and chemesthetic stimuli and that response in this region differentially influences response in regions involved in encoding food (hypothalamus and ventral striatum) when it is evoked by nutritive compared to harmful oral signals. Specifically, the insula exerts more influence over feeding- and reward-related areas in response to receipt of a nutritive taste compared to a harmful taste.

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Poster Session III: Thurs. July 24

Behavioral Evidence of Benzodiazepine-Induced Alterations of the Gustatory Evaluation of Accepted and Aversive Taste Stimuli

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Enhanced palatability has been cited as a basis for benzodiazepine-induced hyperphagia of appetitive stimuli. Using long-term & brief-access tests, we assessed the effect of systemic benzodiazepines on the consumatory responses to sweet, sour, salty, and bitter taste stimuli. Adult male (n=12) & female (n=12) SD rats received either saline or chlordiazepoxide (CDP, 10 mg/kg) injections 20 min prior to testing. Long-term tests assessed licking to either 0.075 M sucrose, 0.03 M citric acid, 0.5 M NaCl, or 0.05 mM QHCl in daily 90 min sessions. Brief-access tests measured licking to the same stimuli across a range of concentrations during 15 s trials. Significant effects were determined by repeated measures ANOVA ($p<0.05$). Microanalysis of licking responses revealed significant CDP-induced increases in measures associated with gustatory evaluation (initial licks, burst size, lick rate) with no effects on variables associated with postingestive influences (meal duration, number of bursts). Brief-access tests confirmed significant increases in licking responses to all tastants following CDP injection. CDP increased licking responses to low sucrose concentrations suggesting an increase in the positive hedonic perception. CDP appeared to decrease the aversiveness of strong salty, sour, and bitter stimuli as indicated by increases in licking to high concentrations. There was no effect of CDP on licking responses to water in long-term or brief-access tests or differential effects between males and females. Our findings provide evidence that GABAergic influences within the gustatory system do not act to enhance the perceived intensity of taste stimuli but rather modulate the hedonic evaluation of appetitive as well as aversive tastants.

Poster Session III: Thurs. July 24

Hindbrain Protein Synthesis Blockade Disrupts Long-Term but not Short-Term Conditioned Taste Aversion Processing

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Conditioned taste aversion (CTA) is an elementary form of associative learning in which animals avoid a novel taste previously paired with visceral toxicosis. CTA paradigms have proven useful for the study of the integration of taste and visceral information as well as memory formation and retention, however most studies have emphasized the role of forebrain structures in processing long-term CTA memory. Further, adequate protocols for

measuring CTA in the short-term have been lacking until recently. Short-term memory is a protein synthesis-independent phase of memory that precedes long-term memory consolidation. Therefore, we investigated the effects of the protein synthesis inhibitor anisomycin on short- and long-term CTA memory within a novel CTA paradigm that permits short-term CTA measurement through rapid generalization testing. As previously documented, anisomycin administration to the forebrain lateral ventricles (240 ug) blocked long-term CTA memory, as rats that had ingested 0.12 LiCl did not show generalization to 0.12 M NaCl on the subsequent test day. Anisomycin delivery to the hindbrain fourth ventricle produced the same results, however, short-term CTA memory was not affected as anisomycin treated rats did show CTA generalization to 0.12 M NaCl within 10 min of 0.12 M LiCl exposure. The results suggest that CTA long-term memory processing may be hindbrain dependent. They also call into question whether hindbrain parabrachial nucleus lesions impair CTA processing through a deficit in short-term taste-visceral association rather than a deficit in the consolidation of the long-term CTA memory trace.

Poster Session III: Thurs. July 24

Gustatory Cortical Activity During a Taste Discrimination Task in Rats

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Neural activities are modulated not only by the features of stimuli but also by the context in which animals receive the stimuli. It is known that taste stimuli evoke prolonged activity in the gustatory cortex (GC) when animals are allowed to taste freely, or when tastants are passively delivered to anaesthetized animals, while transient responses are observed when the animal is licking. To further address the context-specific modulation of taste response, here we examined neural activity in rat GC during a taste discrimination task in which rats have to discriminate taste type and make a quick behavioral choice. Specifically, we trained rats on a two alternative choice paradigm. A taste solution delivered via intra-oral cannula after the rat entered a center nose poke was associated with one of two locations (right or left nose poke); the rats were rewarded for correct response with water. We recorded single unit activities from GC in well trained animals during the task. Many neurons showed event-related activity during delay periods proceeding of taste/reward presentation and during the periods after taste/reward delivery. Firing rates were often suppressed during movement. Some neurons respond to both taste and reward in similar way, which may be reflecting somatosensory input on the tongue, while others produced taste specific responses. Notably, we observed quick and transient activities with a peak of around 200 ms in many taste/reward responsive neurons, suggesting that GC activity can be adapted to the task that animals are engaged.

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Poster Session III: Thurs. July 24

Dynamic and Multimodal Responses of Amygdalar Neurons in Awake Rats

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Recent work suggests that the amygdala and gustatory cortex (GC) work together to code tastes, and that amygdala may 'feed' GC information during taste processing. Here, we present an analysis of amygdalar temporal coding of taste stimuli, and provide strong evidence supporting both of these hypotheses. Exemplars of each of the 4 basic tastes were delivered (7-11 trials per taste) to awake rats through an intra-oral cannula, while the responses of small (2-9) ensembles of single basolateral amygdala (BLA) neurons were recorded. The information available at different time-points following taste administration changed across post-stimulus time, demonstrating that the population of BLA neurons progressed through three epochs of responsiveness. The timing of these epochs was remarkably similar to that previously observed in independent analysis of GC responses, however the inter-regional differences were striking: whereas palatability-specific information appeared only in the 3rd epoch of GC activity (~1 sec following stimulus delivery), in BLA it appeared in the 2nd epoch (~0.25 sec following stimulus delivery). Single BLA neurons typically responded similarly to all 4 tastes for the first 200-250 msec, and then similarly to the pairs of tastes with similar palatabilities (sweet/salty and sour/bitter) for ~750 msec; by the 3rd epoch, responses to only one taste remained. These data strongly suggest that BLA and GC process tastes as a coupled dynamical unit, and that palatability-related information flows from BLA to GC during this process.

Poster Session III: Thurs. July 24

The Effects of Dietary Manipulation on Taste and Preference in C57BL.6J Mice

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Studies with humans illustrate variation in taste ability between obese and lean subjects, although the relationship between obesity and taste is not well understood. We investigated the effects of diet-induced obesity on taste in C57BL/6J (B6) mice raised with a number of different dietary conditions. In experiment one, we measured taste preference (two-bottle tests) in B6 mice given a very high-fat diet (VHFD), normal lab chow (controls), normal chow with ad lib sucrose (0.1 M), or chow with ad-lib ethanol (6%). VHFD mice displayed no preference to 1-10% ethanol relative to controls, which prefer ethanol, while mice raised on ad lib sucrose or ethanol displayed higher preference to ethanol relative to controls. VHFD mice also had attenuated levels of preference and consumption of 0.01 M saccharin or 0.1 M sucrose relative to the other groups. In experiment two, VHFD and control mice were preference tested with concentration ranges of QHCl, saccharin and sucrose. VHFD mice displayed a significantly lower preference and consumption for all concentrations of saccharin and sucrose, but not quinine. In experiment three, VHFD mice displayed lower intake of single concentrations of all of these stimuli, but not water, in 30 min brief-access trials. In short-trial tests, VHFD mice generally possessed lower levels of responsiveness to concentration series of sucrose, saccharin and ethanol, but not NaCl, although the magnitude of these differences was not as great as found in the longer test or two-bottle tests, suggesting a role for post-ingestive effects. In conclusion, B6 mice with diet-induced obesity display a significantly lower preference and licking response for ethanol and sweet stimuli. These findings illustrate substantial effects of dietary manipulation on taste genotype.

Poster Session III: Thurs. July 24

Role of Dietary Zinc Signal in Food Intake Regulation of the Rats

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Zinc is an essential trace element, and regulates a wide variety of physiological functions as an active center of zinc enzymes and so on. It has been reported that zinc deficiency induced anorexia, while zinc supplementation improved anorexia nervosa. The aim of this study is to reveal the role of dietary zinc in food intake regulation, focusing on hypothalamic neuropeptides regulation in food intake of the rats. We have found for the first time that food intake was suppressed in the rats fed zinc deficient diet for 3 days with changing in mRNA expression of hypothalamic neuropeptides. Male SD rats, 4-week old, were used for the food intake regulation analyses. After 3 days' feeding of the zinc deficient diet, zinc solution (3 mg/kg body weight of $ZnSO_4$ saline solution) was adiminstered either orally or intraperitoneally, and measured the food intake and mRNA expression of hypothalamic neuropeptides simultaneously. As a result, we found out that oral zinc administration but not intraperitoneal was clearly effective to increase food intake. Hypothalamic mRNA expression of orexigenic peptides (orexin and so on) was increased, and this effect was disappeared by gastrointestinal vagus nerve disconnection (vagotomy) or by orexin antagonist treatment. These data suggest that orexigenic signal by zinc from the periphery (gastrointestine) to the brain is mediated by the vagus nerve transduction.

Poster Session III: Thurs. July 24

Functional Characterization of Molecular and Cellular Mechanisms Underlying Trigeminal Perception of Creamy Tastants

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The sense of taste is a critical component in the neuronal network that controls dietary preferences. The five basic taste qualities are sweet, bitter, salty, sour and umami. Their individual signal transduction mechanisms have been intensively studied in recent years, providing a substantial insight into the neuronal coding. However, it is still debated whether ''creamy'' also represents an individual quality mediated by the taste system. The perception of creamyness is induced by dietary fats typically enriched e.g. in heavy cream or butter. It has been described that so called supertasters, in contrast to ''normal'' tasters, are able to discriminate different fat contents of food. In concert with that notion, supertasters show increased sensitivity for typical trigeminal stimuli such as capsaicin, temperature and pain. Polyunsaturated fatty acids have been shown to elicit responses in rodent taste receptor cells. However, no mechanism has been proposed for the detection of saturated fatty acids or fats which are abundant in our daily diet. We hypothesize an involvement of lingual trigeminal fibres in the perception of creamyness. We developed a method to form stable fat-in-water emulsions with different saturated dietary fats and fatty acids isolated from milk products. In Ca^{2+}-imaging experiments, dissociated trigeminal neurons of mice selectively respond to application of these emulsions. Our current investigations focus on the molecular mechanisms underlying the perception of creamy stimuli in the trigeminal system of mice. Molecular and biochemical approaches combined with path clamp and Ca^{2+}-imaging recordings will shed light on the relevant signal transduction proteins. The results gathered in this study might be of importance for the food industry and the development of new dietary concepts.

Poster Session III: Thurs. July 24

Psychophysical Effects to Pulses of Orally Presented Zingerone and Piperine: Analysis with a Dynamic Model

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Oral irritants (piperine and zingerone) produce distinct temporal patterns of burning sensation over the time interval that they are presented. Some irritants like piperine produce a tonic (slow rise and then plateau) burn and others like zingerone produce a phasic (faster rise, peak, and then adapting) burn. We implemented a dynamic, mathematical model (McBurney and Balaban, 1994) based upon these two burn profiles. This model predicted how the burn would be affected by two 10-minute pulses of the same irritant separated by a 10-minute rest interval. College undergraduates received either pulsed piperine or pulsed zingerone and they rated the burning sensation every three minutes with magnitude estimates. The rise of burning sensation during each pulse and fall of burning sensation after each pulse were consistent with the model. The peak burning sensation to the second pulse differed unexpectantly though from that of the first pulse. Zingerone produced a sensitization effect as the peak burning sensation to the second pulse was significantly higher than that to the first pulse. On the other hand, piperine produced adaptation or desensitization as the peak burning sensation to the second pulse was significantly less than that to the first pulse. Implications of these results to the practice of dynamic modeling and physiological explanations are discussed.

Poster Session III: Thurs. July 24

Adult Male Rats Avoid Nicotine in a Two-Bottle Free-Access Paradigm

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Many addiction studies in rodents use a limited access paradigm. These types of studies prevent the animals from becoming addicted to the substance on their own schedules. We therefore wanted to develop a two-bottle free-access paradigm for studying the acquisition of nicotine addiction in rats. Adult, male Sprague Dawley rats were given one water bottle containing cherry Kool-Aid® (KA) and another with grape KA. Nicotine was added to the cherry KA in half the animals and to the grape in the other half. Nicotine concentrations of 0.0001-0.01% were tested masked in KA ranging in concentration from 0.01 to 10%. Nicotine preference decreased in a dose dependent manner but increased with increases in KA concentration. The only solution the rats drank in quantities comparable to the average daily human intake was 0.01% nicotine in 0.01% KA (1.13 ± 0.10 mg/kg/day). One nicotine solution was preferred by the rats over the KA solution (0.0001% nicotine in 0.1% KA, $p < 0.001$); however the average daily nicotine intake was only 0.07 ± 0.00 mg/kg/day. The rats were able to associate the nicotine with the flavor of KA to such an extent that they avoided that flavor even when the nicotine was removed ($p < 0.001$ for cherry KA; $p < 0.038$ for grape). These data suggest that rats are sensitive enough to some sensory aspect of nicotine to detect and avoid it at very low concentrations. This learned aversion makes it very unlikely that a two-bottle free-access paradigm will be useful in studying the acquisition of nicotine addiction in adult male rats.

Poster Session III: Thurs. July 24

Effects of Trigeminal Stimulation on Appetite

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Objective: To investigate effects of trigeminal stimulation on hunger and satiety.

Method: Thirty two young subjects (15 males) each participated in two experimental sessions. Three hours prior to arriving at the lab, Ss abstained from eating. Half of the Ss were served 10 portions of 50 ml of tomato soup every 5 min. After each serving Ss indicated their hunger, satiety, liking (of the soup) and wanting (of another 50 ml portion) on VAS scales. The other half of the Ss performed the same tasks, but were served 10 portions of 50 ml of tomato soup spiced with chili. The measurements were repeated one week after the first session, but this time Ss were served the soup variety they did not eat in the first session.

Results: In the course of a session satiety increases and hunger decreases. For the chili spiced soup, hunger and satiety reach ''equilibrium'' after an intake of about 230 ml corresponding to 20-25 min. For ordinary tomato soup, Ss need to eat about 350 ml to experience ''equilibrium'' between hunger and satiety. Up to the ''equilibrium point'' for the spiced soup, satiety scores for the two soups do not differ, but thereafter Ss feel significantly more satiated by the hot soup. For both types of soup, liking and wanting scores decrease with intake and for the hot soup these quantities diverge from the point when hunger and satiety are in equilibrium. A similar divergence between liking and wanting for the ordinary soup sets in around the time when hunger and satiety are in equilibrium for this stimulus. Liking scores are significantly higher for the hot than for the ordinary soup.

Conclusions: Trigeminal stimulation can affect appetite. A lower intake does not have to come at the expense of sensory pleasure, rather, in this case, Ss become more satiated when eating the most pleasurable soup.

Poster Session III: Thurs. July 24

Putative Chemosensory Cells at Strategic Positions in the GI-Tract

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In the gastrointestinal (GI) tract, a variety of digestive processes are continually adapted to the changing composition of ingested foods, which requires a precise chemosensory monitoring of luminal contents. Gustducin-expressing brush cells scattered throughout the GI mucosa are considered candidate sensory cells for accomplishing this task. An especially large cluster of gustducin-positive cells is located exactly at the boundary between the fundic and the oxyntic mucosa of the mouse stomach, at the so-called ''limiting ridge''. In close association with this candidate chemosensory cluster, two populations of enteroendocrine cells were identified: one population containing the satiety regulating hormone ghrelin, the other population comprising serotonin-secreting enterochromaffin cells. The particular arrangement of gustducin-expressing cells and enteroendocrine cells at the limiting ridge suggests a direct interplay between these cell types with immediate implications, not only for digestive processes in the stomach, but also for parameters controlling the satiety status.

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Poster Session III: Thurs. July 24

Sensory Studies on Olive Oil and Ibuprofen's Pharyngeal Pungency

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An extra-virgin olive oil tasting is characterized by a striking pungency predominantly localized in the throat. This pharyngeal sting, elicited by the compound (-)-oleocanthal (OC), is reminiscent of the sting induced by the non-steroidal anti-inflammatory drug (NSAID), ibuprofen (IBU). Because such distinct rostro-caudal sensorial differentiation has not been reported for other oral irritants, we decided to characterize the irritating properties of the two compounds through psychophysical and trigeminal neuron calcium imaging studies, conducted in parallel. First, subjects were asked to rate the irritation triggered by olive oil or a horseradish solution from both their anterior tongue and their throat. At matched pharyngeal irritation intensities, olive oil elicited very little pungency on the tongue while horseradish irritation was very strong in this region. Thus, the principle compounds responsible for olive oil (OC) and horseradish (allylisothiocyanates) pungencies trigger very different irritation sensation profiles, although both excite cultured rat trigeminal neurons in our lab. To verify that the anterior-posterior difference in irritation is not due to an inability of OC and IBU to stimulate the trigeminal nerve, we asked subjects to evaluate nasal irritation when they were separately sprayed into the nare. Both chemicals triggered concentration-dependent nasal irritation. Thus, while OC and IBU are primarily sensed in the throat, which has mixed trigeminal, glossopharyngeal, and vagal innervation, they clearly excite the trigeminal nerve in humans, with very similar sensitivity to cultured trigeminal neurons. These studies suggest a higher expression of OC and IBU sensory receptor(s) in nasal and posterior oral cavities than in anterior oral cavity.

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Taste-Guided Isolation of a Trp Active Compound From the Korean Food Plant Kaennip (*Perilla Frutescens)*

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Several spices and edible plants used in traditional cooking contain interesting bioactive compounds. Among these, we are particularly interested in chemestetic compounds, both for their use in gastronomy and for their medical and pharmacological applications. *Perilla frutescens* Britton (Labiatae) is a native plant of eastern Asia, where it is popular as culinary and medicinal herb (1). The green leaves, named *kaennip* in Korea, are characterized by a strong flavour and a pleasant taste and are used in these countries as ingredient in many dishes. We studied this plant with the aim to isolate natural compounds responsible for its characteristic taste and flavour. The more abundant compound of perilla leaves, obtained by steam distillation or extraction with solvent of freeze dried sample, is perillaketone (1-(3-furanyl)-4-methyl-1-pentanone). We discovered that this molecule is a potent activator of TRPA1 in *in vitro* assays on human cloned receptors. These data are very interesting and they can pave the way to a series of potential perspectives. TRPA1, in fact, is one of the member of the TRP (transient receptor potential) family, ion channels activated by several stimuli (low temperature, pungent natural compounds, environmental irritant) and

involved in pain perception (2). Therefore perillaketone can represent the lead compound for a new class of interesting bioactive compounds, both natural and synthetic.

Acknowledgements: we thank the Italian Ministry for Foreigner Affairs (MAE) for the financial support in the Executive project of exchange Italy-Korea 2007-09. (1) ''Handbook of herbs and spices'' vol. 3, Edited by K. V. Peter, CRC Press, Boca Raton Boston New York Washington, DC 2006.(2) D.E. Clapham, TRP channels as cellular sensors. *Nature*, **2003**, *426*, 517-524.

Poster Session III: Thurs. July 24

An Exquisitely Sensitive PH Biosensor in a Marine Catfish

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The Indo-Pacific catfish, *Plotosus lineatus,* is exceptionally sensitive to minute lowering of the pH in its ambient environment. A transient drop of ~0.05-0.1 pH unit in the seawater (SW) (pH 8.2) bathing the maxillary barbel dramatically activates the innervating facial/trigeminal nerve complex to evoke a phasic burst of action potentials. Increases in pH have no effect; however, bathing the barbel with SW ≤ pH 7.5 or ≥ pH 9 can inactivate the sensor. Upon replacing the barbel flow with pH 8.2 SW, the sensor quickly recovers its sensitivity. The sensitivity of this system to increasing acidity is unparalleled by any known pH biosensor and rivals that of a commercial pH meter. Whether the afferent neural pathway is trigeminal or facial is currently unknown. The facial nerve contains taste fibers that are unresponsive to declining pH, but evoke spikes of relatively small magnitude in response to amino acids. The fibers responsible for the pH effect are insensitive to amino acids and evoke spikes of large magnitude. The molecular nature of the sensor is currently unknown; however, a novel type of acid-sensing (ASIC) channel is hypothesized as distilled water dramatically activates the sensor (i.e. ASIC channels are activated by simply reducing extracellular [Ca^{2+}]). The biological significance of this exquisite sensitivity to slight drops in ambient pH is likely an adaptation for detecting polychaete tube worms, natural prey items. As the polychaete respires, transient drops in SW pH occur at the opening of the worm tube. These pH alterations along with amino acids leaching from the worm enable efficient detection of the prey by the sea catfish.

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Poster Session III: Thurs. July 24

The Role of Solitary Chemosensory Cells in Irritant-Evoked Inflammation of the Nasal Respiratory Epithelium

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The respiratory epithelium of the anterior nasal cavity is richly invested with capsaicin-sensitive fibers of the trigeminal nerve implicated in detection of chemical irritants. Nerve fibers containing substance P and CGRP are abundant in and beneath the epithelium and scattered in the submucosal layers around blood vessels. These fibers possess a variety of ion channels which underlie direct sensitivity to some lipophilic compounds, e.g. capsaicin. Trigeminal chemosensitivity is not, however, limited to receptors on the nerve fibers. Trigeminal fibers also innervate solitary chemosensory cells (also called solitary chemoreceptor cells; SCCs) which are scattered within the nasal epithelium and which are broadly responsive to high concentrations of many substances including most odorants (Lin et al., 2008) and ''bitter''-tasting compounds (Finger et al., 2005). In the present study, we tested whether stimulation of the nasal cavity with a bitter substance, denatonium, results in local neurogenic inflammation similar to that seen after administration of capsaicin. This process entails release of peptides from nerve fibers and opening of endothelial junctions resulting in plasma extravasation. Plasma protein leakage and blood vessel permeability were measured using Evans Blue (EB) and Monastral® Blue. When denatonium (25mM) was applied unilaterally to the anterior nasal cavity, Monastral® Blue-labeling of capillary walls and significant EB plasma leakage was detected predominantly on the injected side. These results suggest that activation of SCCs can evoke neurogenic inflammation of the respiratory mucosa.

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Poster Session III: Thurs. July 24

The Respiratory Response of TRPV1 Knockout Mice to Trigeminal Irritants

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The trigeminal nerve is composed of multisensory neurons which innervate the nasal cavity, the nasopharynx, the oral cavity and the cornea. Although, trigeminal nociceptive fibers are stimulated by a wide variety of chemical irritants, the mechanism of stimulation is known for only of few compounds. TRPV1 channels, for example, are activated by capsaicin. Classic studies have established that exposure to upper respiratory tract irritants result in a systematic alteration in the normal mammalian exhalation pattern which results in a decrease in respiration rate. This patterned respiration rate depression has been used as an indicator of sensory irritation, the ''Alaire Test.'' In the present study, an air dilution olfactometer was used to administer volatile compounds to unanesthetized mice which were restrained in a whole body plethysmograph. Respiration rate depression for female wild type (C57Bl/6J) mice was compared to female TRPV1 knockout mice for a variety of compounds in an attempt to determine if TRPV1 is responsible for the detection of the irritants. TRPV1 knockout mice did not appear to show respiratory rate depression when exposed to cyclohexanone, a known TRPV1 agonist. Knockout mice exposed to eugenol did not show respiratory rate depression to the same degree as the wild type mice. Nicotine seemed to cause similar amounts of respiratory rate depression in wild type and knockout mice. It appears that cyclohexanone is primarily detected by TRPV1, while the detection of eugenol is only partially mediated by TRPV1 and TRPV1 is not involved in the detection of nicotine. It is likely that TRPA1, which has been shown to respond to eugenol *in vitro* and is found on the trigeminal nerve, is responsible for some of the eugenol induced respiration rate depression seen in the knockout mice.

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TRPM5-Expressing Solitary Chemosensory Cells in Mouse Vomeronasal Organ

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Previously, two independent studies have shown that α-gustducin-immunolabeled cells are present in the mouse vomeronasal organ (VNO) (Zancanaro et al., Eur J Neurosci. 11:4473-5, 1999), and the substance P-immunoreactive trigeminal fibers innervate non-sensory epithelium of the VNO (Nagahara

et al. Anat Embryol (Berl). 192:107-15, 1995). However, a role of the α-gustducin-expressing cells in trigeminal sensation had not been determined in the VNO. We have recently identified solitary chemosensory cells in physiological studies using transgenic mice (Lin et al., J Neurophysiol. 99:1451-60, 2008) in which the promoter of the transient receptor potential channel M5 (TRPM5) drives expression of green fluorescent protein (GFP). Here, using the same line of transgenic mice, we found that α-gustducin-expressing cells also expressed GFP, suggesting co-expression of the TRPM5 and α-gustducin in the VNO. Interestingly, most of the TRPM5-positive cells in the VNO were found in the entry duct. These cells were closely apposed by nerve fibers which were positively immunoreactive to substance P, suggesting that the cells are innervated by trigeminal fibers. In physiological experiments using the Ca^{2+} imaging technique, TRPM5-expressing cells responded to stimuli known to activate the trigeminal system. Our results indicate that solitary chemosensory cells in the VNO detect trigeminal stimuli and transmit the signals to the innervated intraepithelial trigeminal nerve fibers.

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Hydroxy-α-Sanshool Activation of Lumbar Spinal Wide-Dynamic Range Neurons

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Hydroxy-α-sanshool (HαS) is an extract of Japanese pepper that elicits tingling and paraesthetic sensations. To study neural mechanisms potentially underlying these sensations, we investigated if N-isobutylalkenylamide (IBA), an HαS derivative, excites wide-dynamic range (WDR) neurons in the lumbar spinal cord since such neurons participate in transmission of nociceptive and possibly paraesthetic information from the skin. Responses of superficial and deep dorsal horn neurons to hind paw stimulation were recorded in rats anesthetized with pentobarbital. Neurons were classified as WDR based on differential responses to graded mechanical stimuli and response to noxious heat and/or irritant chemicals. WDR neurons were then tested with intradermal injection of 10% IBA (in propylene glycol, 1 μl), followed by a second injection of IBA 20 min later. Twenty min later they were tested with topical mustard oil (70%) followed by intradermal capsaicin (3.3 mM). 21 of 23 WDR neurons responded robustly to the initial IBA injection over a prolonged (>10 min) time course consistent with tingling sensation. Responses to the second IBA injection were significantly lower ($p < 0.01$), consistent with desensitization of tingle sensation in humans. Responses to vehicle (propylene glycol), when present, were weaker compared to IBA and returned to baseline within 2 min. 11/12 units responded to capsaicin and 14/17 to mustard oil applied topically to the receptive field. Our results are consistent with a recent report that IBA activates thermoTRP channels TRPV1 and TRPA1 in sensory neurons, and support the possibility that the tingle and paraesthetic sensations of IBA are conveyed partly by WDR neurons projecting in ascending somatosensory pathways.

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Spatiotemporal Dynamics of Odor Representation in the Trigeminal Ganglion *in vivo* Visualized by Voltage Sensitive Dye Imaging

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Chemosensation from the mammalian nasal cavity is predominantly mediated by two independent neural systems, the olfactory and the somatosensory (trigeminal) system. Optical imaging techniques have thus far provided significant knowledge regarding the functional organization of information processing at the level of the olfactory bulb. In contrast, due to the difficulty in accessing trigeminal ganglia somata and nerve fibers experimentally, a direct visualization of evoked activity in the trigeminal ganglion *in vivo* has been almost impossible. This leaves many questions about the trigeminal representation of odor related stimuli very much unexplored. In order to investigate the population coding of olfactory signals within the trigeminal ganglion, we established a preparation that allows the high-resolution recording of optical signals arising from a large region of the rat trigeminal ganglion *in vivo*, using voltage sensitive dye imaging. Stimuli were individually delivered by a specialized custom-made olfactometer. Tested substances include CO_2 as a pure pain activator, as well as odorants believed to have a strong trigeminal component and classical olfactory stimuli. Our data indicate a prototypical activation pattern related to a painful stimulus. Stimulation with Ethanol, an odorant with a strong trigeminal component produced an activation that showed high similarity to this "pain"-pattern. Moreover the Ethanol map included unique activation spots that might code for odor identity. In contrast classical olfactory stimuli elicited activation patterns clearly distinct from such "pain"-pattern.

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Molecular Signature of and Trigeminal Neural Pathway from Solitary Chemoreceptor Cells

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Solitary chemoreceptor cells (SCCs) in non-neural epithelia of nasal cavity and vomeronasal organ are involved in trigeminal chemosensation. SCCs express several intracellular signaling molecules present in bitter taste receptor cells such as T2R, G protein α-gustducin, and phospholipase C-β2. Here we report that SCCs express T1R3, a component of sweet/umami taste receptors, and TRPM5, an essential channel in taste signaling cascade. Also, both attractive (T1R3) and aversive (T2Rs) receptors were co-expressed in SCCs. This is quite different situation observed in taste receptor cells. Transgenic mice expressing a trans-synaptic tracer, wheat germ agglutinin (WGA), in SCCs under the control of mouse T1R3 gene promoter/enhancer revealed the WGA protein transport to a subset of neurons in the trigeminal ganglion. Furthermore, in the brainstem WGA immunoreactivity was detected in several nuclei such as the spinal and principal trigeminal nuclei,

intermediate region of nucleus of solitary tract, parvocellular reticular formation, and trigeminal motor nucleus, which are known as central targets of trigeminal neurons. The afferent neural pathway conveying the chemosensory information from SCCs is clearly visualized as part of trigeminal sensory pathways.

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Quantifying Mechanical Stimuli in Rat and Human Nose Models During Breathing

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Direct in vivo measurement of the nasal cellular or neural responses to mechanical stimuli as the result of airflow during active breathing remains a challenge. In vitro approaches offer easier access to the epithelium, but the question remains whether the experimental setup adequately recreated a realistic physiological range of the stimulus. Using computational fluid dynamics, we attempted to calculate the mechanical stimuli at the nasal wall in human and rat under various breathing conditions and compare to the simulated results of an experimental setup similar to Grosmaitre et al., 2007. In humans, the static pressure (**P**) and shear stress (**S**) (unit: Pa) at the nasal wall is calculated to be in the range of **P** -4.4– 15.0 (**S** 0 –1.9) during restful inhalation (230 ml/s) and **P** -94.2 –300 (**S** 0 – 15.6) during sniffing (1310 ml/s). Peak values of **S** occur at walls near the nasal valve. In the olfactory region, the values drop to **P** 23.5–143 (**S** 0.02-4.86) during sniffing. In rats, the range becomes **P** -0.3-39 (**S** 0.01-1.1) and **P** 0-554 (**S** 0.005-12) during restful breathing (2.55ml/s) and strong sniffing (10ml/s) respectively. In the septal organ and olfactory region, the values reduce to **P** 60-100 (**S** 0.7-1) and **P** 80-200 (**S** 0.1-0.9) respectively during sniff. A micropipette was also simulated with a 4um opening, placed in a 2 mm water layer and 4 - 1000 um over the tissue sample at 45 to 90 angles. A pressure of 20 psi was applied to eject water through the pipette and the P and S exerted on the tissue were calculated as a function of distance away from the tip. The peak values were shown to be over 1 order of magnitude higher than those exerted by natural breathing and sniffing. In conclusion, optimal placement of pipette is necessary to recreate the physiological mechano-stimuli range in an in vitro situation.

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Odor Thresholds and Respiratory Effects of Sulphur Dioxide

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Sulphur dioxide (SO_2) is an environmental and occupational pollutant causing irritation of the upper airways and the eyes. Despite these known health hazards, little is known about the concentrations causing either olfactory perceptions or sensory irritation. The aim of the study was to determine (a) the odor threshold of SO_2 and (b) identifying a concentration affecting the depth of breathing in human volunteers. A flow olfactometer was used to determine the individual odor thresholds of SO_2 and to deliver nine concentrations (0.06 to 12 ppm) to measure their effects on breathing depth. 39 subjects, stratified by age and gender were investigated. Written informed consent was obtained prior to the experiments. The local ethics committee approved the study protocol. The depth of breathing was determined by means of respiratory inductive plethysmography (breathing belt) during 5 inhalations of SO_2 at each concentration. The median odor thresholds was 1 ppm (IQR=1.3 ppm), neither significantly influenced by age nor gender. The results of the analysis of breathing depth showed that there is a quadratic dose-effect relationship between concentration and breathing depth ($F = 7.9$, $p < .01$). At low concentrations (up to 0.5 ppm) the breathing depth of the participants was reduced, highest around 1 to 2 ppm, and once again decreased at higher concentrations. This relationship can be described by an inverted u-function. Combining the odor threshold and the breathing depth results - 50% of the participants had an odor threshold above 1 ppm – we restricted the analysis on the four highest concentrations and found almost linear decrease of breathing depth to 90% of the control value. In conclusion, this weak effect on breathing depth might be a first hint for sensory irritations at SO_2 concentrations above 2 ppm.

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Genomic, Expression, and Functional Analyses of Olfactory Receptors in the Silkworm *Bombyx Mori*

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The chemical senses such as olfaction and gustation play an important role in sexual and feeding behavior in insects. Odorant perception by insects is primarily mediated by olfactory receptors (ORs) that are expressed on the dendrites of olfactory neurons housed within chemosensilla. Taste perception is mediated by gustatory receptors (GRs) in gustatory neurons. Genome projects have revealed genes for ORs and GRs in various insect species including *Drosophila melanogaster* (62 ORs/68 GRs), *Anopheles gambiae* (79 ORs/72 GRs) and *Apis mellifera* (170 ORs/13 GRs). We herein report identification of OR and GR genes from genome of the silkmoth, *Bombyx mori*. RT-PCR experiments revealed that many of the *Bombyx mori* OR (BmOR) genes were expressed in the antenna of adult moths and in the antenna and maxilla of larvae. We performed functional characterization of ORs that were expressed in larvae using a *Xenopus* oocyte expression system. Various odorants were applied to oocytes expressing each BmOR and the *Bombyx mori* ortholog of the Or83b family. We found several ORs that showed responses to behaviorally-relevant odorants in a combinational fashion. Our results provide insight into molecular mechanisms about how the silkworm uses olfaction to search foods.

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Deconstructing Insect Odorant Receptors

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In insects, each olfactory sensory neuron (OSN) expresses between two and three members of the olfactory receptor (OR) gene family, which are part of a novel class of ligand-activated nonselective cation channels. The functional OR consists of a heteromeric complex, comprising at least one variable odorant-binding subunit and one constant subunit, part of the highly conserved OR83b family. DEET, the most widely used topical insect repellent, acts by inhibiting a subsets of heteromeric insect ORs that require the OR83b co-receptor, masking the host odor. In order to probe the function of this novel class of proteins and investigate the mode of action of DEET, we carried out alanine-scanning mutagenesis on the OR83b co-receptor on residues conserved in five insect species across 450 million years. OR83b mutants were expressed in OSNs of the fruitfly *Drosophila melanogaster* and tested for function using single sensillum recordings. To further describe alterations in the OR, the mutants were expressed in heterologous cells and their channel properties analyzed. Understanding how the insect OR heteromer functions will have an impact on the control of insect-borne diseases and the design of better insect repellents.

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A Drosophila Odorant Receptor Distinguishes Ligand Enantiomers with High Selectivity

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We have functionally expressed and characterized a *Drosophila* odorant receptor, 85a (DmOr85a), using the *Xenopus* oocyte heterologous expression system and two electrode voltage clamp electrophysiology. Functional responses to known agonists are observed only when DmOr85a is expressed with the general co-receptor, DmOr83b, but additional exogenous proteins are not required. The DmOr85a/83b complex expressed in *Xenopus* oocytes exhibits a ligand response profile similar to what has been reported in *in vivo* studies; responding to ethyl 3-hydroxybutyrate, hexanol, and ethyl butyrate, but not methyl benzoate or benzaldehyde. Furthermore, the rank order of potency is comparable between assay systems. These results support the utility and accuracy of the *Xenopus* oocyte system. Ethyl 3-hydroxybutyrate activates DmOr85a/83b with an apparent EC_{50} of 104 ± 18 μM. This compound contains a chiral center, so we asked whether DmOr85a/83b is able to distinguish between the enantiomeric structures. Oocytes expressing the DmOr85a/83b complex responded to (S)-ethyl 3-hydroxybutyrate with slightly greater sensitivity (EC_{50} = 58 ± 10 μM) than to the racemic mixture. Surprisingly, DmOr85a/83b was nearly unresponsive to (R)-ethyl 3-hydroxybutyrate. At an extremely high concentration (10 mM), the (R) enantiomer was able to activate DmOr85a/83b at approximately 5% the maximal response elicited by the (S) enantiomer. Thus, DmOr85a/83b displays at least 3000-fold selectivity for (S)-ethyl 3-hydroxybutyrate over (R)-ethyl 3-hydroxybutyrate. This result supports the role of individual odorant receptors in making fine distinctions among closely related ligands.

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Hidden Markov Models and Sequence-Structure Correlates to Identify Active Sites in Olfactory Receptors

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GPCRs are signaling proteins that traverse the cell membrane and are responsible for intracellular signaling events. Olfactory receptors (OR), which constitute the largest super-family of olfactory receptors, are GPCRs. They are responsible for interactions with odor molecules following which ORs are activated (the activation is possibly structural) and result in motivating a signal transduction cascade, eventually leading to olfaction. Computer modeling of ORs and GPCRs provide a glimpse into their structures at a molecular level and have to potential to help identify facets and features and active sites that might be responsible for odor-binding. Several programs describe the use of Hidden Markov Models (HMM) to identify transmembrane (TM) domains in GPCRs. In creating computer models of olfactory receptors, we have used HMMs to identify the TMs and also to ascertain the location of intra- and extra-cellular loops. The use of HMMs has allowed us to correlate the pseudogenecity (from genome studies) of mammalian olfactory receptors with the disruption of their transmembrane structure. Through mammalian olfactory repertoire-wide surveys of both functional receptors and pseudogenes, we will statistically identify sites that are possibly responsible for olfactory receptor function.

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Relationship Between Receptor Code and Odor Quality in Twelve Odorants

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It is generally accepted that odor quality is encoded by a combination of activated olfactory receptors (ORs). However, it is little known whether or not odorants activating a similar subset of ORs present a similar odor quality. Therefore, we compared the similarities of twelve odorants both in the receptor code and in the perceived odor quality. Responsiveness to the test odorants was examined in isolated olfactory sensory neurons (OSNs) of mice by calcium imaging assay. Out of 1143 OSNs examined, 110 responded to one or more test odorants, and were classified into 40 different response profiles. Similarities of the receptor code were estimated by the overlap of OSN responses in all possible pairs of odorants and analyzed by multidimensional scaling (MDS) and hierarchical cluster analysis. Meanwhile, similarities of perceptual odor quality among twelve odorants were evaluated by human sensory test. The data of the perceptual similarity was also applied to MDS and hierarchical cluster analysis to examine which odorants show a similar odor quality. In both statistical analyses, the classification of odor quality was consistent with that of the receptor code. These results confirmed that a combination of activated ORs encodes odor quality, and further demonstrated that odorants sharing more OSN responses are more similar in odor quality.

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A Human Olfactory Receptor for Waxy, Fatty and Rose Odors

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Perception of thousands of odors by a few hundreds of olfactory receptors (ORs) results from a combinatorial coding, in which one OR recognizes multiple odorants and different odorants are recognized by different combinations of ORs. Moreover, some odorants may act both as agonist or antagonist depending on the OR. This dual agonist/antagonist combinatorial coding is in agreement with behavioral and psychophysical observations of mixture perception. To date, no relationship has been demonstrated between odorant structure, their activity on OR and their odor quality. In the present study we asked if odorant ligands of a human OR that share common structural features could also share common odor quality descriptors. We previously described the odorant repertoire of a human OR named OR1G1, identifying both agonists and antagonists (Sanz et al., 2005). We used these activity data to perform a 3D-Quantitative Structure Activity Relationship (3D-QSAR) study of these ligands using Catalyst/HypoGen software (Accelrys Inc.). We obtained a double-alignment model explaining previously reported experimental activities and permitting both to predict the antagonist effect of some compounds and to identify new potent agonists. These predictions were experimentally validated by functional characterization of the OR1G1 heterologously expressed in HEK293 cells using calcium imaging upon odorant stimulation. Thereafter, we evaluated the statistical link between OR1G1 response to odorants, 3D-QSAR categorization of OR1G1 ligands and their olfactory description. We showed

that OR1G1 recognizes distinct groups of odorants, one of which shares both 3D structural and odorous characteristics. We especially underlined the involvement of OR1G1 in the coding of fatty, waxy and rose odors in humans.

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The Cellular Basis of Odor Mixture Representation in a Mammalian System: Peripheral Interactions

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In animal systems, including zebrafish, honey bee, spiny lobster, and rat, odor mixture interactions may occur at different levels, both peripherally between receptor neurons and centrally between principal neurons in the olfactory bulb. However, the precise cellular mechanism for such interactions is largely unknown because of limitations in the spatial resolution across populations of neurons. Studies *in vitro* using mammalian dissociated olfactory receptor neurons suggest that competitive and non-competitive binding inhibition at identified receptors may account for mixture interactions in the periphery (Touhara, K, *Neurochem Int*, 2007; Duchamp-Viret P, Duchamp A, Chaput MA, *Eur J Neurosci*, 2003). We tested the hypothesis that mixture interactions occur *in vivo* by ligand binding interactions at the receptor level. We used a transgenic mouse in which the receptor neurons express synaptopHluorin, a reporter of synaptic transmission, whenever presynaptic activation occurs upon odor stimulation of specific receptors in the nose (Bozza T, McGann JP, Mombaerts P, Wachowiak M, *Neuron*, 2004). By fluorescence imaging of identified glomeruli in the olfactory bulb, we obtained spatial maps of receptor neuron activation in response to odor mixture pairs, including eugenol and methyl isoeugenol, for which there has been previous evidence of receptor antagonism. Results indicate that, within detection limits, for particular glomeruli independently activated by either component of the binary mixture, both suppression of one component by another and moreover, synergism between the two components, may occur at the receptor level.

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Poster Session III: Thurs. July 24

Development of Card Type Olfactory Identification Test

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A variety of olfactory identification tests (UPSIT, Sniffin' Stick, Smell Diskettes and OSIT-J) have been proposed and developed in recent years. We had been working on the development of OSIT-J (Odor Stick Identification Test for Japanese), and succeed in commercialization in 2006. This test kit is much easier to use comparing to traditional method ''T & T olfactmeter''. It has, however, occurred the request for shorter time and simpler procedure for examination. In order to fulfil this request, we newly developed card type odor identification test (We named this kit ''Open Essence''). We printed capsuled odorant on the paper, and folded and pressed with each other. The size of folded paper is name card one, and there ware twelve kinds of odorants. The twelve odorants and alternatives are same as OSIT-J, and naturally compatible with OSIT-J. ''Open Essence'' have big advantages compared to OSIT-J, as follows. (1) Patients are able to examin by their selves, in their pace. This means medical doctor takes no time for examination. (2) This kit is single-use, and no tool for rubbing is required. This guarantees total cleanliness and no contamination of odorants.

Poster Session III: Thurs. July 24

Clinical Usefulness of the Card Type Olfactory Identification Test for Japanese Patients With Olfactory Disturbance

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The card type olfactory identification test kit (Open Essence) is a new test of olfactory function recently developed for Japanese. It is consisted of twelve odors and alternatives same as OSIT-J. We evaluated this test kit in relation to Japanese standard olfactory test (T&T olfactometer) and OSIT-J for the Japanese patients with olfactory disturbance. Significant correlations were found between the score of Open Essence, the average recognition threshold of T&T olfactometer and the OSIT-J score, respectively. The examination time of Open Essence is shortest in these three tests. We conclude that Open Essence is useful for evaluating olfactory disturbance in Japanese people.

Poster Session III: Thurs. July 24

A Short Olfactory Test Based on the Identification of Three Odors

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Introduction: Numerous psychophysical tests of olfactory function have been developed during the last 30 years. However, although most tests provide accurate results testing typically requires time which is not available in clinical routine. Aim of the present study was to investigate results from a test based on the identification of 3 odors only.

Material and Methods: A total of 500 subjects (patients with olfactory loss plus healthy controls) were included. They received 1) detailed olfactory testing including extensive tests for phenyl ethyl alcohol odor thresholds, odor discrimination, and odor identification, and 2) the 3-item odor identification test, the so-called ''q-sticks''. Results from the q-sticks were analysed with regard to the discrimination of anosmia from hyposmia/normosmia.

Results: On a group level the q-sticks clearly separated between anosmic, hyposmic, and normosmic subjects. In addition, as predicted, q-sticks scores were significantly higher in women compared to men, and in younger compared to older subjects. With regard to a q-sticks score of 0 the new test had a very high specificity of 96% and a moderate sensitivity of 66%. These figures were 59 and 98% for a score of q-sticks score of 2.

Discussion: Although the q-sticks must not be seen as a replacement of more extensive and, therefore, more accurate olfactory tests, they allow to identify anosmia with a very high specificity. Considering the test's portability, ease of administration, longevity, and possibility to be used over and over again, it can be expected to find its way into the clinician's diagnostic armamentarium.

Poster Session III: Thurs. July 24

Repeatability of the San Diego Odor Identification Test (SDOIT) and Comparison With the Brief Smell Identification Test (B-SIT)

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The objective of this study was to describe the SDOIT repeatability and to compare the SDOIT to the B-SIT. Ninety participants, 33 men and 57 women aged 50 to 70 years completed this 2-visit olfaction study. During visit 1, a brief health questionnaire was completed and the SDOIT and B-SIT were administered according to standard protocols[1,2,3]. The order of test administration was randomized. An average of 3 weeks later (range 2-5 weeks), participants returned to re-take the SDOIT. Changes in health status were recorded. The SDOIT score was the total number of odorants correctly identified out of 8 odorants presented, and olfactory impairment was defined as correctly identifying <6 odorants [4]. The B-SIT score was the total number of odorants correctly identified out of 12 odorants presented, and participants correctly identifying <9 odorants were categorized as abnormal [3]. The SDOIT repeatability was high (concordance correlation coefficient = 0.85, 95% confidence interval (CI) 0.79-0.91). The same score was obtained on retest for 73% of participants, while 18% improved (8 by 1 unit, 7 by 2, and 1 by 3) and 9% declined (7 by 1 unit and 1 by 3 units). Most changes in SDOIT score could not be explained by changes in health, medications, or smoking. Test retest agreement was 96% for the SDOIT; 4% improved from impaired at visit 1 to unimpaired at visit 2. Overall, SDOIT impairment classification and B-SIT abnormal classification agreed in 96% of participants (kappa = 0.81, 95% CI 0.63-0.99). In conclusion, the SDOIT showed good test repeatability. Agreement for impaired/abnormal olfaction was demonstrated for the SDOIT and the B-SIT. Simple measures of olfactory impairment may be useful in epidemiological studies of olfaction.

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[1]Murphy C, Anderson JA, Markison S. Psychophysical assessment of chemosensory disorders in clinical populations. In: Kurihara K, Suzuki N, Ogawa H, eds. Olfaction and Taste XI. Tokyo, Japan: Springer-Verlag Tokyo; 1994:609-613. [2]Morgan CD, Nordin S, Murphy C. Odor identification as an early marker for Alzheimer's disease: impact of lexical functioning and detection sensitivity. J Clin Exp Neuropsychol. 1995;17:793-803. [3]Doty RL. The Brief Smell Identification Test™ Administration Manual. New Jersey. Sensonics Inc. 2001. [4]Murphy C, Schubert CR, Cruickshanks KJ, Klein BEK, Klein R, Nondahl DM. Prevalence of Olfactory Impairment in Older Adults. JAMA. 2002; 288(18):2307-2312.

Poster Session III: Thurs. July 24

Methodological Factors in Odor Detection by Humans

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Measured odor thresholds depend on both subject sensitivity and method. Yet, threshold- methods have received relatively little systematic attention in olfaction. We measured psychometric, i.e., proportion correct detection vs. concentration, functions for acetic acid. A 2-out-of-5, forced-choice procedure was used. Stimuli were precisely controlled using an air-dilution olfactometer. Stimulus-concentration was measured via gas chromatography/mass spectrometry, using solid phase micro extraction to enhance analytical sensitivity. The design had four factors, all randomized or counter-balanced: 1) practiced subjects vs. unpracticed subjects (between subjects); 2) 15-second inter- trial interval (ITI) vs. 30-second ITI (within subjects); 3) re-sampling allowed (i.e., subjects could smell each of the 5 stimuli presented during a trial as many times as they wished) vs. not allowed (within subjects); 4) concentrations presented in ascending order (lowest concentration first, moving up to the highest concentration, then starting again at the lowest concentration after a break) vs. random order (within subjects). A five-way ANOVA (the above four factors plus stimulus concentration) revealed a significant main effect of concentration, demonstrating the expected dose-response relationship. Further, performance was better with ascending order of presentation, and better when subjects were allowed to re-sample. The other main effects, as well as most interactions, failed to reach significance. These results highlight the importance of methods for measured thresholds, and have implications for laboratory practice.

Poster Session III: Thurs. July 24

A Novel Psychophysical Method for Estimating Olfactory Rapid Adaptation in Humans

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Sensory adaptation is a reduction in sensitivity or responsiveness resulting from continuous stimulation. In this presentation we describe a novel method for estimating psychophysical rapid adaptation in human olfaction. The method employs stimulus conditions derived from an analogous psychophysical technique in audition. The premise of the technique is that extended presentation of an odorant will produce adaptation, *decreasing* receptor sensitivity and *increasing* threshold for a *simultaneous* target odorant presented briefly at various time-points after adapting stimulus onset; *where both the adapting odorant and the target odorant are the same*. To test this procedure, we used a liquid-dilution olfactometer to estimate thresholds for brief (600 ms) presentations of vanilla odor; 11 volunteers (9 females; ages 18-21) served as subjects. The adapting odorant concentration for each subject was set to twice the baseline threshold for the 600-ms target (i.e., the same level *relative* to threshold). To evaluate rapid adaptation, we compared thresholds for targets presented simultaneously with the adapting stimulus as a function of the relative delay between the onset of the adapting stimulus and the onset of the target. As predicted from the analogous auditory studies, thresholds for the target stimulus increased in an orderly manner with increases in onset delay (i.e., as the adaptation process progressed). The results suggest that olfactory rapid adaptation is measurable psychophysically within 100-200 ms after stimulus onset, far faster than previous estimates employing intermittent stimulus conditions. These estimates are also consistent with physiological measures of adaptation in olfactory receptor neurons.

Poster Session III: Thurs. July 24

The Multiple Intensity Odor Identification Test (MIOID)

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The Multiple Intensity Odor Identification Test (MIOID) provides a measure of odor identification ability designed to increase test specificity when assessing patients with hyposmia. The test utilizes a traditional multiple-choice format common to most odor identification tests. The odorants are presented at multiple intensities with the assumption that patients with hyposmia will have improved odor naming ability when given more intense odorants. Patients who have difficulty naming odors due to non-sensory limitations (e.g., impaired cognition) should not benefit from the intensity manipulation. Performance on the odor naming portion of the test is highly correlated with performance on the Sniffin' Sticks Odor Identification Test ($r = 0.71$, $p < 0.001$). Odor intensity-mediated changes in performance were

observed in clinical populations known to have impaired olfactory functioning. In addition, performance on the odor identification portion of the MIOID test was correlated with a measure of memory function in a clinical population. However, this relationship was moderated by odorant intensity. The data suggest that the MIOID maintains the high sensitivity associated with odor identification tests while adding more specific information that is useful in determining the relative influence of sensory versus cognitive impairments on odor identification ability. These results are discussed in terms of clinical and research implications as well as future test development of the MIOID.

Poster Session III: Thurs. July 24

Test-Retest Reliability of the Olfactory Detection Threshold Test of the Sniffin' Sticks

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The aim of the present study was to investigate the test-retest reliability of the olfactory detection threshold subtest of the Sniffin' Sticks test battery, if administered repeatedly on four time points. The detection threshold test was repeatedly conducted in sixty-four healthy subjects. On the first testing session the threshold test was accomplished three times (T1 = 0 min, T2 = 35 min, T3 = 105 min), representing a short-term testing. A fourth threshold test was conducted on a second testing session (T4 = 35.1 days after the first testing session), representing a long-term testing. The average scores for olfactory detection threshold for n-butanol did not differ significantly across the four points of time. The test-retest reliability (Pearson r) between the four time points of threshold testing were in a range of 0.43 – 0.85 ($p < 0.01$). These results support the notion that the olfactory detection threshold test is a highly reliable method for repeated olfactory testing, even if the test is repeated more than once per day and over a long-term period. It is concluded that the olfactory detection threshold test of the Sniffin' Sticks is suitable for repeated testing during experimental or clinical studies.

Poster Session III: Thurs. July 24

Testing Persons' Sensitivity for Odor and Sensory Irritation Detection and Recognition

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Persons' sensitivity for odor and sensory irritation detection and substance recognition have been tested by the method of constant stimuli (dynamic olfactometry) on university young students (n=12), persons selected from the general women population with special report chemical sensitivity (CS-persons, n=10) and non-CS controls (n=20). Lower concentrations of two chemical substances (pyridine and formaldehyde) were applied in the studies. CS persons showed a lower false alarm rate for both odor detection and substance recognition in comparison with Non-CS control and university students while the later two groups have similar false alarm rates. Persons' detectability is mainly determined by their odor sensitivity in the joint odor and sensory irritation detection task, especially at lower concentrations. This principle is fit for all kind of subjects. CS persons do not showed their heightened sensitivity for odor, the detection thresholds for odor were quite same for all three groups. CS persons showed lower recognition threshold for pyridine, which could not verify their self-reported sensitivity to formaldehyde. Persons' sensitivity for sensory irritation is difficult to determine and their irritation detection thresholds excess the giving concentration levels. A sensitivity index from Luce's choice theory (eta) showed a small individual sensitivity difference for odor perception whereas large individual sensitivity difference for sensory irritation perception. Another sensitivity index from signal detection theory (d') showed that the sensitivity difference between CS and non-CS is too small, which can not confirm CS is a functional problem.

Poster Session III: Thurs. July 24

The Role of the Insula in Taste and Orthonasal Olfaction of Food Odors

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Between-study comparisons indicate that sensation of taste and smell produces overlapping activation in the insula. We used event-related fMRI to investigate sensory representation and top-down modulation of taste and orthonasal olfaction in the same subjects. During scanning subjects received a taste, an odor (orthonasally presented food), a tasteless solution or odorless air while performing a detection task (attention) or during passive sensing (no attention or top-down processing required). Passive sensation resulted in responses in respective primary sensory regions; the insula and overlying operculum were activated by taste, but not smell, whereas piriform cortex was activated by smell, but not taste. Overlapping sensory representation of taste and smell was observed in caudal orbitofrontal cortex (OFC), caudomedial OFC, anterior cingulate cortex and striatum. Attention to taste (i.e., trying to detect a taste in the absence of taste) resulted in activation of mid-dorsal and anterior insula, while attention to smell (trying to smell in the absence of odor) increased activity in piriform cortex, and in the ventral insula (VI), replicating prior studies. A conjunction analysis showed overlapping attentional effects in bilateral anterior insula and overlying operculum. This region showed sensory responses to taste, but not to odors. Response to odors in VI ($r = .77$-$.80$, $p < 0.005$), but not in the piriform ($r = .004$-$.07$, $p = .37$-$.84$ (n.s.)), was correlated with sweetness ratings of the odors. These results show that primary taste and olfactory regions principally respond to stimulation in their respective sensory modality and that orthonasal olfactory coding in the insula reflects attentional modulation or encoding of the perceived sweetness of food odors.

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Poster Session III: Thurs. July 24

Response-Time Measures of Gustatory-Olfactory Flavor Integration

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How does information arising in the human gustatory and olfactory systems combine and interact in flavor perception? To help answer the question, we presented subjects on each trial with a brief pulse of one of three flavorants (the gustatory sucrose; the retronasal olfactory citral; sucrose/citral mixture) or water. Stimuli were delivered to the mouth through a computer-operated, automated flow system that controlled the stimulus's duration (0.5 s) and volume (0.5 ml). Subjects responded by pressing a button as quickly as possible when they detected any of the three flavorants (but not water), thereby providing measures of simple response times (RTs). In the experimental condition, the four possible types of trials – water and three flavorants – were

interleaved within a session. In each of the three control conditions, two possible types of trials – water plus one of the three flavorants – were interleaved within a session. The results extend our earlier findings (Burger et al., AChemS, 2007) in two ways. First, in general, the results show patterns of RT across flavorants that were similar in the experimental and control conditions, implying that subjects did not attend selectively to one flavor component or the other. Second, in general, responses were faster (RTs were smaller) to the mixture than to either of the individual components presented alone. Together, these findings provide further evidence for the integration of information from gustation and retronasal olfaction in rapid perceptual responses to flavor mixtures.

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Poster Session III: Thurs. July 24

The Relationship Between Bold Response to a Taste/Olfactory Mixture and Actual Eating Behavior Differs for Young and Older Adults

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Nutritional problems in older adults are often related to changes in weight, appetite, and the overall enjoyment derived from eating. Chemosensory functioning, hunger perception, and reward processing are all important factors involved in eating behavior that may be affected by aging. The purpose of this analysis was to investigate associations between eating behavior, defined here as caloric intake, and fMRI activation in response to a pleasant flavor in young and older adults. An event-related fMRI paradigm was used to measure brain activity during administration and hedonic evaluation of a taste/olfactory mixture (sucrose and citral). During the follow-up session, participants were presented with a lunchtime meal consisting of various food options (cheese pizza, snacks, and dessert) and energy intake was assessed. A regression was run on brain activity in response to citral/sucrose using the amount of energy consumed (kcal) as a predictor. A region of interest analysis was also run on fMRI data and fit coefficients were correlated with energy intake. The resulting associations between energy intake and brain activity differed for young and older adults. Young adults who consumed more had less activity in the left amygdala, left piriform cortex, and bilaterally in the parahippocampal gyrus and insula. Older adults who consumed more had more activity in the right amygdala, right entorhinal cortex, right piriform cortex, and bilaterally in the parahippocampal gyrus and hippocampus. Quantifying relationships between eating behavior and neural processes related to reward, energy homeostasis, and taste and olfactory processing in young and older adults may increase our understanding of age-related nutritional problems and changes in eating behavior.

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Poster Session III: Thurs. July 24

Labels about Health Benefits Modulate Insular Responses to Flavors

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We investigated whether labeling flavorful drinks with health-related descriptors can affect the drinks' perceived hedonic value and neural encoding. During fMRI scanning, subjects were presented with a flavorful stimulus concomitant to one of two possible auditory descriptors (''healthy'' or ''treat''). Unbeknownst to subjects, we manipulated the auditory descriptors in order for the same flavor stimulus to be associated with both labels at different selected trials. A repeated measures analysis of variance showed that subjects produced significantly higher pleasantness ratings to the same flavorful drink when it was labeled ''treat'' compared ''healthy''. The magnitude of this effect was inversely related to sensitivity to reward, measured by the Behavioral Activation Scale (BAS). When BAS scores were included as a covariate, the effect of the label increased from $F = 2.6$ to $F = 13.8$, indicating that subjects with lower sensitivity to reward are more likely to rate the drink labeled treat as more pleasant than the drink labeled healthy. Next we compared brain response to perception of the drink when it was labeled ''treat'' vs. ''healthy''. Preferential response to ''treat'' labels was observed in the anterior cingulate cortex and ventral striatum and the strength of this relationship increased when BAS scores were entered as a covariate. These same regions also responded to receipt of a highly palatable milkshake and the magnitude of the response was positively correlated with milkshake pleasantness. These findings indicate that labeling a drink as a treat can cause neural and behavioral responses to shift towards those of a prototypical treat stimulus but that this effect is strongly modulated by sensitivity to reward, which is a stable personality trait linked to dopamine function.

Poster Session III: Thurs. July 24

Expectation, Experience and Expertise - How to Cope with Incidental Findings in Neuroimaging Studies in Taste and Olfaction

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Objectives: The number of brain imaging studies in the science of taste and olfaction is increasing. As in other disciplines, there is growing awareness for the need to have policies to handle incidental findings. We present our experience of five years of brain imaging and introduce our in-house policy in managing incidental findings.

Methods: We imaged 317 women and 205 men (age range: 9-79 years, mean 32, median 26) in 21 smell and taste studies. Studies comprised functional and morphometric magnet resonance imaging (MRI) examinations. Subject numbers varied between 8 and 122 per study, the median being 19 participants. In all subjects we acquired a high resolution scan with a voxel size between 1 and 2 mm. These images were not intended to fulfill diagnostic criteria but allowed for exclusion of gross pathology. All scans were reviewed by a neuroradiologist.

Results: 87% of the anatomical scans had good quality, 6.5% acceptable quality and 6.5% were rated as bad. 5% of the subjects had known brain pathologies due to the design of one study. In 83% of the participants no pathology was found. In 3.7% further diagnostic imaging was suggested as the available scan raised suspicion of a potential pathology. One vestibular schwannoma and one cavernoma were found and were referred for further counseling. The remaining findings had no medical consequences: 3% chronic vascular lesions, neuroepithelial cysts (1.6%), empty sella, benign bony defects, and pituitary gland cysts to name the most frequent.

Conclusions: In our series nearly 4% had ambiguous findings necessitating further diagnostics. This suggests that the brain scan acquired during a brain imaging study should be looked at with expertise. Local policies should be in place to cope with unexpected findings as they invariably occur.

Poster Session III: Thurs. July 24

Individual Differences in the Acquisition of Odor Preferences: Behavioral and Electrophysiological Correlates

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Whereas some aspects of olfactory hedonism in humans are present from birth, others form during development and throughout adulthood Although hedonic representations have a strong innate component, there are growing evidences for plasticity at multiple levels of the olfactory system. In particular, the hedonic representation of smells is not fixed and may be modified with learning and experience. Through an associative learning procedure whereby a neutral or novel smell is associated with an unconditioned stimulus (US, i.e. pleasant taste) the smell may acquire the positive hedonic valence of the US. Although such acquisition of odor preferences has been documented in the past, there is still a need to clarify both the inter-individual variability that sustained such processes and the neural correlates of such modulation of odor pleasantness. The present study set out to examine these questions. Twenty-four participants (9 men, mean age = 21.59 ± 2.39) were tested under 3 experimental sessions. In session 1, participants were to sniff two odorants (anise and chocolate) and to estimate odor intensity and odor pleasantness. In session 2, odorants were randomly presented in an associative learning procedure with either water or a pleasant sweet solution. The third session was identical to the first one. Olfactory evoked potentials were recorded continuously during the study using a 64-channels EEG system (Micromed ®). Results showed a very large variability across subjects: 4 subjects showed a significant decrease of odor pleasantness ($p < .001$), 14 subjects showed no significant changes of odor pleasantness ($p > .05$) and 6 subjects showed a significant increase of odor pleasantness after learning ($p<.001$). Olfactory evoked potentials will be compared across groups of individuals and will highlight the neurophysiological substrates underlying this variability across subjects.

Poster Session III: Thurs. July 24

An Fmri Validation Study Using Independent Component Analysis (ICA)

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FMRI is a non-invasive method to visualize stimulus processing in the brain. Analysis of acquired data is normally performed using hypothesis-driven analyzing tools. This means that a search is performed over the data to detect signal changes which follow the experimental paradigm in their temporal course. This search is based on the assumption of a typical signal course induced by stimulation. However, in certain cases the time course of neuronal activity cannot always be predicted. ICA is a data-driven method. This means that an *a priori* hypothesis about the paradigm'ss time course is not necessary. ICA might therefore be a useful adjunct in the analysis of fMRI data. The aim of this study was to compare the results of ICA for the detection of cortical signal changes within an fMRI data set to the results found using a standard, hypothesis-driven method. Functional images were obtained from 22 volunteers using a 3T MRI scanner. We used an intranasal CO_2 event-related stimulation paradigm. Images were analyzed using SPM2 and GIFT. Detected activities were compared between the two methods. Using the hypothesis-driven analyzing tool we detected activation in brain areas known to be involved following chemical stimulation of the nasal mucosa: orbitofrontal cortex, association cortex. In addition we found activations in areas specific to the processing of painful and aversive stimuli. Activation of these areas could also be shown by analyzing the data with the data-driven model. Our results indicate that ICA is suitable for analyzing fMRI data, of which no *a priori* hypothesis is known. Using ICA it may be possible to identify cortical activations in fMRI data which do not follow the typical haemodynamic response function.

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Poster Session III: Thurs. July 24

Functional Characterization of Human Bitter Taste Receptors

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Bitter taste is an aversive reaction that likely evolved to protect an individual from potentially toxic molecules. Human bitter taste is mediated by a family of 25 G protein-coupled receptors (T2Rs), which are believed to recognize hundreds of different bitter molecules. Until now, 8 T2Rs have been deorphaned with a limited number of bitter molecules. We have undertaken a systematic functional expression approach to deorphan human T2Rs. We evaluated over 200 bitter molecules based on their taste thresholds and feasibility in a calcium imaging assay. About 120 bitter molecules were used to test the 25 human T2Rs. We observed positive responses for 22 human T2Rs transiently transfected into HEK293 cells. Most of the 22 deorphaned T2Rs are broadly tuned and recognize structurally diverse ligands. Furthermore, most of the bitter molecules activated more than one T2Rs with different affinities. Our findings indicate a certain degree of 'promiscuity' among the human bitter receptors in the recognition of bitter stimuli. Dose-response analysis suggests that certain bitter receptors may mediate 'dominant' responses to specific stimuli. These results give us a more complete picture of how the T2Rs work and allow us to identify and target the right receptors to develop modulators of bitter taste elicited by different bitter substances.

Poster Session III: Thurs. July 24

Regulation of Bitter-Evoked Calcium Release Signals in Mouse Taste Cells

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Taste stimuli activate distinct signaling pathways in taste receptor cells. Some taste stimuli are detected via G-protein coupled receptors that cause calcium release from intracellular stores, while other stimuli depolarize taste cells to cause calcium influx through voltage gated calcium channels. We have found that activation of each of these pathways generates unique calcium signals within taste cells (Hacker et al., 2008), however any potential differences between the calcium-release mechanisms in response to discrete taste stimuli have not been investigated. The goal of this study is to characterize the evoked calcium responses generated in taste cells in response to different bitter compounds. Using calcium imaging, we measured the contribution of calcium-release channels on the endoplasmic reticulum (ER) to the intracellular calcium increases in response to compounds such as denatonium, cyclohexamide and caffeine. We also determined the role of the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) in the refilling of the ER and how blocking the SERCA pumps affects subsequent calcium responses. Recently, we identified the presence of two SERCA isoforms (2b and 3) and IP_3R1 in addition to IP_3R3 in mouse taste cells, indicating

that multiple proteins contribute to the regulation of calcium stores. Currently, we are determining if there are physiological differences in the stimulus-induced calcium release that correlates with the expression of these different proteins.

Poster Session III: Thurs. July 24

Nicotinic Acetylcholine Receptors (NACHRS): Novel Bitter Taste Receptors for Nicotine

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Peripheral mechanisms for nicotine bitter taste transduction were probed using physiologic, pharmacologic and genetic tools. In cDNA from rat fungiform and circumvallate lingual epithelium we identified α3, α4, α7, β2, and β4 nAChR subunits. In rats, Nicotine (Nic; 1-20 mM) and acetylcholine (ACh; 1-10 mM) elicited dose-dependent increases in the chorda tympani (CT) taste nerve responses. CT response to 10 mM Nic or 10 mM ACh was inhibited by nAChR blockers, mecamylamine (Mec; 0-0.5 mM) or dihydro-β-erythroidine (DHβE; 0-0.5 mM) in a dose-dependent manner, however, their CT response was indifferent to 0.5 mM atropine, a muscarinic AChR blocker. While the inhibition of rat CT response in the presence of Mec+DHβE was additive, the response was never entirely blocked. Since behavioral and neural responses to bitter tastants are Trpm5-dependent, Trpm5 wild type (WT) and knockout (KO) mice were also studied. KO mice, although indifferent to quinine, respond behaviorally to nicotine as WT, even if trigeminal input was diminished. In both genotypes, Nic elicited a dose-dependent increase in the CT response that was incompletely inhibited by 0.3 mM Mec. The CT response was smaller in KO relative to WT mice. At 20 mM Nic, the tonic CT response in KO mice was 40% smaller relative to the WT mice and in both genotypes Mec inhibited the response by 40%. We conclude that the tonic Nic CT response depends on nAChR-dependent and -independent mechanisms and that the nAChR-dependent pathway is mostly independent of Trpm5.

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Identification of Compounds that Selectively Block Bitter Taste Mediated by Human T2R8

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Among five taste qualities recognized by humans, bitter taste is most commonly perceived as unpleasant and undesirable. It is believed that bitter taste developed as a means to recognize potentially toxic and/or harmful chemicals that could be present in food sources. However, not all bitter tasting molecules are harmful. Studies suggest that consumption of some vegetables that taste bitter might help in preventing certain forms of cancer. Many pharmaceuticals taste bitter to humans, which may restrict their use. This points to a need to identify novel ways to reduce or block bitter taste. However, no universally effective approach has been identified. All known means, such as encapsulating of a bitter product or masking bitter taste with sugar, have limitations. We undertook a different (molecular) approach. Human bitter taste is mediated by 25 bitter receptors (hT2Rs or TAS2Rs). Binding of a bitter molecule to one or several of these receptors is the initial step resulting in bitter sensation. We developed a method to express human T2Rs in HEK293 cells and study their function using intracellular calcium release as the readout. Our high throughput screening identified a molecule that selectively activates only one of the human bitter receptors, hT2R8. The EC50 in the assay for this hT2R8 agonist (0.7 mM) is in good agreement with its bitter taste threshold (1-2 mM). Our screening also identified compounds that selectively inhibit hT2R8 activity in this assay. Taste tests revealed that these hT2R8 antagonists have no taste on their own and can significantly reduce the intensity of the bitter taste of the hT2R8 agonist (from moderate-strong down to barely detectable). The relative potencies of these antagonists in the taste test correlate very well with their ability to inhibit hT2R8 activity in the *in vitro* assay. We thus provide the first examples of compounds that can block bitter taste by selectively binding to specific human bitter receptors. Developing such bitter receptor antagonists represents a new paradigm for reducing bitter taste.

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Responses to Quinine-HCl and $CaCl_2$ in Morphologically Identified Frog Taste Cells

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Recent studies have demonstrated that type II cells in taste buds of mice possess G protein-coupled receptors of bitter taste compounds. Since conventional synapses with afferent fibers have been found in type III cells but not in type II cells in rodents and frogs, it is unclear how bitter taste information from type II cells is communicated to afferent nerve fibers. There are Ca^{2+}-sensitive fibers (Ca^{2+}-fibers) and quinine (a bitter substance)-sensitive fibers (Q-fibers) in the frog glossopharyngeal nerve. Ca^{2+}-fibers do not respond to quinine-HCl (Q), while Q-fibers do not respond to $CaCl_2$. These findings suggest that Q and $CaCl_2$ taste stimuli are received by different subsets of taste cells. In this study, to investigate taste signal processing in the frog taste organ, we applied Ca^{2+} imaging and patch-clamp recording to bullfrog (*Rana catesbeiana*) taste disc slice preparations. We used patch pipettes filled with Calcium Green-1 dextran for Ca^{2+} imaging and cell type identification. Depolarization with high K^+ (70 mM) resulted in an increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in only type III cells. Application of 10 mM Q to the apical portion of taste cells elicited an inward current and $[Ca^{2+}]_i$ increase in type Ib and II cells but not in type III cells. Application of 40 mM $CaCl_2$ to the apical portion of taste cells elicited an inward current and $[Ca^{2+}]_i$ increase in type III cells but not in other types of cells. The present results suggested that Q taste signals are transmitted from type Ib and II cells directly to Q-fibers via unconventional synaptic mechanisms and that $CaCl_2$ taste signals are transmitted from type III cells to Ca^{2+}-fibers via conventional synaptic mechanisms which normally require voltage-gated Ca^{2+} channels.

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T1R3 is a Receptor for the Taste of Calcium and Magnesium

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Calcium and magnesium are essential for survival but it is unknown how animals detect and consume enough of these minerals to meet their needs. To investigate this, we exploited the PWK/PhJ (PWK) strain of mice, which

avidly ingests calcium, in contrast to the C57BL/6J (B6) and most other inbred strains. We found that the PWK strain's avidity extends to $MgCl_2$ but not to representative sweet, sour, salty, bitter or umami taste compounds. A genome scan of B6 x PWK F_2 hybrid mice linked a component of the strain difference in avidity to distal chromosome 4. Studies with congenic and knockout mice showed this linkage can be accounted for by alleles of the taste receptor gene, *Tas1r3*. Most notably, calcium and magnesium solutions that were avoided by wild-type B6 mice were preferred by B6 mice null for the *Tas1r3* gene. Oral calcium elicited less electrophysiological activity in the chorda tympani nerve of *Tas1r3* null than wild-type mice. Comparison of the sequence of *Tas1r3* in 40 inbred mouse strains identified a V689A substitution in the 4th transmembrane domain of T1R3 that may be responsible for the PWK strain's avidity for calcium and magnesium. Our results imply that, in addition to its established roles in the detection of sweet and umami compounds, T1R3 may function as a gustatory calcium-magnesium receptor.

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A Taste for Calcium

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Systemic calcium homeostasis is essential for survival and it is tightly regulated within a narrow range. The extracellular calcium sensing receptor (CaSR) detects small fluctuations of Ca^{2+} and is expressed in those tissues that are involved in Ca^{2+} regulation such as parathyroid chief cells, kidney, bone and intestine. CaSR belong to the class 3 of the G-protein coupled receptor superfamily, which includes metabotropic glutamate receptors (mGluR), $GABA_b$, GPRC6A and taste receptors (T1R1/T1R3). Ca^{2+} and polyvalent cations are not the only molecules that activate CaSR by binding to its flytrap-like domain, also amino acids and peptides can interact with other allosteric sites of the receptor. The aim of this study was to examine the expression of CaSR in gustatory tissue as a specialized sensor for dietary Ca^{2+}. Taste and non-taste tissue was analyzed by normal and real-time quantitative PCR and results were confirmed by immunohistochemistry. CaSR was localized within taste cells of circumvallate, foliate and fungiform papillae. We speculate that dietary Ca^{2+} and CaSR agonists can activate the receptor in taste type 2 cells and induce responses in gustatory nerves. Cell type distribution will be discussed as well.

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Glossopharyngeal Nerve Transection does not Impair Unconditioned Avoidance of all Bitters Equally

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Previously, we found that the majority of bitter-selective neurons in the rat brainstem had foliate receptive fields (RFs), suggesting glossospharyngeal (GL) innervation. Neurons with more anterior RFs also responded to bitter stimuli; however these cells typically responded best to salts or acids and bitter activity appeared limited to ionic stimuli (i.e. quinine & denatonium). Given that the neurons responsive to nonionic bitters (PROP & cycloheximide) were mainly those receiving GL projections, we hypothesized that this nerve was necessary for avoidance of these stimuli. Thirty male SD rats were divided into 3 surgery groups: bilateral GL transection (GLX), chorda tympani transection (CTX) or SHAM, and post-surgical avoidance functions generated for 4 bitter stimuli (quinine, denatonium, PROP & cycloheximide) using a brief-access test. GLX significantly compromised avoidance for all stimuli compared to the other surgery groups ($p < .02$) while the CTX group did not differ from SHAM for any stimulus. Contrary to our hypothesis, GLX had a greater effect on ionic than nonionic bitters (1.0 vs. 0.3 $\log_{10}$ units, $p < .006$). One possibility is that input from the GL and CT is largely redundant for nonionic bitters, although to date gustatory neurons with anterior tongue RFs have not been found to respond significantly to cycloheximide. Alternatively, while most NST neurons activated by nonionic bitters had foliate RFs in the previous experiment, a few nasoincisor duct-responsive cells were also found, suggesting that the GSP may be more important for this task than originally anticipated.

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Taste Nerve Responses to Amino Acids and Bitter Compounds in Zebrafish and Medaka Fish

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The stimulatory effectiveness of amino acids and bitter compounds on the external gustatory receptors of the zebrafish (*Danio rerio*) and medaka fish (*Oryzias latipes*) was investigated. The extracellular facial nerve recordings were performed using a glass suction electrode. In zebrafish, the taste nerve (ramus mandibularis of the facial nerve) responded most strongly to L-Ala and L-Pro, moderately to L-SerL-TyrL-CysH, Gly, denatonium, caffeine and quinine, and weakly to other amino acids (L-Arg, L-His, L-Glu-Na, L-Lys, L-Leu, L-Asp-Na, L-Val, D-Ala). Cross-adaptation with 1 mM L-Ala which depressed the taste response to 1 mM L-Pro, L-Ser, L-Tyr, L-CysH and Gly, indicated that these amino acids might stimulate common gustatory receptor(s). In the case of bitter compounds (caffeine, denatonium, quinine), continuous application of one of them did not depress the responses to the others. In medaka fish, L-Pro, denatonium, caffeine and quinine showed strong stimulatory effectiveness, but the other amino acids including those which were stimulative in zebrafish were slightly or hardly effective. Cross adaptation studies suggested that the taste response to bitter compounds might be mediated by different transduction mechanisms with one another, as shown in zebrafish. We found that the gustatory systems of zebrafish and medaka fish have different profile of stimulative amino acids with each other. Comparing the profiles of stimulative amino acids derived from nerve recordings and the ligand specificity of T1Rs identified in these fish (Oike et al., 2007), amino acids might react with common taste receptors both in zebrafish (zfT1R2a/3, zfT1R2b/3) and medaka fish (mfT1R2c/3). Bitter compounds used in our study might stimulate distinct receptors with one another in both species.

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A Rapid and Reliable Method for Measuring Chorda Tympani Nerve Responses in Mice

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The mouse has become an important model system for analyzing how food-related stimuli are transduced and processed in the peripheral taste system. To this end, many investigators perform molecular or pharmacological manipulations of the peripheral taste system, and then assess the functional consequences of these actions by recording responses of peripheral taste nerves to lingual stimulation. One of the most commonly studied taste nerves, the chorda tympani (CT), relays information from taste cells in the anterior lingual epithelium to the nucleus of the solitary tract. The standard approach for accessing the CT nerve, referred to as the "mandibular approach," is challenging in mice because of their diminutive size. Here,

we describe an easier and more reliable approach. One can simply place an electrode against the CT nerve as it passes through the middle ear cavity, and obtain strong electrophysiological responses without cutting or desheathing the nerve. This ''middle-ear'' approach was originally described by Cheal (1977), but only in a cursory manner. To illustrate the efficacy of this approach, we show concentration-response functions for four prototypical taste stimuli (NaCl, sucrose, citric acid and QHCl) in C57BL/6J (B6) mice, and then compare the responses of B6 and FVB/NJ mice to several preferred taste stimuli (sucrose, SC45647 and Polycose).

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T2R Gene Family Expression in the Human Tongue: A Comparison Between Normal Healthy Subjects and Patients with Taste Disorders

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Genes associated with bitterness are called the T2R gene family. We evaluated gene expression of the T2R family in the human tongue in normal healthy subjects and in patients with taste disorders. The healthy subjects were 54 people. Their ages ranged from 20 to 73 years. The subjects with taste disorders were 51 patients. Their ages ranged from 25 to 88 years. A sample was collected by scraping the foliate papillae of the tongue and total RNA was extracted using TRIzol (Invitrogen). Then, a reverse transcription reaction was performed for total RNA using Super Script III, and PCR was performed using Ex Taq (Takara). Electrophoresis was performed using a 2100 Bioanalyzer (Agilent). Gene expression was evaluated in 10 genes: T2R3, 8, 9, 10, 13, and 16 and THTR4, 5, 9, and 11. When the frequency of gene expression was compared between healthy subjects and the patients with taste disorders, T2R3, 8, 9, and 10 and THTR4 and 5 showed significantly decreased frequencies of expression in the patients with taste disorders. When evaluated with regards to the causes of the taste disorders, the expression of T2R3, 8, 9, and 10 and THTR5 were significantly decreased in those patients with decreased serum zinc levels. The patient group with taste disorders related to bitterness tended to show lower gene expression compared to healthy subjects. Especially T2R3, 8, and 9 and THTR4 and 5 showed statistically significant decreases. Patients with taste disorders showed a decreased expression of taste genes in the tongue. In particular, in patients diagnosed with zinc deficiency, expression of taste genes was decreased. It was suggested that a decreased expression of taste associated genes could be involved in the mechanism of taste disorders in humans.

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Trigeminal sensitivity and Olfactory Function in Patients Before and After Septoplasty

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Endonasal surgery can affect olfactory and trigeminal function. This study investigated to what extent this occurs in clinical practice. We studied 15 patients who under went septoplasty and 17 normosmic controls. Patients were tested just before and circa 2 months after the surgery, this interval was matched in the control group. Investigation included olfactory testing using the ''Sniffin' Sticks'' odor identification test and determination of trigeminal detection thresholds of CO2 and CO2 induced pain. Furthermore, we determined the duration of CO2 stimuli at which participants experienced a certain intensity level. This latency was significantly correlated with thresholds for CO2 and CO2 induced pain. In addition, the obtained latencies correlated between those for the right and left nostril, also exhibiting a correlation with age, women were more sensitive than men. The two groups did not show significant differences in terms of olfactory function, although patients tended towards lower scores. Furthermore, there was no significant difference between the two groups regarding detection of trigeminal thresholds for CO2 and CO2 induced pain and for intensity ratings of stimuli administered at both threshold levels. Patients undergoing septoplasty exhibited significantly greater latencies before indicating a certain level of intensity the stimulus had reached. This was most pronounced for higher stimulus concentrations. Thus, while the study is still ongoing, results from the present investigation indicated no major effect of septoplasty on intranasal trigeminal function, although the septoplasty group generally exhibited a lower sensitivity towards CO2-induced pain.

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Sensory Perception of Cooling Ingredients by Different Ethnic Groups

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Cooling Sensates™ substances derived from (l)-menthol are novel ingredients that can intensify or extend the flavor impact of chewing gum, confectionary, and oral care products. In ongoing studies, we examined the sensations of cooling, heat/burning, bitterness, and tingling from mono-(l)-menthyl succinate, mono-(l)-menthyl glutarate and (l)-menthyl lactate in young adults residing in the U.S. who self-described themselves as South Asian (n = 23), East Asian (n = 24) or Caucasian (n = 45). Subjects rated three concentrations (75, 150 and 300 ppm) of each compound using 15-cm line scales at four time points over a 10-min period (0, 2.5, 5 and 10 min after tasting). They also indicated the locations of each sensation in the mouth, nose and throat. The intensity of all attributes was maximal directly after tasting ($p < 0.0001$) and decreased with time ($p < 0.0001$). (l)-Menthyl lactate produced stronger sensations than the other compounds ($p < 0.0001$) with cooling as the predominant attribute, followed by mild heat/burning and tingling. Bitterness was barely detectable in any of the samples. At time 0 and 300 ppm, Asians (South and East combined) perceived more heat/burning from all three compounds than did Caucasians ($p < 0.05$-0.01), and this effect dissipated by 5 min. As compared to Caucasians, Asians also perceived heat/burning from all compounds in more locations ($p < 0.05$), which might have contributed to higher heat/burn ratings in the Asian group. These data suggest that the perception of heat/burning from cooling ingredients may vary by ethnicity. Future studies should address the basis of these perceptual differences and whether they influence hedonics. Genetic taste sensitivity to 6-n-propylthiouracil (PROP) was not related to the perception of the samples.

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Perceived Air Quality and Symptoms from Exposure to Volatile Organic Compounds Emitted from Microorganisms and Damp Building Materials

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In two studies, we examined whether acute exposure of human subjects to volatile organic compounds (VOCs) emitted from damp building materials and molds grown on the materials would evoke symptoms similar to those reported in moldy buildings. Mold commonly found in indoor air was inoculated on both pinewood and particleboard placed in a cultivation chamber. The emissions fed an exposure chamber (study 1) for whole-body exposure or a hood (study 2) for head-only exposure. Subjects had exposures of 60 and 10 min, respectively, from study 1 to study 2, and rated air quality and symptoms before, during, and after exposure. Total VOC and selected VOCs (3-methyl-1-butanol, 2-heptanone, etc.) were brought to levels typically found in "moldy buildings" (study 1) or to levels 10-100 times higher (study 2). At the levels of study 1 (TVOC=1.2 mg/m^3), subjects could discern no odor. Exposure caused no significant increase in symptoms, either at 30 or 60 min of exposure, or 15 min after exposure. Because the subjects could discern odor at the levels delivered in study 2, we ran exposures with and without a nose clip. Without the clip, subjects reported deterioration of air quality (stuffy air, smell, unpleasant smell). They also reported somewhat elevated symptoms related to the nose and skin. No such outcome occurred with the nose clip. Although the conditions represent only a small fraction of those that may normally occur, the outcome points towards the role of olfaction in such circumstances. Since these subjects would have had few of the concerns that often accompany exposures in the real world, we take their reaction at the higher exposure to indicate the intrinsic importance of olfaction to symptoms in moldy buildings.

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Poster Session III: Thurs. July 24

Expecting Negative Effects of Odor Exposure Influences Various Stages of Olfactory Information Processing: An Unpleasant Odor Associated with Sensory Irritation (Part I)

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Background: Do expectations that odors have adverse health effects influence "early" (perception of odor *characteristics*) or "late" (odor *interpretations*) olfactory processing? This was measured using Olfactory Event Related Potentials (ERPs) in response to an unpleasant odor (H_2S).

Method: During the acquisition phase of a conditioning paradigm, subjects learned that H_2S had a *danger* connotation in one context (CS+), where the odor was always followed by a sensory irritating CO_2 pulse (US). The same odor had a *safe* connotation in another context (CS-). Here, the odor was never followed by CO_2. A visual stimulus predicted whether or not the upcoming odor was a CS+ (and thus followed by the US) or a CS-. In this manner, H_2S was perceived either as a safe signal in one condition, or as a warning signal for irritation in the other condition. ERPs and intensity and annoyance ratings of H_2S from the test phase were compared between these two conditions.

Preliminary results (N=19): A main effect of Expectancy on the "early" N1 and "late" P2 latency was found: latencies were shorter in the CS+ context. Additionally, an effect of Expectancy on the P2 amplitude was found: the amplitude was higher in the CS+ context. Although not significant (yet), intensity and annoyance ratings were higher in the CS+ condition.

Conclusion: Both early encoding of olfactory information, as well as the more interpretational phases of odor perception can be influenced by expectations about the effects of exposure. Such expectations might be based on learned associations between an odor and a previously encountered negative consequence.

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Poster Session III: Thurs. July 24

Expecting Negative Effects of Odor Exposure Influences Various Stages of Olfactory Information Processing: A Pleasant Odor Associated With Sensory Irritation (Part II)

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Background: Using classical conditioning and olfactory Event Related Potentials (ERPs), Bulsing et al., (2008; see this abstract book) showed that learned associations between an unpleasant odor and a previously encountered negative consequence (a sensory irritating CO_2 pulse) influence both "early" (encoding of stimulus *characteristics*) and "late" (odor *interpretations*) phases of olfactory processing. Here, it was investigated whether a *pleasant* odor (PEA) could also become associated with a negative consequence, leading to altered perception.

Method: During the acquisition phase, subjects (N=30) learned that PEA had a *danger* connotation in one context (CS+), where the odor was always followed by a CO_2 pulse (US). The same odor had a *safe* connotation in another context (CS-). Here, the odor was never followed by CO_2. A visual stimulus predicted whether or not the odor was a CS+ (and thus followed by the US) or a CS-. In this manner, PEA was perceived either as a safe signal in one condition, or as a warning signal for irritation in the other condition. ERPs and intensity and annoyance ratings of PEA from the test phase were compared between the two conditions.

Results: A main effect of Expectancy on the "early" N1 amplitude (not N1 latency) was found: amplitudes were higher in the CS+ condition. No effect of Expectancy on the "late" P2 peak was found. The odor was rated as equally intense during both contexts, but as more annoying in the CS+ condition.

Conclusion: Even if an odor has a positive hedonic valence it can become associated with negative consequences and alter early olfactory encoding.

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Poster Session III: Thurs. July 24

Odor and Irritation from Complex Mixtures of Aromatic Hydrocarbons and their Main Constituents

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Detection thresholds for odor, ocular chemesthesis, and nasal chemesthesis, the last assessed via nasal localization, were measured in 22 subjects. The materials comprised two mixtures: a complex aromatic hydrocarbon fluid (CAH) composed predominantly of alkylated naphthalenes, and a complex aromatic hydrocarbon fluid with low naphthalene content (CAH-LN); and their three main constituents, naphthalene (Naph), 1-methylnaphthalene (1-MN), and 2-methylnaphthalene (2-MN). Stimuli were prepared as serial dilutions in mineral oil (chemesthetic tests) or silicone oil (olfactory tests). Following a forced-choice paradigm, subjects sampled each concentration from low to high between 20 and 28 times, generating complete psychometric functions. Odor thresholds, analytically verified, lay between 3.5 μg/m^3 (Naph) and 21.1 μg/m^3 (CAH-LN). Ocular detection occurred at concentrations about 5 orders of magnitude higher, between 178 mg/m^3 (CAH-LN) and 550 mg/m^3 (2-MN). Whereas most subjects were able to detect the

chemicals via the eye, only about 1/3 of them could do so via nasal localization. For those who could, nasal localization thresholds lay close to those for ocular detection. Overall, individual differences in sensitivity were significantly smaller for chemesthesis than for olfaction, and the slopes of the psychometric functions were much steeper. The results have significance for the understanding of individual differences in chemoreception. The human psychophysical testing gave answers somewhat at variance with those obtained from the respiratory depression assay for sensory irritation in mice.

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Poster Session III: Thurs. July 24

Human Performance to Detect and Lateralize Olfactory, Olfactory-Trigeminal, and Trigeminal Substances

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Objectives: It is questionable if humans are able to lateralize pure odorants. Only few substances excite selectively the olfactory system. One of them is hydrogen sulphide (H_2S). The aim of the detection study was to quantify the human sensitivity in response to stimulation with low and high concentrations of H_2S as well as in response to the olfactory-trigeminal substance isoamyl acetate (IAA) and the trigeminal substance carbon dioxide (CO_2). Based on the results of the detection study we carried out a lateralisation experiment to test the human ability to lateralize the different substances (H_2S, IAA, and CO_2).

Methods: We tested healthy subjects (H_2S and CO_2 (n=20), IAA (n=23)). We used two concentrations of H_2S: 2ppm and 8ppm, 17.5% v/v IAA, and 50% v/v CO_2. The odorant stimulation was performed using an olfactometer. All experiments were carried out based on an event-related design paradigm. After every stimulus subjects were asked if the stimulus contained the H_2S, the IAA, or the CO_2-stimulant. In the lateralisation experiment the participants were asked to discriminate between the H_2S, IAA, and CO_2 stimuli perceived either from the left or from the right nostril.

Results and Conclusion: We found that humans can detect H_2S in low concentration with moderate sensitivity. Subjects showed a high sensitivity in response to stimulation with 8ppm H_2S, 50% v/v CO_2, and 17.5% v/v IAA. The lateralisation experiment revealed that subjects can lateralize H_2S neither in low nor in high concentrations. In contrast to that, subjects possess the ability to lateralize IAA and CO_2 stimuli. These results demonstrate that humans are able to lateralize odorants that excite the trigeminal system, but they are not able to lateralize odorants that stimulate the olfactory system exclusively.

Poster Session III: Thurs. July 24

Brain Substrates of Congruent Integration Between Good Odors and Pungent Trigeminal Stimuli

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Multiple sensory treatments are cross-modal processes whereby one sensory modality influences information processing from another modality. The chemical senses provide special window onto multiple sensory integration: odors are mixtures of various compounds stimulating both the olfactory and trigeminal systems. Although brain structures involved in the integration of odorants and trigeminal stimuli have been documented, there is still a need to clarify whether the congruency between both types of stimulation represents a prominent factor in such integration. The present study set out to examine this question. Twenty-four participants were tested under 5 conditions: [rose], [orange], [CO2], [rose+CO2] (incongruent), [orange+CO2] (congruent). Responses were assessed by fMRI (1.5T -Siemens Sonata; slices: 36; FOV: 19.2cm; Matrix: 64x64; TR: 3 sec; TE: 35 ms; FA: 90°; Voxel size: $3 \times 3 \times 3.75$ mm). Stimuli were delivered to the subjects using a Burghart OM6b olfactometer (6 l/min); after each block, participants were to estimate intensity and pleasantness of the stimuli: whereas rose and orange were assessed as pleasant, CO2 was perceived as unpleasant ($p < .05$). Moreover, whereas the incongruent mixture was perceived as unpleasant, the congruent mixture was perceived as pleasant ($p < .05$). Pre- and post-processing of the imaging data was performed using SPM5. Contrasting the congruent condition with the incongruent condition revealed activations in various regions including the striatum and cingulate cortex, the thalamus and the hippocampus. Taken together, theses activations may reflect the reward value of the congruent integration (striatum and cingulate cortex), the convergence of trigeminal and olfactory stimuli (thalamus) and the reactivation of semantic associations between orange and CO2 (hippocampus).

Poster Session III: Thurs. July 24

Mechanisms of Chloride Uptake in Frog Olfactory Receptor Neurons

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In olfactory transduction about 70% of the odorant-induced receptor current is carried by excitatory Cl^- efflux. This requires Olfactory Receptor Neurons (ORNs) to have an exceptionally high intracellular Cl^- concentration to make this anionic current excitatory. The Na-K-2Cl cotransporter 1 (NKCC1) is expressed in mammalian ORNs, and has been postulated to be the principal mechanism by which these neurons accumulate Cl^-. To determine whether NKCC1 is important for amphibian olfactory transduction, we used the suction pipette technique to record from *Rana pipiens* ORNs. We found that a 30 minute application of bumetanide, a NKCC blocker, produced a 50% decrease of the odorant-induced current compared to the control group. Similar effects were observed when intracellular chloride concentration was decreased by bathing ORNs for 30 minutes with a low Cl^- solution. Both manipulations only affected the chloride component of the odorant-induced current. In bumetanide treated ORNs the chloride current could be rescued by lowering external Cl^- to reestablish the chloride gradient indicating that the chloride channel was functional and the decrease of the odorant-induced current was just due to a decrease in the chloride gradient. These results suggest that in amphibians, NKCC1 is also important for proper olfactory transduction, and plays a key role in Cl^- accumulation in ORNs. Preliminary data investigating the cellular localization of NKCC1 indicate that the co-transporter is located at the cell body of ORNs and possibly also at the cilia and the dendritic knob.

Poster Session III: Thurs. July 24

Developmental Expression of the Hyperpolarization-Activated Cyclic Nucleotide-Gated Channel in Olfactory Sensory Neurons

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Odor receptors (ORs) are implicated in the formation of olfactory bulb glomeruli (i.e. Mombaerts et al., 1996; Wang et al., 1998; Feinstein and Mombaerts, 2004), and recent studies suggest that G-protein activation, independent of OR activity, is sufficient to induce olfactory sensory neuron (OSN) axon coalescence. Axon targeting/sorting is perturbed in mice deficient in adenylyl cyclase III (ACIII). However, mice lacking Gαolf or the cyclic-nucleotide-gated (CNG) channel have normal OSN axon coalescence and glomerular formation, suggesting that CNGA2 may not be an early target of cAMP. This prompted us to ask if an alternative channel, the hyperpolarization-activated CNG channel (HCN) (Surges et al., 2006), could be a target for cAMP during development of the olfactory pathway. In the hippocampus HCN subunits are implicated in developmental mechanisms (Brewster et al., 2007); differential sensitivity of the HCN subunits to cAMP may provide an explanation for how HCN channels influence axon targeting in response to cAMP (Lynch and Barry, 1991). To assess a possible role of HCN in development of the olfactory pathway we used PCR, immunoblots, in situs and immunohistochemistry. We focused on spatial and temporal expression patterns of HCN subunits when axons are coalescing to form glomeruli (E13-P4). We show HCN1, 2, and 4 are present in OSNs by E13. Initially HCN subunits are present in both "immature "(GAP43+) and "mature" (OMP+) neurons. By E17, expression of HCN primarily co-localizes with mature OSNs. These data suggest that these subunits are present during the time period implicated in glomerular formation and thus offer preliminary evidence that they may be involved in axon coalescence and the formation of glomeruli.

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Poster Session III: Thurs. July 24

ATP-Induced ATP Release Via Purinergic Receptor Stimulation in Mouse Olfactory Epithelium

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ATP acts as a neurotrophic factor and evokes synthesis and release of neurotrophic factors in the central nervous system. In olfactory epithelium (OE), purinergic (P2) receptors are located on all cell types, including the basal progenitor cells. We tested the hypothesis that ATP activation of P2 receptors induces ATP release. We used two fluorescent markers that bind to ATP, quinacrine and *N*-methylanthraniloyl (MANT-ATP), to identify endogenous ATP stores in neonatal Swiss Webster mouse OE slices. We observed co-localized punctate labeling from both markers throughout the OE. To monitor the release of ATP, we measured the decrease of fluorescence over 400 s, in the presence (50 μM) and absence of exogenous ATP, and expressed it as a percentage of control (t = 0 s) fluorescence. In the apical region, in control (0 ATP) conditions, the fluorescence decreased to 68.0 ± 3.8% (MANT-ATP) or 54.3 ± 3.9% (quinacrine). Compared to control, ATP significantly decreased the loss of fluorescence to 46.0 ± 3.4% (MANT-ATP; $p = 0.0007$) or 23.7 ± 3.1 % (quinacrine; $p < 0.0001$). In the basal region of the OE, ATP significantly decreased the loss of fluorescence compared to control conditions, from 28.5 ± 3.0 % (control) to 16.7 ± 1.5 % (ATP; $p < 0.0001$). Pre-treatment with the nonspecific P2 receptor antagonist PPADS (5 min; 25 μM), reduced the loss of fluorescence compared to control (52.1 ± 2.2 % vs. 28.5 ± 3.0 %). Further application of ATP did not have a significant effect on the loss of fluorescence (58.6 ± 3.3 %; $p = 0.1$). We conclude that P2 receptor activation by ATP leads to ATP release in neonatal mouse OE. ATP-induced ATP release could stimulate P2 receptors on basal cells and promote proliferation, suggesting that ATP may have a role in neuroregeneration.

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Poster Session III: Thurs. July 24

Biophysical Properties, Morphology and Gap Junction Coupling of Olfactory Ensheathing Cells

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Olfactory ensheathing cells (OECs) wrap axons of olfactory sensory neurons (OSNs) and promote axon growth in cell culture, and when transplanted, in animal models of spinal cord injury. **We hypothesize that OECs communicate among themselves and with OSNs to regulate OSN axon growth and incorporation into olfactory circuits.** With this hypothesis as guide, we began studying the biophysical and communication properties of OECs using whole-cell voltage-clamp in acute mouse olfactory bulb slices, and with immunohistochemistry. We found both linear current profiles and voltage- and time-dependent currents in OECs, showing that they are a heterogeneous population. Hyperpolarization-activated inward currents were blocked by 100 μM barium, identifying them as inward-rectifier potassium (K^+) channels. Outward currents were partially blocked by 20 mM tetraethylammonium and 100 μM aminopyridine, K^+ channel blockers. Dye-fills of OECs revealed a complex morphology with fine interdigitations and long lamellae surrounding axon bundles. Approximately 15 % of OECs were dye-coupled to 1-9 other OECs, suggesting that coupling is regulated and may have a functional role. OEC markers colocalized with connexin43, a known mediator of glial gap junction coupling which likely mediates coupling among OECs. OECs seem to better promote axon growth in the olfactory nerve than when transplanted to sites of injury; studying them in their normal environment will help understand the mechanisms accounting for this difference. We present a characterization in normal conditions that establishes a foundation for studying OEC phenotypes in conditions of synchronized OSN regeneration after a lesion to the olfactory epithelium, in order to find candidate mechanisms involved in the role of OECs as regulators of circuit formation.

Poster Session III: Thurs. July 24

Cellular Basis for the Mammalian Olfactory Response to Nicotine

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Smoking behavior is regulated by sensory stimuli independent of the pharmacological effects of nicotine (NIC) (Rose et al., 1993). A better understanding of sensory mechanisms underlying smoking behavior may help to develop better smoking alternatives. We used biophysical methods to characterize NIC sensitivity and response mechanisms of olfactory sensory neurons (OSNs). In view of substantial differences in the olfactory receptor repertoire between rodent and human (Mombaerts, 1999), we studied

biopsied human OSNs, cultured human olfactory cells (CHOCs, Gomez et al., 2000) and rat OSNs. NIC induced increases in intracellular calcium $[Ca^{2+}]_i$ in mammalian OSNs in a dose-dependent manner. Most rat OSNs displayed stereoselectivity, with neurons responding to either enantiomer alone or both. Freshly biopsied and primary cultured human olfactory neurons were less stereoselective. Nicotinic cholinergic antagonists, mecamylamine and hexamethonium had no effect on the nicotine responses of rat or human OSNs. Removal of extracellular Ca^{2+} inhibited the response of rat OSNs to NIC by 96.5±1.3%. In primary CHOCs, the NIC-induced increase in $[Ca^{2+}]_i$ was abolished by the removal of extracellular Ca^{2+}. The NIC response was abolished in 4 of 5 OSNs in rats by MDL12,330A, an adenyl cyclase blocker. Our results show that 1) the odor of NIC is not dependent on nicotinic cholinergic receptors; 2) stereoselectivity supports the presence of at least two distinct NIC-responsive receptors and response modulation patterns suggest at least 3 NIC -sensitive populations of OSNs; 3) increases in $[Ca^{2+}]_i$ in response to NIC depend on extracellular Ca^{2+}; and 4) the data support the hypothesis that the NIC response is mediated by cAMP-dependent Ca^{2+} influx.

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Poster Session III: Thurs. July 24

Muscarinic Acetylcholine Receptors in Rat Olfactory Receptor Neurons and Other Nasal Epithelial Cells

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Rhinitis is a common chronic disease in the USA (E.Weir, 2003). A more complete understanding of the regulation of the nasal mucosa is essential for rational therapeutic strategies. Muscarinic acetylcholine receptors (mAChR) regulate nasal glands and blood vessels which affect nasal secretion and congestion. Since mAChRs may play an additional role by causing olfactory loss during and after rhinitis, we investigated modulation of olfactory sensory neurons (OSNs) by mAChRs. Using freshly dissociated rat OSNs, we found sensitivity to 50uM carbamylcholine(CCh), a non-selective AChR agonist. CCh caused a concentration dependent increase in intracellular calcium,[Ca 2+] i in some rat OSNs (n=9/119) as well as other cells which were not OSNs (n=36/140). OSNs responded to acetylcholine and muscarinic agonists in a dose-dependent manner (0.1-10uM). These responses were slightly dependent on the presence of extracellular Ca 2+. Neither of the nicotinic receptor antagonists, mecamylamine nor hexamethonium, blocked responses to carbamylcholine, discounting a role for olfactory nicotinic receptors in the responses to carbamylcholine. These responses, however, were completely inhibited by the non-selective mAChR antagonist 20uM atropine. Atropine also completely blocked responses to 10 uM oxotremorine M, a muscarinic acetylcholine receptor agonist. Muscarinic receptor agonists exhibited different efficacies on OSNs: Acetylcholine = carbamylcholine = oxotremorine M > bethanechol = pilocarpine > McN 343 (all tested at 50μM). While not confirmatory, this is consistent with the presence of M1 and M3 mAChR subtypes in nasal epithelial cells. These findings suggest that autonomic activity in the nasal epithelium may modulate olfactory sensitivity.

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Poster Session III: Thurs. July 24

A Role for Retinitis Pigmentosa GTPase Regulator (RPGR) in Olfactory Sensory Neurons

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Cilia are microtubule-based structures that project from the surface of most mammalian cells. Olfactory sensory neurons (OSNs) terminate in a dendritic knob containing multiple basal bodies from which sensory cilia project into the nasal mucosa. These cilia compartmentalize the signaling molecules necessary for odorant detection. Despite ever-increasing knowledge of intraflagellar transport components, the mechanisms regulating protein sorting/entry into cilia are poorly understood. Recently, we reported that LCA patients and mice with mutations in the basal body protein, CEP290, exhibit severely abnormal olfactory function due to selective mislocalization of the olfactory G-protein, G_{olf}. Here, we investigate another basal body protein, Retinitis Pigmentosa GTPase Regulator (RPGR), in the olfactory epithelium (OE). Complex alternative splicing of the *RPGR* gene results in multiple protein isoforms, with the two most prominent being $RPGR^{1\text{-}19}$ and $RPGR^{ORF15}$. Staining with two exon-specific antibodies revealed a differential localization of these two isoforms; $RPGR^{1\text{-}19}$ exclusively localized to OSN cilia and $RPGR^{ORF15}$ localized primarily to dendritic knobs. Immunoprecipitation from OE showed that RPGR is in complex with basal body and ciliary proteins. Electro-olfactogram recordings from isoforms-selective RPGR-knockout mice uncovered an anosmic phenotype. Surprisingly, ciliary localization of select components of the olfactory signaling machinery appeared unaltered in this mouse. Further investigations are required to determine the precise mechanism of the olfactory dysfunction in mice with altered RPGR function. Together, our data reveal the expression of multiple RPGR isoforms in OE, which are likely part of a multiprotein complex regulating OSN ciliary function.

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Poster Session III: Thurs. July 24

Is the Rat Olfactory System Sensitive to the Metabolic Status?

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Converging evidence indicates a strong relationship between odor and food intake at different levels of the olfactory system. The olfactory bulb (OB) integrates various metabolic informations to modulate odor processing (Pager et al.,1974). The olfactory mucosa (OM) expresses numerous neuropeptides or hormones related to food intake, such as orexins (OX) or leptin and their receptors (Caillol et al., 2003, Baly et al., 2007), suggesting modulations of the olfactory message at the first level. Furthermore, the transcriptional profile of the olfactory mucosa is modified by fasting (Badonnel et al., 2007). We explored if the olfactory system exhibits an adaptation following long-term alterations of metabolic status. We investigated changes in olfactory behaviors (hidden cookie test) and in transcriptomic levels (orexins, leptin, insulin (Ins) peptides and receptors measured by qPCR) in OM and OB of different rat strains in relation with changes in circulating metabolite and hormonal levels. Four-month-old genetically obese Zucker rats were compared with their lean counterparts or to age-matched inborn obesity resistant Lou/C. The data showed significant strain differences for most studied parameters. Both food intake, body weight, triglycerides, leptin and insulin circulating levels were significantly increased in Zucker rats. These rats displayed higher performance in olfactory-mediated food-finding behavior. Transcriptomic parameters were different among the strains in the OB, where OX, OXR2 and Ins mRNA were up-regulated in Lou/C. Our data indicate that the metabolic status modulates metabolic peptides and receptors expression at least in the OB in association with modification in olfactory behaviors. It outlines the influence of hormones as acting partners of the settings of the olfactory message.

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Two Anorectic Peptides, Insulin and Leptin, Alter Spontaneous and Odor-Evoked Activity of Rat Olfactory Sensory Neurons

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In mammals, the olfactory function is modulated by the status of satiety, which is mainly signalled by peptide hormones. However, the underlying mechanisms linking olfaction and food-intake are poorly understood. The present study investigates the influence of insulin and leptin, two anorectic peptides, on the functional properties of rat olfactory sensory neurons (OSNs), in vitro. First, the firing activity of OSNs was recorded in an intact epithelium by patch-clamping the dendritic knobs. Insulin and leptin dramatically increased OSN excitability by augmenting the spontaneous mean firing frequency in 96% (n=24) and 75% (n=24) of the cells, respectively. When the activity was electrically-evoked, perfusion of insulin or leptin shortened the latency to the first action potential by 27.5% and 34.2%, respectively, and decreased the interspike intervals by 13.5% and 13.8%, respectively. Second, the peptide effects on odorant-induced activities were analyzed. By using electroolfactogram (EOG) recordings, insulin and leptin were shown to decrease the global response to isoamyl acetate stimulation to 46% and 38%, respectively. Patch-clamp recordings from some OSNs were consistent with the EOG data. Indeed, peptides significantly reduced the inward transduction current evoked by isoamyl acetate under voltage-clamp, and decreased the duration of the odor-elicited receptor potential under current-clamp. The results suggest that insulin and leptin may decrease the global signal to noise ratio of the OSNs' response to odors and consequently, modulate the impact of the primary sensory message on the olfactory bulb.

Poster Session III: Thurs. July 24

The FXG: A Novel FMRP-Containing Granule Expressed in Olfactory Nerve Terminals

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FMRP is an RNA binding protein whose loss leads to Fragile X syndrome (FXS), the most common inherited mental retardation and single gene cause of autism. As FXS is characterized by hypersensitivity to sensory stimuli, including olfactory input, we examined the expression of FMRP and its homologues, FXR1P and FXR2P, in the developing, mature and regenerating rodent olfactory system. In agreement with earlier studies, FMRP is localized in the cell bodies and proximal dendrites of virtually all neurons. However, we also observe FMRP in discrete granules (Fragile X Granules; FXGs) that, within the olfactory bulb, are selectively expressed within the olfactory nerve layer and glomeruli. FXGs are also expressed in a subset of other brain regions, including frontal cortex and area CA3 of the hippocampus. Immunoelectron microscopy shows that FMRP is localized at presynaptic terminals and in axons in these granule-rich regions. While FXGs are prominent only during development in most brain regions, they persist in olfactory glomeruli in mature animals and are transiently upregulated during regeneration of adult olfactory circuits. All FXGs contain FXR2P, while region-selective subsets harbor FMRP and/or FXR1P. Genetic studies show that FXR2P is essential for FXG formation, while FMRP regulates FXG number and developmental expression. These data suggest that Fragile X proteins and local translation play a distinct, presynaptic role during discrete developmental epochs in defined circuits of the mammalian CNS. Moreover, the neurological defects in FXS, including olfactory hypersensitivity, could be due in part to the loss of FMRP function in presynaptic and/or axonal compartments in these distinct neuronal circuits.

Poster Session III: Thurs. July 24

Differences in Matrix Metalloproteinase-9 Expression in Two Olfactory Injury Models

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The olfactory system has a remarkable capacity for neural regeneration and recovery after both peripheral and central injury, yet the mechanisms underlying recovery are poorly understood. We previously reported that matrix metalloproteinases (MMPs), enzymes that regulate the extracellular matrix, are elevated during critical times following nerve transection injury. In this study, we compared MMP-9 levels in two injury models: nerve transection, a central injury to olfactory axons projecting to the olfactory bulb, and methyl bromide gas exposure, a peripheral injury that kills olfactory neurons directly. We measured MMP-9 levels in the olfactory bulb of mice at different recovery time points after injury using Western blot. We also monitored glial fibriallary acidic protein (GFAP) and olfactory marker protein (OMP) to detect astrocytic activation (gliosis) and reinnervation by mature olfactory neurons, respectively, in the olfactory bulb. In the nerve transection model, MMP-9 expression was detected within hours and peaked at day 1. In the methyl bromide model, MMP-9 expression was delayed and peaked at day 5. In both models, GFAP levels increased by day 1, reflecting the presence of gliosis, and remained elevated for several weeks. OMP levels began to decrease by day 1, indicating degeneration of olfactory neurons. By day 10, OMP levels in the nerve transection injury model begin to recover, reflecting reinnervation of the olfactory bulb. However, in the methyl bromide gas injury, OMP levels had not yet recovered by 3 weeks. This is the first report demonstrating a difference in the expression of MMP-9 for two types of neural injury, central vs. peripheral, suggesting that MMP-9 may play an important role in specific components of neuronal injury and recovery processes.

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Poster Session III: Thurs. July 24

Genetic Ablation of Atruncated TRKB Isoform (TRKB.T1) Increases Adult Olfactory Bulb Neurogenesis

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The addition of new neurons in the adult olfactory bulb (OB) has been proposed as a mechanism to enhance plasticity with specific benefits for olfactory discrimination. Currently the signaling mechanisms regulating adult OB neurogenesis are poorly understood. We have recently demonstrated that brain-derived neurotrophic factor (BDNF) and its receptor TrkB are critical regulators of neuroblast migration from the SVZ to the OB in the adult animal. In our current study, we assess the role of the truncated form of TrkB (TrkB.T1) as a potential mediator of adult neurogenesis. TrkB.T1 binds BDNF with high affinity but lacks the functional kinase domain of the full length TrkB receptor. Thus, TrkB.T1 is predominantly thought to function as an endogenous dominant negative that sequesters BDNF and hinders activation and downstream signaling of the full length TrkB receptor. To assess the role of TrkBT.T1 in adult neurogenesis we used a recently developed mouse linein which the truncated TrkB receptor type I isoform has

been genetically ablated (TrkB.T1 knock-out). We demonstrate that loss of TrkB.T1 leads to increased migration and survival of new neurons in the adult OB. In addition, we provide *in vitro* evidence for secondary effects of TrkB.T1 in the regulation of cellular proliferation. Based upon these and previous data from our lab, we present an interactive model in which TrkB.T1 may dynamically regulate BDNF-TrkB signaling, and thus play a functional role in altering rates of adult neurogenesis *in vivo*.

Poster Session III: Thurs. July 24

Successful Olfactory Transplantation in Mice: Comparison of Methods

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Impaired olfactory function leads to a decrease in the quality of life for many patients. Often clinicians have few, or no, treatment options to offer these patients. This study investigates new treatment strategies based on different methods of transplanting the olfactory epithelium directly onto the olfactory bulb (OB). Strips of olfactory epithelium obtained from donor GFP (green fluorescent protein) mice were transplanted to sites in both the olfactory bulb and cerebral cortex of recipient wild type mice. Transplant tissue and survival within the host brain was confirmed by the presence of GFP positive cells. Transplant failure often occurs when there is donor tissue expulsion. Survival rates were determined for two transplant methods, blade and rod, along with size of donor tissue, transplant site and different holding times used in an attempt to prevent tissue expulsion. For OB transplants, the success rate for the rod method was higher than that for the blade method. The success rate for the rod method was decreased when the holding time was increased from 0.5 to 5 minutes. In cortex, both the rod and blade methods yielded higher success rates than in the OB. The success rate in the OB transplants also decreased when the size of donor tissue was increased from 0.25 to 1mm. We conclude that optimal conditions for transplantation in the OB are achieved using the rod method and that increasing the holding time may result in damage to the OB.

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Poster Session III: Thurs. July 24

Expression of IGSF8, A Novel Cell Adhesion Molecule, in the Developing Mouse Olfactory Pathway

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Precise targeting of axons to their correct targets during development is critical for neuronal wiring. The mouse olfactory system is an excellent model system to study axon guidance due to the complex trajectory of axonal projections between the olfactory epithelium (OE) and olfactory bulb (OB). The roles of cell adhesion molecules (CAMs) in neuronal migration, target recognition and synapse formation are well documented in the CNS. Despite the burgeoning evidence of the roles of various growth promoting and inhibitory factors in olfactory development such as netrins, ephrins, slits and semaphorins, the function of various CAMs remains largely unknown. We therefore hypothesized that CAMs may participate in maintaining the topography in the olfactory system (OS). Thus, our aim is to determine whether CAMs play a role in olfactory axonal navigation from the OE to the OB. Using a commercially available oligo array we screened a small set of genes consisting of CAMs and genes involved in synaptogenesis at different developmental stages. Using these arrays we identified several novel genes in the OE and OB as candidate guidance cues. One gene, IgSF8 belongs to the immunoglobulin superfamily (IgSF) and likely mediates cell-cell interactions. We have confirmed the expression of IgSF8 in the olfactory sensory neuron (OSN) axons of the olfactory nerve using immunohistochemistry. IgSF8 protein expression was observed in the OS as early as embryonic day (E)13. Protein levels in the olfactory bulb increase postnatally, concomitant with increases in OSN axons within the developing OB. Expression was equally prominent in the developing spinal cord and retina in developing axon tracts. These data lead us to hypothesize that IgSF8 may be more broadly involved in axon targeting in the developing nervous system.

Poster Session III: Thurs. July 24

Requirement of Slits and Robo-2 in the Segregation of Basal Vomeronasal Neuron Axons to the Accessory Olfactory Bulb

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The establishment of proper connectivity in the nervous system is essential for its function. In both the main and accessory olfactory systems, axons of chemosensory neurons form stereotypic connections with higher-order neurons in the CNS, allowing sensory stimuli to be translated into neural information. The formation of these connections is essential for olfactory function and relies on axon guidance molecules to direct pathfinding axons to their correct targets. The mechanisms involved in directing the formation of precise connections between sensory neurons in the vomeronasal organ (VNO) and their target field, the accessory olfactory bulb (AOB), are not yet fully understood. We are interested in defining the axon guidance cues that can promote the targeting of basal VNO neuron axons to the posterior AOB. We have examined the pattern of expression of Slit family members and their receptors, Robos, in the accessory olfactory system. We have shown that Robo-2 expression is restricted to basal vomeronasal neurons and that Slits are expressed in the AOB. To evaluate the role of Slits and Robos in this system, we have analyzed vomeronasal projections in mice lacking Slit family members or Robo-2. We have shown that ablating expression of Robo-2 *in vivo* in vomeronasal neurons leads to mistargeting of basal vomeronasal neuron axons to the anterior region of the AOB. Similar defects are observed in Slit mutant mice demonstrating that Slit-Robo-2 interactions are required for the accurate segregation of vomeronasal projections within two specific regions of the AOB.

Poster Session III: Thurs. July 24

Neuroglian and FGFR Interactions in Development of the Glia-Rich Axon Sorting Zone in the Moth Olfactory Pathway

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In the olfactory pathway of the moth *Manduca sexta*, olfactory receptor axons (ORNs) are sorted into target-specific fascicles in a discrete glia-rich region of the nerve called the Sorting Zone (SZ). The first ORN axons to arrive near the olfactory lobe induce glial proliferation and migration, which populates the SZ; upon encountering SZ glia, later arriving axons separate from their neighbors, dramatically change directions, and leave in new fascicles. In co-cultures of ORN axons and SZ glia, the growth cones of ORN axons become more complex shortly after contact with the glia. Immunocytochemical data have shown that fibroblast growth factor receptors

(FGFRs) are activated on the glia, epidermal growth factor receptors (EGFRs) are activated on ORN axons, and the cell adhesion molecule neuroglian becomes tightly anchored in axonal and glial membranes. To test the hypothesis that interaction between neuroglian molecules on axonal and glial membranes elicits activation of the FGFRs on glial cells, we have injected PD173074, a specific blocker of FGFR activation, into developing animals at the onset of ORN ingrowth into the olfactory lobe. The number of SZ glia decreases, consistent with an effect on glial proliferation or survival, and the behavior of Fasciclin-II^{+} axons in the SZ is disordered, as expected if the SZ glia network is disrupted. We also are using time-lapse imaging and immunocytochemistry to examine in co-cultures of SZ glia and ORN axons the effects of neuroglian and FGFR activation on axonal growth cone behavior, axon outgrowth, and glial morphology and movement. Finally we are labeling SZ glia by dye-filling in slice preparations or with an antibody against a *Manduca* GABA transporter to study the effect of blocking FGFR activation on SZ glia morphology.

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Poster Session III: Thurs. July 24

Identification of Mitral/Tufted Cell-Specific Transcriptional Enhancer Upstream of Mouse *Tbx21* Gene

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The mitral/tufted cells are excitatory projection neurons in the olfactory bulb, which relay the odor information coming from the olfactory epithelium to various areas of the olfactory cortices. In spite of their functional importance in odor information coding and processing, there are few molecular and genetic tools that can be used for specific manipulation of the mitral/tufted cells. Tbx21 (T-box 21) belonging to the T-box gene family was first identified as a transcription factor regulating the differentiation and function of T cells. In the nervous system, Tbx21 is specifically expressed in the mitral/tufted cells of the olfactory bulb. In the present study, we performed a promoter/enhancer analysis of the mouse *Tbx21* gene by comparing nucleotide sequence similarities with *Tbx21* gene from other mammalian species and generating various transgenic mouse lines with a fluorescent protein reporter. Consequently, we identified a cis-regulatory enhancer element (307 bp) at ~ 3 kb upstream of the transcription start site of *Tbx21* gene, which is both necessary and sufficient for mitral/tufted cell-specific transgene expression. Furthermore, fine morphology and presynaptic activity of the mitral cells could be visualized by transgenic expression of photoconvertible fluorescent protein Kaede and exocytosis-monitoring reporter synaptopHluorin, respectively, under the control of *Tbx21* gene enhancer. Thus, this enhancer will be used as a powerful genetic tool for future studies on the development and function of the mitral/tufted cells.

Poster Session III: Thurs. July 24

Role of Trkb in Dendritic Development of Mitral Cells in Mouse Olfactory Bulb

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Mature mitral cells in the mammalian olfactory bulb (OB) have a single primary dendrite that arborizes within a single glomerulus. Here, we focused on mechanisms regulating the development of mitral cell dendritic arbors within a glomerulus. First, we visualized the development of glomerular tufts of mitral cells in mice using intracellular lucifer yellow injections. We found that dendritic tufts in a glomerulus significantly increase their total length and number of branching points during early postnatal days, especially from postnatal day (P) 3 to 10. Second, using immunohistochemical analyses, we found that the truncated isoform of TrkB (TrkB.T1) is localized at the tip of mitral cell dendrites including dendritic tips in the glomerular tuft, while full-length TrkB (TrkB.FL) was expressed by thick dendritic trunks of mitral cells. Interestingly, TrkB.T1 expression in glomeruli was high during early postnatal days, but disappeared by P10. Third, to examine the role of TrkB in dendritic development, we cultured mitral cells and treated them with neurotrophins: BDNF, NT-3, and NT-4. In cultured mitral cells at 1 day *in vitro* (DIV), localization of TrkB.T1 at the tip of neurites was observed, while TrkB.FL was seen in whole neurites. When treated with BDNF or NT-4 from 0 to 4 DIV, mitral cells significantly increased the number of primary neurites and branching points, as well as their total neurite length compared with untreated controls. NT-3 treatment did not have a significant effect. Our findings strongly suggest that TrkB.T1 expression during dendritic development plays a significant role in the elaboration of mitral cell glomerular dendritic arbors, consistent with our working hypothesis that neurotrophins are determinants of OB circuitry.

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Poster Session III: Thurs. July 24

Functional Insight into the Role of Phosphoinositide-3-Kinase in Mammalian Olfactory Receptor Neurons

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Phosphoinositide-3-kinase (PI3K) activity can modulate the response of acutely dissociated rat olfactory receptor neurons (ORNs) to complex odorants, potentially through regulating cyclic nucleotide signaling (Spehr et al., Neuron, 2002). The same pan-selective PI3K antagonists, Wortmannin and LY294002, can modulate the response of ORNs to complex odorants monitored in the intact rat olfactory epithelium (OE) through loose-patch recording from dendritic knobs, and do so in a manner consistent with the release of inhibition as in the initial finding. PI3K-dependent enhancement of the odor evoked response shifts the dose-response curve up to one log unit, suggesting strong modulation. Activation of P2Y purinergic receptors, also thought to be coupled to phosphoinositide signaling, with ATP or UTP failed to modulate odor responses in a PI3K-dependent manner, suggesting PI3K-dependent activity is mediated through odorant receptors rather than through purinergic modulatory receptors known to occur in mammalian ORNs. Gamma isoform-specific inhibitor I (Calbiochem) has the same effect as the pan-selective antagonists, suggesting the modulation is mediated through G-protein coupled receptors (GPCRs). AS252424, another gamma isoform-specific blocker, and TGX-221, a beta isoform-specific blocker also known to couple through GPCRs in other cells, can both modulate the calcium signal in acutely dissociated rat ORNs evoked by complex odorants, providing further evidence for mediation through GPCRs. Western blot analysis to date confirms the presence of at least PI3K-gamma in a ciliary membrane preparation of the rat OE. We conclude that the response of rat ORNs to complex odorants is mediated in part through PI3K-dependent activity coupled to the activation of odorant receptors.

Poster Session III: Thurs. July 24

Phosphoinositide 3-Kinase Mediated Signaling in Lobster Olfactory Transduction

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Recent work has suggested a potential role for phospholipid signaling in olfactory transduction in both invertebrate and mammalian olfactory receptor neurons (ORNs) through the involvement of phosphoinositide 3-kinases (PI3Ks) (Brady et al., 2006; Spehr et al., 2002; Zhainazarov et al., 2001). Class I isoforms of PI3K convert phosphatidylinositol-4,5-bisphosphate (PIP_2) to phosphatidylinositol-3,4,5-trisphosphate (PIP_3) in response to extracellular stimuli. The β and γ isoforms of class I PI3Ks are activated by G protein-coupled receptors (GPCRs) and thus may be relevant to olfactory signal transduction. Here, we show that western blotting with isoform-specific antibodies revealed a protein extracted from the outer dendrites of lobster ORNs that is immunoreactive with an antibody directed against the mammalian PI3Kγ isoform. We subsequently identified two class I PI3Ks in lobster olfactory tissue cDNA library by RT-PCR and sequencing. The lobster PI3K co-immunoprecipitated with both G_{α} and $G_{\beta\gamma}$. Odorant-evoked PI3K activity could be detected using a protein-lipid overlay assay in the protein extracted from the outer dendrites. Finally, a potent, PI3Kγ-specific inhibitor, AS-252424, reduced the odor-evoked output of lobster ORNs recorded *in situ*. Collectively, these findings implicate the involvement of a PI3K similar to the mammalian PI3Kγ isoform coupled via G protein activation (Wu et al., 2007) in lobster olfactory transduction.

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Poster Session III: Thurs. July 24

Phosphoinositide Metabolism is Essential for Regulating the Output of Lobster Olfactory Receptor Neurons

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Transient receptor potential (TRP) channels often play a role in sensory transduction, including chemosensory transduction. TRP channels, a common downstream target of phosphoinositide signaling, are known to be modulated by exogeneous phosphatidylinositol 4,5-bisphosphate (PIP2), phosphatidylinositol 3,4,5-trisphosphate (PIP3), and/or DAG. Lobster olfactory receptor neurons (ORNs) express a TRP-related, Ca^{2+}/Mg^{2+} permeable, non-selective, sodium/calcium gated, cation channel. Here we report that phosphoinositides are essential to maintain the function of the lobster channel. Chelation of endogenous PIP2 with either an anti-PIP2 antibody or by electrostatic screening with polyvalent cations, or hydrolysis of PIP2 by activation of endogenous PLC, accelerated rundown and/or blocked the channel. Exogenous PIP3 activated the channel independently of intracellular sodium and/or calcium. Exogenous non-hydrolysable DAG analogs failed to change the gating parameters of the channel, suggesting the channel was insensitive to DAG. Electrophysiological recording from lobster ORNs in situ coupled with phosphoinositide binding assays in conjuction with a panel of pharmacological tools targeting the key components of both phosphoinositide and DAG metabolism (phosphoinositide 3-OH kinase, phospholipase C, phosphoinositide 4-kinase and DG-kinase) was used to measure if and how changes in lipid concentration correlate with ORN output. PIP2 depletion suppressed both the odor-evoked whole cell current and the odor-evoked discharge of ORNs, and did so independently of DAG production. Collectively, our results demonstrate that accurate turnover of phosphoinositides is essential for regulating the output of lobster ORNs, at least in part through their action on the olfactory TRP-related ion channel.

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Poster Session III: Thurs. July 24

The Roles of Phosphodiesterases in Shaping the Odor-Evoked Responses of Olfactory Sensory Neurons

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The cilia of olfactory sensory neurons (OSNs) are cellular compartments specialized for odor detection. Phosphodiesterase (PDE) activity in the cilia has long been hypothesized to account for rapid OSN response termination by degrading odor-induced cAMP. Two PDEs, PDE1C and PDE4A, have been found in OSNs. PDE1C is enriched in the cilia, while PDE4A is localized throughout the cell including the dendrite from where the cilia emanate, but is excluded from the cilia. We knocked out *pde1c* and *pde4a* genes in mice and measured the electroolfactogram (EOG) from the mutant mice. Lack of PDE activity in the cilia was expected to slow response termination, and might also lead to larger responses with quicker onset. Surprisingly, disrupting the cilial PDE, PDE1C, resulted in reduced EOG amplitude, slower response onset kinetics, and accelerated response termination. Prolonged response termination was only observed in mice that lack both PDE1C and PDE4A, whereas disrupting PDE4A alone did not affect OSN responses. As PDE4A does not localize to OSN cilia, the rapid termination still observed in $PDE1C^{-/-}$ mice but lost in the double knockout mice implies that PDE4A can contribute to OSN response termination by degrading cAMP in the dendrite. Computer modeling suggested that cAMP diffusion out of the cilia followed by degradation by PDE4A could be sufficiently fast to account for rapid termination. Together these data suggest that one of the major functions of the cilial PDE, PDE1C, is to allow high sensitivity of OSNs, while PDE4A serves to constrain cilial cAMP. The activity of either PDE is sufficient for rapid removal of cilial cAMP following stimulation. These observations provide a new perspective in the compartmental control of second messengers as well as in modulation of olfactory signal transduction.

Poster Session III: Thurs. July 24

MYR-RIC8A Enhances $G\alpha_{15}$-Mediated Ca^{2+} Response of Vertebrate Olfactory Receptors

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The determination of ligand specificities of odorant receptors (ORs) will contribute to the understanding of how odorants are discriminated by the olfactory system. To date, some ORs have paired with their cognate ligands using Ca^{2+} imaging, one of the most commonly utilized reliable methods for detecting activation of GPCRs in heterologous cells. However, most of ORs have been failed to be expressed functionally in heterologous cells and to assay their ligand binding because they are poorly translocated to the cell surface. Recently, RTP1 and Ric8B were identified as factors that help solve the problem. Here, we employ myristoylation sequence-conjugated mutant of Ric8A (Myr-Ric8A), guanine nucleotide exchange factor for Gαq, as a signal amplifier. As a result, co-expression of Myr-Ric8A

greatly enhanced $G\alpha_{15}$-mediated Ca^{2+} responsiveness of endogenous β_2 adrenergic receptor and three ORs heterologously expressed in HEK293 cells. Co-expression of Myr-Ric8A and RTP1 enables us to de-orphanize MOR139-3 as a receptor for *m*-cresol using Ca^{2+} imaging. Further investigation revealed that MOR139-3 had a broad molecular receptive range that included not only aromatic compounds such as eugenol but also aliphatic compounds such as 2-octanol. Our results suggest that Myr-Ric8A should be helpful in functional characterization of ORs in heterologous cells using Ca^{2+} imaging.

Poster Session III: Thurs. July 24

The Na^+/Ca^{2+} Exchanger Inhibitor, KB-R7943, Potently Blocks a Presumptive TRPC Channel Homolog Implicated in Lobster Olfactory Transduction

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The absence of specific pharmacological tools targeting TRP channels caused us to continue to search for specific pharmacological blockers of the lobster olfactory sodium-gated channel (SGC), a presumptive TRPC channel homolog involved in olfactory transduction. Given recent work (R. Kraft, Biochem. Biophys. Res. Comm. 361:230, 2007) showing that the Na^+/Ca^{2+} exchanger inhibitor, KB-R7943, potently blocks TRPC channels, we investigated this probe as a specific blocker of the lobster SGC. KB-R7943 reversibly inhibited the odorant-evoked discharge of both phasotonic and bursting lobster olfactory receptor neurons (ORNs) in a dose-dependent manner. KB-R7943 (50μM) completely and reversibly inhibited the odorant-evoked whole-cell current. KB-R7943 reversibly blocked the SGC in both outside- and inside-out patch recordings in a dose- and voltage-dependent manner. KB-R7943 decreased the channel open probability without changing single channel conductance. Another blocker with a greater selectivity for the Na^+/Ca^{2+} exchanger, SN-6 (10μM), had no effect on either the odorant-evoked discharge of the ORNs nor on the SGC recorded in inside-out patches, suggesting that KB-R7943 was acting on the channel directly and can be considered a potent inhibitor of the lobster olfactory SGC channel.

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Poster Session III: Thurs. July 24

Expression of *TRP* Channel Genes in the Antenna of the Malaria Mosquito *Anopheles Gambiae*

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The malaria vector mosquito, *Anopheles gambiae*, utilizes heat as well as odors as crucial cues in its host-seeking behavior. In an attempt to understand the molecular and cellular basis of thermosensation in *An. gambiae*, we carried out reverse transcriptase-PCR amplifications with primer pairs targeting *Anopheles* homologs of *Drosophila transient receptor potential* (*TRP*) channel genes. Here we report that several *TRP* channel genes were consistently detected in the antenna of *An. gambiae*. Fluorescent *in situ* hybridization experiments revealed that they were expressed in a discrete and stereotypic subset of antennal neurons consistent with the view that these *TRP* channels are involved in the host-relevant thermo-detection associated with this mosquito sensory appendage. Our results offer insight into an important molecular aspect of mosquito host seeking and may facilitate the on-going effort to reduce malaria transmission by *An. gambiae*.

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Poster Session III: Thurs. July 24

Analysis of G Proteins in the CO_2 Response of *Drosophila*

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Carbon dioxide (CO_2) is an important chemical signal for many insect species. In *Drosophila melanogaster*, a population of neurons in the antenna is dedicated to the detection of CO_2. Recent work identified two receptors, Gr21a and Gr63a, which are expressed in these neurons and are necessary and sufficient for CO_2 detection. Gr21a and Gr63a belong to a large family of seven-transmembrane-domain chemoreceptor proteins. Given their heptahelical structure, we attempted to determine whether G proteins are necessary for the CO_2 response. Overexpression of constitutively active forms of Gαi and Gαo did not affect the electrophysiological response to CO_2. However, overexpression of constitutively active forms of Gαq and Gαs decreased the CO_2 response. To further investigate the roles of Gαq and Gαs, we used RNAi to knock down expression levels and competitive inhibitor peptides to decrease the activity of these two proteins. While disrupting Gαs did not have an effect on the electrophysiological response to CO_2, both RNAi and inhibitors to Gαq decreased the CO_2 response. We then generated a *Gαq* deletion line using P-element excision. Flies heterozygous for this deletion showed a decrease in CO_2 response. Flies heterozygous for a *Gαs* deletion have a normal response to CO_2. When ectopically expressed, Gr21a and Gr63a can confer a CO_2 response to neurons that normally are insensitive to CO_2. However, the response is lower than that of the endogenous CO_2 neuron. Co-expression of Gαq, but not Gαs, with Gr21a and Gr63a increased this CO_2 response. Taken together, our data suggest that Gαq acts either directly or indirectly in CO_2 response.

Poster Session III: Thurs. July 24

Evidence for the Role of Inspiration in Retronasal Olfactory Responses Measured by the Electroolfactogram

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Electroolfactograms were recorded from the dorsal and lateral regions of the olfactory epithelium during orthonasal and retronasal olfactory stimulation. For retronasal stimulation, odorants were injected into the retronasal space with a cannula inserted up the trachea. Single and multiple odor pulses were used in rats overdosed with pentobarbital.As we previously reported, hydrophobic non-polar odorants (such as myrcene and vinyl cyclohexane) were the most effective stimuli during the expiratory phase of retronasal stimulation. However, when we measured the responses to inspiration after expiration of odorants, there were increased responses to odorants of intermediate hyphobicity and polarity. Examples of such odorants were ethyl butyrate, hexanone, and hexanal. A very polar odorant (methyl benzoate) was not effective in either phase of retronasal stimulation even though it produced large orthonasal responses. We speculate that the intermediate-polarity odorants did not effectively enter the olfactory space with expiratory air flow, but a small volume of those odorants was held in the non-olfactory region near the external nares and was pulled into the olfactory region during inspiration. Very polar odorants, by contrast, would be sorbed out of

the airstream before reaching the anterior part of the nose. These data suggest different trajectories for air flow within the nasal cavity between orthonasal and retronasal olfaction. They also suggest that the expiratory phase may favor strong responses from more than only the non-polar odorants. They are further evidence for the differences in perception orthonasally vs. retronasally in human psychophysical studies.

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Poster Session III: Thurs. July 24

Odor Concentration-Detection Functions in Humans for Homologous N-Acetates

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Models of quantitative structure-activity relationships (QSARs) for odor potency constitute important tools for understanding basic and applied aspects of human olfaction. The present study is part of a project that aims to define human olfactory sensitivity towards a variety of volatile organic compounds (VOCs), in a QSAR context, via concentration-detection (i.e., psychometric) functions rather than single odor detection thresholds. Stimuli included ethyl, butyl, hexyl, and octyl acetate. Vapors were presented via an 8-channel vapor delivery device, designed to provide optimal odor sampling conditions, and were quantified by gas chromatography. Subjects ($n \geq 16$) from both genders, normosmic and nonsmokers, used a three-alternative, forced choice procedure against carbon-filtered air blanks, in an ascending concentration approach. A sigmoid (logistic) function accurately modeled odor detectability both at the group and individual level. Two parameters defined each function: C, the concentration detected at halfway between chance and perfect detection (i.e., the odor threshold), and D, the function steepness. The thresholds obtained were lower than most previously reported but shared with them a similar trend along the homologous series. Steepness of the averaged individual functions increased slightly but significantly with carbon chain length. Variability in odor thresholds across participants was relatively low: close to one, and always lower than two, orders of magnitude. No gender differences emerged. The outcome supports the notion that a QSAR based on a solvation equation holds promise to describe and predict the absolute olfactory potency of VOCs, now comprehensively defined as full psychometric odor functions, and not just as single, relative threshold values across vapors.

Poster Session III: Thurs. July 24

The Effect of Different Odorants on Rapid Olfactory Adaptation in Humans

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In an accompanying presentation (Smith, Gamble and Heil) we introduce a new psychophysical technique for estimating olfactory rapid adaptation (RA) in humans. That study demonstrated that RA to vanilla odor can be measured within 100-200 ms following stimulus onset. In this work we compare RA, measured using the same technique, to three different odorants, vanilla, coconut and 2-propanol. As described in the accompanying presentation, we used a liquid-dilution olfactometer to estimate thresholds for brief target odorant presentations. Twenty-five college-aged volunteers served as subjects. The adapting odorant concentration was set to twice the baseline threshold for the 600-ms target. To evaluate RA, we compared thresholds for targets presented simultaneously with the same adapting odorant as a function of the relative delay between the onset of the adapting stimulus and the onset of the target. RA measured for each odorant reflected the characteristic RA onset, with thresholds for the target stimulus increasing in an orderly manner with increases in onset delay, though the rate of threshold increases varied with odorant type. The estimated time constants for the RA onset were 400 ms for propanol, 300 ms for vanilla extract and 150 ms for coconut extract. While the observed differences in RA time constants were not statistically significant, they are suggestive of differences in mechanism. One possible explanation for this variance may be the relative trigeminal quality of the odorants, where propanol and other alcohols, including the base for vanilla extract, activate trigeminal receptors as well as, or in place of, olfactory receptors.

Poster Session III: Thurs. July 24

Cross Adaptation of Green Odors with or 1-7 Agonists

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A set of straight chain aldehydes ranging in length from seven carbons to ten carbons have been identified to excite olfactory receptor (OR) I-7 in mice {Zhao, 1998 58}; {Araneda, 2000 59}. The greatest number of neurons are activated when exposed to octanal (C8), and very little excitation occurs when exposed to hexanal (C6). The receptor is responsive to (C10) and lower to undecanal (C11). C8, C10, and C11 all exhibit a citrus-like quality, while C6 is green. Homology modeling has revealed striking similarities between the human, mouse and rat I-7 receptors. Studies have shown that similar quality odors can cause cross-adaptation. This study examines the cross-adaptation of these four compounds. It would be expected that the greatest cross-adaptation would occur between C8 and C10 odors which both excite the I7 receptor. Little to no cross adaptation should occur for C6 and C8, C10, and C11. Stimuli were polypropylene squeeze bottles containing a single perfume blotter dipped in approximately one inch of odorant dissolved in poly(ethylene glycol). Bottles were retrofitted with a teflon ball placed at the tip so subjects could place the squeeze bottle against the nose. Subjects were presented with three bottles and asked to rate the first and third bottles in the series for perceived intensity. A subject adapted to the second bottle by taking five deep breathes, each lasting approximately three seconds. Self adaptation was evident in all four aldehyde conditions. There was strong cross adaptation between octanal, decanal, and undecanal. With the strongest cross-adaptation between decanal and undecanal.

Poster Session III: Thurs. July 24

Undecanal as an Antagonist of Bourgeonal at Isointense Levels

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Potential antagonism between odors is of interest when trying to predict the odor quality of a mixture. In a study by Spehr et al. (2004) it was shown that the odorant Undecanal acts as an antagonist to Bourgeonal when presenting them serially. Would this antagonism also be portrayed as asymmetry in the odor mixture quality? First, we attempted to replicate the study by Spehr et al., although comparing isointense odors of intermediate intensity. Fifteen men and fifteen women were presented triads of odorants in quick succession with Undecanal or control odorants (n-Butanol, Veilex) presented in the second position. The participants were asked to indicate the intensity of the odors in the second and third position in comparison to a value of the first odor, which was set by the experimenter. The results of the study show that

Undecanal did not inhibit the intensity of Bourgeonal more effectively than the control odorants. The data also suggest that women seem to perceive suprathreshold levels of Bourgeonal as more intense than men. A second study underway investigates the symmetry of odor mixture quality following simultaneous presentation of Bourgeonal and Undecanal.

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Poster Session III: Thurs. July 24

Modulation of Olfactory Perception: Antagonists of Butane-2,3-Dione

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Previously reported studies have shown a that some odorants, that have a similar structure than eugenol, can activate or inhibit the eugenol mouse olfactory receptor according to the nature and the position of the chemical group (allyl or aldehyde for example). These studies indicate that a molecular structure analogy between odorants might be a key parameter to find interactions (agonists and antagonists). Our objectives was to find in humans, using psychophysical methods, supporting evidences for mechanisms at the level of olfactory receptors responsible for perceived aroma-aroma interactions by identifying antagonists of specific odorants (e.g. butane-2,3-dione) on the basis of molecular structure analogy and olfactory properties. Such antagonists could then be used to avoid off-odour perception in food. Psychophysical methods were used to investigate in humans the interactions between odorants in binary mixtures, based on perceived odour intensity and quality. A new method based on complete adaptation was specifically developped to easyly screen for receptor antagonist. An odorant with a fruity character, 3-methyl butyl propanoate, was found to mask the buttery notes of butane-2,3-dione. we showed that the masking effect was evidenced without cross-adaptation or de-adaptation between 3-methyl butyl propanoate and butane-2,3-dione. We show that interactions responsible for this observed masking are most likely occurring in the processing of the olfactory signals by the nervous system rather than by direct interactions, e.g. antagonism, on specific odor receptors.

Poster Session III: Thurs. July 24

Self–Rating and Olfactory Function

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Introduction: Substantial anecdotal and some scientific evidence indicates that a majority of women believe their sense of smell is heightened during pregnancy. However, evidence supporting heightened olfactory sensitivity during pregnancy is limited and inconclusive. Previous studies have reported that accurate self-ratings of olfactory (and gustatory) function are limited to some clinical populations. This study was designed to examine the relationship between self-report and odor sensitivity in healthy young women (non-pregnant and pregnant) and men with no reported smell dysfunction.

Methods: Nineteen non-pregnant and 18 pregnant women (1st trimester) and 19 males rated their sense of scale on a 9–point Lickert scale and odor thresholds for phenyl ethyl alcohol were established using a standard staircase procedure.

Results: There was no correlation between self-rating and odor threshold in any of the groups. Although pregnant women rated their sense of smell higher than non-pregnant females, who rated their sense of smell marginally higher than males, there were no differences among the groups in odor thresholds. A preliminary signal detection analysis revealed no difference in d', nor in trial-by-trial confidence ratings between pregnant and non-pregnant women. Confidence ratings were higher for hits than for false alarms regardless of pregnancy status.

Conclusion: These data support the hypothesis that normosmic individuals are inaccurate in their assessment of their olfactory sensitivity. They further suggest that pregnancy may not affect olfactory sensitivity per se. Inflated self-ratings during pregnancy may reflect changes in cognitive odor information processing. These data are part of an on-going longitudinal study of olfactory sensitivity across the three trimesters of pregnancy.

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Poster Session III: Thurs. July 24

Odor Representation Through the Lens of Odor Identification

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One hundred eighty two persons sought to identify 53 everyday objects by smell. The subjects, males and females, ranged from their teens to their eighties. The outcome had much in common with previous studies that showed effects of age and sex on performance, though now with a level of detail not previously available. The outcome also showed the improvement in performance of offering potentially confusable choices derived from free identification. Importantly, the results from so many persons allowed treatment of free identification as a "confusion" task where the analysis entailed use of the same name, correct or incorrect, for different objects as an index of confusion. The confusion matrix virtually eliminates subjectivity in scoring. The matrix of items-by-items allows multidimensional representation of how close or far apart any two items lie in perceptual/semantic space. By inventive graphics, we can show actively how the internal representation of odors changes as a person ages or goes from ad lib naming to naming via choices. We can also show how a given odor object looks compared with another. In three-dimensional space, some look short, squat, and others tall, lean. These representations afford a way to choose odorants for tests of aptitude. We have, at present, numerous tests of identification to assess impairment. In general, because of ceiling effects, these fail to select for exceptional performance. We seek to correct this imbalance, with an eye towards identification for either choice of experts or evaluation of persons with complaints about environmental odors, the latter group quite "odor vigilant." Epidemiological results imply that many in the US population find certain everyday odors aversive.

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Poster Session III: Thurs. July 24

Eye Closure Enhances the Olfactory Discrimination Ability but not the Olfactory Sensitivity of Human Subjects

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In a previous fMRI experiment we investigated the effects of the conditions eyes-open and eyes-closed in complete darkness on the activation of cortical areas. We found that ocular motor and attentional cortical areas where activated during the eyes-open condition. On the contrary, sensory brain areas, especially olfactory and gustatory brain areas, were animated without external stimulation, just by eye closure in complete darkness. These results support the hypothesis of two different states of mental acitivity: an "exteroceptive" state characterized by attention and ocular motor activity (eyes-open condition) and an "interoceptive" state characterized by

multisensory activity (eyes-closed condition). Therefore the hypothesis of the current study was that olfactory performance of human subjects differs regarding to the eyes-open and eyes-closed conditions. Especially it was hypothesized that subjects have a higher olfactory sensitivity and ability to discriminate odors when smelling with their eyes closed compared to eyes open. Olfactory sensitivity to n-butanol and olfactory discrimination performance was investigated using two subtests of the Sniffin' Sticks test battery. Fifty-three healthy human subjects (27 females, 26 males) were tested under the conditions eyes-open and eyes-closed. The order of both conditions was pseudo randomized. We found that eye closure significantly enhances the ability to discriminate odors but does not influence olfactory sensitivity. It is suggested that eye closure does only effect higher olfactory processes like olfactory discrimination but does not influence peripheral olfactory processes like the olfactory threshold. This needs to be considered during studies investigating the olfactory and the gustatory system.

Poster Session III: Thurs. July 24

Dummies Versus Air Puffs: Most Efficient Stimulus Delivery Depends on Odor Volatility

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In nature odors diffuse from a source into a laminar boundary layer (diffusion) to be carried away by air currents (bulk flow). At a distant receiver the odor arrives in packages and the concentration of odor molecules in these packages depends on its volatility. For low volatile odors the concentration in the packages, possibly, is too low for detection, whereas the concentration in the laminar boundary layer may be sufficient for detection. Thus, diffusion becomes increasingly important with decreasing volatility while bulk flow outranks diffusion for highly volatile odors. In neurophysiological experiments, bulk flow is commonly simulated by injecting an odor puff into a constant air stream (air-delivered stimulation). In behavioral assays, odors of low volatility are presented by using dummies (dummy-stimulation; e.g. Brandstaetter et al., [2008] Naturwissenschaften). In the present study, we compared the effectiveness of dummy- and air-delivered stimulation by measuring neuronal responses in carpenter ants (*Camponotus floridanus*) to odors of different volatility. Neuronal activity in olfactory receptor neurons was monitored by electroantennography and responses in antennal (olfactory) lobe neurons by calcium imaging. As olfactory stimuli we used *C. floridanus*' alarm pheromone (undecane; high volatility), the releaser component of its trail pheromone (nerolic acid; medium volatility), and a behaviorally active C23 alkene (cis-9-tricosene; low volatility). Air-delivered stimulation elicited strong neuronal responses to highly volatile odors, whereas dummy-stimulation was particularly efficient with odors of low volatility. Thus, dummy-stimulation is especially advantageous when studying the animals' detection and processing of low volatile odors.

Poster Session III: Thurs. July 24

The OLFACT (Olfactory Function Assessment by Computerized Testing) Test Battery

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The OLFACT™ Test Battery consists of a set of four tests to measure olfactory function in a clinical setting. The battery includes a threshold test, a 15 item odor discrimination test, a 40 item odor identification test, and an odor memory test. Stimuli for all the tests are generated by the same platform (OLFACT™ olfactometer). Presentation of the stimuli and recording and scoring of responses are under computer control. Instructions for taking the various tests are also presented by the computer, resulting in minimal involvement by the test administrator in the testing process. Response parameters, e.g., correct/incorrect, latency, etc., are stored in a data file which can be downloaded for data analysis. The threshold test is based upon a series of binary dilutions of n-butanol, starting at 4%. A standard single staircase with reversal procedure is employed with stimuli offered in a three-alternative forced choice paradigm (3AFC; one n-butanol dilution, two blanks). The odor discrimination test consists of 15 trials which again use the 3AFC (two odors the same, one different). The odor identification test consists of 40 items. Each odor is presented in a 4AFC paradigm; each of the four choices consists of a label and a picture. The odor memory test assesses both semantic and episodic memory by first presenting 10 odors, followed after a short period of time by a second presentation of the 10 original odors mixed with 10 new odors. Each odor must be identified using the 4AFC paradigm as well as "old" or "new". Standardized norms by age and sex are in the process of being developed for all the tests.

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Poster Session IV: Fri. July 25

Immunocytochemical and Ultrastructural Studies of Purinergic Signaling in Rodent Taste Buds

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Our laboratory has shown that classical synapses and synaptic proteins are associated with Type III cells. No classical synapses, however, have been found associated with Type II cells, yet it is generally accepted that Type II cells transduce bitter, sweet and umami stimuli. Recent studies indicate that Type II cells release ATP as a neurotransmitter. The goal of the present study is to learn more about the nature of purinergic contacts in rodent circumvallate taste buds by examining immunoreactivity to antisera directed against: 1) the purinergic receptors P2X2 and P2Y4, and 2) the hemichannel proteins pannexin and connexin-43. Our results indicate that P2X2-like immunoreactivity (-LIR) is present in virtually all intragemmal nerve processes. Both Type II and Type III cells form contacts with these nerve processes. Large, atypical mitochondria are present in Type II cells at the contacts with P2X2-LIR nerve processes. The classical synapses formed by Type III cells are onto P2X2-LIR nerve processes. P2Y4-LIR is present on both Type II and Type III cells. Using immunoelectron microscopy we have found that Pannexin-1-LIR is present in both Type II and Type III cells. Connexin-43-LIR colocalizes with the Type II cell markers TRPM5- and IP3R3-LIR. Connexin-43-LIR colocalizes with a large subset of pannexin-1-LIR cells, but not with the Type III cell marker, serotonin. The results of our studies suggest that subsets of both Type II and Type III cells may release ATP. The presence of P2Y4 receptors on both Type II and Type III cells suggests that both cell types may respond to stimulation by ATP. Our observation that Type III cells form classical synapses onto P2X2-LIR nerve fibers suggests that nerve fibers receive input via ATP from Type II cells and vesicular neurotransmitters from Type III cells.

Poster Session IV: Fri. July 25

Knocking Out P2X Receptors Prevents ATP Release from Taste Buds

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ATP is a neurotransmitter in taste buds. In response to sweet, bitter, and umami taste compounds, Receptor (Type II) cells release ATP via gap junction hemichannels believed to be composed of pannexin 1. ATP released from Receptor cells diffuses to nearby gustatory sensory afferents where it activates purinergic P2X2 and P2X3 receptors. Key data underlying this understanding of how taste buds respond to gustatory stimulation include: (1) by using ATP biosensor cells, Receptor (Type II) cells were identified as the source of ATP secreted during taste stimulation, (2) mice lacking P2X2 and P2X3 receptors (P2X2 and P2X3 double knockout, or "DKO") are seriously deficient in taste behavioral responses, and (3) taste-evoked responses in chorda tympani and glossopharyngeal nerves are virtually absent in DKO mice. We have used ATP biosensor cells and a luciferin/luciferase-based ATP assay to test whether transmitter release in DKO mice is normal. Surprisingly, we discovered that intact circumvallate taste buds and isolated Receptor (Type II) cells from DKO mice fail to release ATP. Functional imaging indicates that cellular responses to gustatory stimuli (*i.e.*, release of stored intracellular Ca^{2+}) are normal in DKO taste cells. These unexpected results suggest that there is a failure of ATP release mechanisms in the DKO animals. Immunostaining for pannexin 1 in taste buds of DKO mice is indistinguishable from that in wild type mice, suggesting that the failure is not due to the absence of the gap junction hemichannel. Experiments are underway to attempt to explain how absence of P2X2 and P2X3 receptors leads to a failure of ATP release from taste cells. DKO mice courtesy of Roche Palo Alto. Supported by NIH/NIDCD grants 5R01DC000374 (SDR), 5R01DC007630 (SDR), R01DC007495 (SCK, TEF), and P30DC04657

Poster Session IV: Fri. July 25

Firing Rate-Dependent ATP Release from Mouse Fungiform Taste Cells with Action Potentials

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Recent reports have highlighted the role of ATP as a key neurotransmitter from taste cells to gustatory nerve fibers. Among the taste cells, synapses are observed only in Type III cells, some of which express putative sour receptors. However, sweet, bitter and umami receptors are expressed in Type II cells. Recently, the reports with ATP biosensors suggested that Type II cells are able to release ATP through hemichannels; one report indicated that action potential-like pulses induced ATP release from taste cells. In this study, we tried to detect tastant-evoked ATP release from single taste cells with action potentials of mouse fungiform papillae. The action potentials were recorded with the electrode basolaterally attached to a single taste cell. The electrode solution was collected and applied for luciferase assay to determine the ATP just after an increase in the firing rate was observed in response to a taste compound. To identify Type II and Type III cells, we used gustducin-GFP and glutamic acid dehydrogenase 67-GFP mice, respectively. When Type II cells increased the firing rate in response to saccharin, quinine or glutamate, ATP was detected in the electrode solution. The amount of ATP increased in a firing rate-dependent manner. When Type III cells responded to HCl, ATP was below the detection limit of the luciferase assay. The results suggest that the amount of ATP released from single taste cells differ with the response properties, or that Type III cells release another neurotransmitter.

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Poster Session IV: Fri. July 25

Voltage-Gated Sodium Channels Expressed in Taste Bud Cells

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Taste bud cells transmit information from apical taste receptors to basolateral nerve fibers. Following taste receptor activation, taste cells depolarize, activate voltage-gated sodium channels, and fire action potentials. Initial cell depolarization is likely mediated by TRPM5 in sweet, bitter, and umami cells, and by the candidate sour receptor, PKD2L1, in sour cells. Using double label immunohistochemistry, TRPM5 was positioned immediately beneath tight junctions to receive calcium signals originating from sweet, bitter, and umami receptor activation, while PKD2L1 was positioned at the taste pore to sense sour tastants in saliva. The molecular identities of the voltage-gated sodium channels that sense and propagate receptor-mediated signals is unknown. Using mouse taste bud and lingual epithelial cells collected by laser capture microdissection, SCN2A, SCN3A, and SCN9A voltage-gated sodium channel transcripts were found to be specifically expressed in taste tissue by RT-PCR analysis. SCN3A and SCN9A were expressed in TRPM5 cells, while SCN2A was expressed in both TRPM5 and PKD2L1 cells. We conclude that voltage-gated sodium channels are positioned to sense depolarizing signals from TRPM5 and PKD2L1. SCN2A, SCN3A and SCN9A channels likely account for the tetrodotoxin-sensitive sodium currents in taste receptor cells.

Poster Session IV: Fri. July 25

Expression of Adenosine Receptor in Mouse Taste Buds

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ATP released from taste cells is a crucial signaling molecule which activates taste nerves via P2X purinergic receptors (ligand-gated ion channels). Taste cells themselves express a variety of P2X and P2Y receptors as well as the ectoATPase, NTPDase2. Therefore release of ATP by taste cells is likely to result in extracellular accumulation of adenosine within taste buds via action of the ATPase and non-specific phosphatases. In other systems, adenosine modulates cellular activity and responsiveness. Four different adenosine receptors, A1, A2A, A2B, A3 receptors, have been cloned and characterized. However, the expression of adenosine receptor subtypes has not been examined in the gustatory organs. In this study, the expressions of adenosine receptor subtypes were examined by RT-PCR and in situ hybridization in mouse gustatory papillae. These analyses showed that A2B receptors were expressed in mouse taste bud cells. These results suggest that extracellular ATP could play a dual role, one as an agonist for P2 receptors and another as precursor of adenosine. The effects of extracellular ATP remain to be determined in exploring the function of taste buds. Further investigation by in situ hybridization is underway for other adenosine receptors. Support: NIH grants to T.E.F.

Poster Session IV: Fri. July 25

Sour Taste Stimuli Evoke ATP Release from Taste Buds

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ATP is a key transmitter in taste buds. Upon bitter taste stimulation, Type II taste cells release ATP via hemichannels, likely comprised of Pannexin-1. In contrast to bitter transduction, sour transduction appears to involve primarily Type III taste cells. To determine whether ATP is released from taste tissue in response to sour stimuli, we used a luciferin/luciferase assay to detect ATP release following apical application of acidic stimuli to taste bud bearing epithelium isolated from circumvallate (CV) papillae. Both citric acid and HCl (10-20 mM) resulted in significant ATP release. To insure this release was from taste cells and not epithelial cells, we applied the same stimuli to apical membranes of non-taste bud bearing lingual epithelium. In the absence of taste buds, lingual epithelium released substantially less ATP in response to sour stimuli. The cation channel TRPM5 is present in Type II taste cells and is involved in bitter transduction. The protein is not evident in Type III cells, thus we tested whether mice lacking TRPM5 release ATP upon stimulation with tastants. Taste buds of TRPM5 knockout mice fail to release ATP following stimulation with bitter compounds, but do release ATP following application of sour stimuli. ATP release in response to both bitter and sour stimuli is substantially reduced in the presence of the specific pannexin hemichannel blocker carbenoxolone (5 μm). In summary, both bitter and sour stimuli evoke release of ATP from taste tissue and a common step in both pathways appears to be the release of ATP via pannexin hemichannels.

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Poster Session IV: Fri. July 25

Presynaptic (Type III) Cells in Mouse Taste Buds Sense Sour Taste

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Although candidate molecular receptors for sour taste transduction are found in subsets of taste cells, mechanisms of sour transduction remain unclear. Taste buds contain two types of cells that directly participate in chemosensory transduction– receptor (Type II) cells, and presynaptic (Type III) cells. Receptor cells express G protein-coupled taste receptors and respond to sweet, bitter and umami taste stimulation. Presynaptic cells form synapses and appear to sense salty and sour (acid) taste (Tomchik et al., 2007). Using Ca^{2+} imaging on isolated taste cells and biosensor cells to identify neurotransmitter release (Huang et al., 2005, 2007), we now show unambiguously that presynaptic (Type III) cells respond to acid taste stimulation with an influx of Ca^{2+} and release of serotonin (5-HT). In sharp contrast, acid taste stimulation does not elicit Ca^{2+} influx in receptor cells nor does it stimulate them to secrete neurotransmitter (ATP). Further, by recording responses evoked by acetic acid titrated to different pH levels in isolated cells and from taste buds in lingual slices, we show that the most effective stimulus for acid taste is the membrane-permeant acetic acid molecule (protonated CH_3COOH), and not pH (*i.e.*, H^+) *per se*. Collectively, the data indicate that presynaptic cells are the taste bud cells that respond to sour taste and secrete neurotransmitter, and support the notion that the proximate stimulus for sour taste is intracellular acidification, not extracellular protons (Lyall *et al* 2001).

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Poster Session IV: Fri. July 25

Taste Function in pkd1l3 Knockout Mice

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Recent studies have suggested the involvement of the polycystic kidney disease-1 and -2 like genes, *Pkd1l3* and *Pkd2l1*, in sour taste transduction. In a heterologous system, acids produce responses in cells which co-express the *Pkd1l3* protein (PKD1L3) and the *Pkd2l1* protein (PKD2L1), but not cells which express the individual proteins. *In vivo*, disruption of taste cells expressing PKD2L1 eliminates acid taste responses. However, no previous data exits on taste responses in the absence of PKD1L3. In order to assess the importance of PKD1L3, we genetically engineered knockout mice with a disrupted *Pkd1l3* gene and examined taste function of these mice using behavioral and neurophysiological approaches. We measured preference ratios for concentration series of citric acid, HCl, NaCl, inosine monophosphate, quinine, sucralose, KCl, $CaCl_2$, NH_4Cl, $MgCl_2$, and ethanol in 48-h two bottle tests. In separate groups of LiCl-conditioned mice, we measured taste thresholds for either NaCl or citric acid. We found no significant differences in behavioral taste responses between *Pkd1l3* knockout animals and wild-type controls. Additionally, electrophysiological recordings of taste-evoked activity in both the chorda tympani and glossopharyngeal nerves reveal that knockout mice have unaltered responses to a variety of taste stimuli, including acids. In conclusion, disruption of *Pkd1l3* does not alter behavioral and neural taste responses. Further evidence is needed to confirm the roles of PKD1L3 and PKD2L1 in taste function.

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Poster Session IV: Fri. July 25

The Effect of GABA on Human Taste Sensations and the Influence of Food Components on the Activity of Glutamate Decarboxylase, GABA Synthesizing Enzyme

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γ-Aminobutyrate (GABA) is synthesized from L-glutamate by glutamate decarboxylase (GAD). Mammals express GAD67, one of the GAD isoforms, in the type III taste bud (Nakamura et al., *Chem. Senses*, **32**, J19 (2007)), where the participation of GABA in taste signal transduction is strongly suspected. In our study, we have found that the presence of GABA not only influences the human taste sensations, but also affects how food components interacting with the GAD would alter the taste sensations. We found that GABA by itself has sour and bitter tastes. However the five taste sensations were examined in the presence GABA, the significantly enhanced were umami and salty tastes and the mildly was sour taste. These results suggest that GABA influence the human taste sensations. By

examining the interactions between GAD and the extracts of various food components such as spices, teas, fungi, algae and sprouts, we found that some extracts affected GAD activity. Our present results suggest that GABA is involved in the taste mechanism and its production can be influenced by the food taken daily. It is highly probable that some food components may alter the taste sensations via GAD activity.

Poster Session IV: Fri. July 25

Mouse Taste Cells Co-Release the Neurotransmitters, Serotonin and Norepinephrine

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ATP and serotonin (5-HT) are transmitters secreted from taste receptor (Type II) and presynaptic (Type III) cells, respectively. Norepinephrine (NE) has also been proposed as a transmitter or paracrine hormone in taste buds (Herness et al., 2002; Dvoryanchikov et al., 2007). RT-PCR and immunostaining show that a subset of taste cells possesses high affinity NE transporters. Depolarizing pieces of lingual tissue containing taste buds with high K^+ elicits NE release. Yet, to date, the identity of the taste cells that secrete NE is unknown. We have used cellular biosensors (Huang et al., 2005, 2007) to identify taste bud transmitters and the cells that release them. Here, we used CHO cells stably transfected with α1A receptors and loaded with Fura2 (''NE biosensors'') to detect NE secretion. Biosensors respond to ≥10 nM NE with a reliable Fura2 signal. NE biosensors alone are not affected by KCl or taste stimuli. However, we recorded robust responses from NE biosensors positioned near taste buds when the taste buds were stimulated with KCl (50 mM) or a mixture of tastants (cycloheximide, 10 μM; saccharin, 2 mM; denatonium, 1 mM; SC45647, 100 μM). NE biosensor responses evoked by stimulating taste buds were reversibly blocked by prazosin, an α1A receptor antagonist, verifying that the signals arise from secreted NE. NE is released only from presynaptic and not taste receptor cells. Biosensor cells showed that no NE was released when Ca^{2+} in the bath was replaced with Mg^{2+}. Presynaptic taste cells also secrete 5-HT in a Ca-dependent manner upon stimulation. Thus, the present findings suggest that many presynaptic taste cells co-release two neurotransmitters, norepinephrine and serotonin.

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Poster Session IV: Fri. July 25

Immediate and Ongoing Inhibition of 5-HT Re-Uptake have Contrasting Effects on Human Taste Thresholds

Lucy F. Donaldson, Ellen McRobie and Samantha O'Driscoll

This study compared the effects of acute and chronic 5-HT reuptake inhibition on human taste thresholds. Bitter and salt recognition thresholds were determined in 26 healthy volunteers at the tip of the tongue at each of four experimental sessions. Different concentrations of quinine and NaCl solutions were presented to each subject in a pseudorandom order. Each was presented a minimum of 5 times before and after drug or placebo in two double-blind within-subjects experiments. Psychophysical taste functions were constructed to calculate bitter and salt taste threshold before and after each intervention: Intervention 1 (n = 21), a 5 minute application of either SSRI (paroxetine, 2 mg/ml) or placebo to the tongue; Intervention 2, systemic SSRI (paroxetine (20mg)) or inactive placebo - thresholds determined at 30 minutes (n = 11), 2 hours (n = 26) and 4 hours (n = 11). Lingual SSRI increased bitter threshold (54 ± 34%) significantly more than placebo (-39 ± 26%, p = 0.03). Systemic SSRI tended to increase bitter thresholds at 30 minutes (+17 ± 29%, ns). There was a significant decrease in bitter threshold at 2 hours (-43 ± 13% change SSRI, -11 ± 15% placebo, $p < 0.01$), as we have previously reported. There were no significant effects of lingual or systemic SSRI on salt thresholds at any time. These data show that acute inhibition of 5-HT reuptake at the taste bud increases bitter thresholds whereas longer inhibition (2 hours) decreases bitter thresholds, with no change on salt thresholds. These results suggest that the immediate effect of 5-HT re-uptake inhibition may be to inhibit taste signalling. In contrast, more prolonged reuptake inhibition enhances taste signalling in humans. These temporally distinct effects may represent changes in the effects of 5-HT on taste receptor cells over time.

Poster Session IV: Fri. July 25

Edible Taste Strips as a Novel Method for Evaluating Disturbances in Human Taste Function

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Edible taste strips composed of pullulan and methylcellulose allow the delivery of precise amounts of tastants to the oral cavity. The goal of this study is to determine the efficacy of edible strip technology in identifying human taste disturbances. One population of subjects was treated with an oral application of 0.12% chlorhexidine, which decreases both salty and bitter taste responses in humans. The performance characteristics of edible taste strips containing suprathreshold levels of sodium chloride were then examined after a chlorhexidine rinse. Test subjects reported on average a forty to fifty percent decrease in the intensity of salt taste perception after chlorhexidine treatment for the five different suprathreshold presentations of salt tastant. A second population of subjects was treated with an oral rinse that was extracted from *Gymnema sylvestre* leaves. These extracts are enriched in gymnemic acids, which block human sweet taste function. *Gymnema sylvestre* extracts were incorporated in edible strips, and gymnemic acid content was estimated by TLC. *Gymnema* strips were then dissolved in water for presentation to subjects. After *Gymnema* treatment, our population of subjects reported on average a seventy percent decrease in the intensity of sweet taste perception at all suprathreshold levels of sweet tastant. These results provide evidence that edible strips are useful for storing taste modifiers for subsequent presentation to subjects. Most importantly, edible taste strips are a reliable method for rapidly evaluating disturbances in human taste function in the clinic, or at remote locations. Supported by NIDCD R44 DC007291, and a Return of Overhead Research Incentive Grant from Temple University.

Poster Session IV: Fri. July 25

Development of a High Throughput (HT) *in vivo* Operant Taste Discrimination Assay

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Recent advances in the molecular and cellular biology of taste signaling pathways have made possible the development of recombinant cell lines and HT technologies for the discovery of novel tastants and taste-modifying compounds. Cell based assays provide rapid indication of the potential for compounds to affect taste, but assessment of taste efficacy ultimately must be performed in behaving organisms. An *in vivo* HT taste assay therefore would provide substantial advantage over cell-based screening technologies.

We describe here a method and apparatus, the Microtiter Operant Gustometer (MOG), for a HT taste discrimination assay using rats. Taste stimuli (solution, suspension, or solid) are presented in a 96-well plate located beneath the floor of the MOG and accessed by licking through a retractable aperture in the floor. The first lick of each trial produces two retractable levers from the front panel that control a food pellet dispenser. The taste of the sample determines which lever is correct for the animal to obtain the food reward. At the end of a trial, the 96-well plate advances to align the next well with the aperture. Experimentally MOG, rats sampled from all 96 wells in a 90-minute session with keen interest and discriminated 300 mM sucrose from water, quinine, citric acid, and NaCl with >90% accuracy. The HT capacity permitted simultaneous dose-response evaluation of 5 nutritive and non-nutritive sweeteners, as well as a primary screen of a sweet tastant library. The MOG introduces the first technology for *in vivo* HT discovery and evaluation of novel molecules for taste.

Poster Session IV: Fri. July 25

Examination of *N*-PROP Recognition Thresholds with Edible Taste Strips

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Edible taste strips composed of pullulan-hydroxypropyl methylcellulose polymers readily incorporate bitter tastants such as 6-propyl-2-thiouracil (*n*-PROP). The goal of this study was to examine taste recognition thresholds for *n*-PROP in individuals who could detect this bitter tastant, and to identify potential olfactory components of *n*-PROP release from edible strips. Taste recognition thresholds for *n*-PROP were examined by a three strip procedure that utilized a single series ascending method of limits. Stimuli were presented in triads where only one of three samples contained tastant. In our population, 90 percent of subjects could detect *n*-PROP as bitter. The range for *n*-PROP taste recognition thresholds occurred over two log units, with an upper range of 140 nanomoles. These recognition thresholds are over one order of magnitude lower than those reported for *n*-PROP with aqueous tests. A similar threshold range was observed for the bitter tastant quinine when quinine was incorporated into strips. Next, the volatility of dissolved taste films on the tongue was examined in order to identify potential olfactory components of tastant release. The same group of subjects was tested for their ability to detect *n*-PROP with their nasal passages occluded. Taste recognition thresholds for half of the subject population were identical in both the absence and presence of nose clamps. The remaining subjects detected *n*-PROP at the next higher or lower amount. These results indicate that edible taste strips primarily measure gustatory cues. The results also demonstrate that edible strip technology is a highly sensitive and promising method for examining taste blindness in humans.

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Poster Session IV: Fri. July 25

The Use of Edible Taste Strips for Measuring Taste Recognition Thresholds

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Edible taste strips composed of pullulan-hydroxypropyl methylcellulose (HPMC) polymers allow the delivery of precise amounts of tastants to the oral cavity. Taste strips composed of 90% pullulan and 10% HPMC exhibit no background sweet, sour, salty, or bitter taste. Studies are underway to determine norms for taste recognition thresholds as a function of age in decades and sex by utilizing taste strip technology. Recognition thresholds for sweet taste were examined by two separate protocols. One method was a modification of the three-drop procedure that used a single series ascending method of limits. This approach allowed the presentation of taste stimuli in triads where only one sample contained tastant. The second approach utilized a two-alternative staircase method where recognition thresholds were determined by the successful completion of four reversals by the subject. Both approaches yielded similar recognition thresholds for sweet taste. With taste strips, overall recognition thresholds for sweet taste are nearly two orders of magnitude lower than results obtained from a traditional aqueous taste test by either of the two methods described above. In addition, recognition thresholds for sweet taste increased with subject age, with taste recognition thresholds similar for both males and females. Also, the variability among our subject population was considerably smaller with edible taste strips. These results indicate that edible taste strips are a highly sensitive method for examining taste recognition thresholds in humans. This new means of presenting taste stimuli should have widespread applications for examining human taste function.

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Poster Session IV: Fri. July 25

Experience Induced Increases in Taste Discrimination for Sweeteners and Monosodium Glutamate (MSG)

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Experience with a fructose solution induces an increased taste discrimination ability for glucose solutions, and experience with MSG in food induces increased discrimination for MSG solutions. Both induction effects reverse after treatment is stopped (Kobayashi & Kennedy, 2002; Kobayashi et al., 2006, Gonzalez et al., 2008). We are further characterizing the induction and probing the mechanism(s) with other sweeteners and umami stimuli, using the experimental design shown to be appropriate by Gonzalez et al., (2008). All sweetener concentrations are isosweet, and the umami concentrations isointense, to the original fructose treatment and glucose or MSG test concentrations, as determined by a gLMS scale and magnitude matching. Experience with glucose, fructose, and Na-cyclamate significantly increased discrimination for glucose, while experience with water, acesulfame-K and MSG in solution did not. The effect of Na-cyclamate on glucose discrimination was significantly greater than the effects of sugar experience. Experience with umami solutions significantly increased discrimination for MSG, while experience with water, glucose, acesulfame-K, and Na-cyclamate did not. Experiments still in progress suggest that experience with Na-cyclamate generalizes, i.e. increases discrimination of other sweeteners. The differential effects of Na-cyclamate and acesulfame-K on glucose discrimination support a peripheral mechanism. It is known that Na-cyclamate binds to the T1R3 sweet receptor subunit (Xu et al., 2004), which has been proposed to serve a modulatory function (DuBois, 2004). Our overall data support a role for Na-cyclamate binding to T1R3, with a positive modulatory effect, in the sweet taste induction, but not in umami taste induction.

We thank Biology 040, 2006 students for assistance.

Poster Session IV: Fri. July 25

Citric Acid Modulates Discrimination of Sweetness Intensity in Sucrose Solutions

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Previous research has demonstrated that citric acid can suppress the perceived sweetness of sucrose. However, there are multiple potential interactions between taste compounds of linear suppression, the added taste might lead to masking or inhibition, which would change the shape of the psychometric function. The present study aimed to determine whether the addition of citric acid also changes the discrimination threshold (equivalent to the slope of the psychometric function) of sweetness in sucrose solutions. Twenty participants compared pairs of solutions, indicating which was perceived as tasting sweeter. In the sucrose-only condition, each pair consisted of one solution at a concentration of 42 mM while the other ranged from 0 mM to 94 mM. In the sucrose/citric-acid mixture condition, the same concentrations of sucrose were used as in the sucrose-only condition, but the concentration of citric-acid was kept constant at 3mM for all solutions. We calculated a discrimination threshold for each condition, and found that the threshold for sweetness discrimination was more than twice as high in the mixture condition as in the sucrose-only condition ($p < .05$) – that is, the psychometric function was significantly shallower. These results indicate that the addition of citric acid significantly decreases the ability to discriminate between different concentrations of sucrose. Thus, interactions between sucrose and citric acid can be non-linear and the perceptual consequences of mixing them cannot be described solely in terms of enhancement and suppression.

Poster Session IV: Fri. July 25

Prop Taste Insensitivity is Associated with Decreased Ability to Detect Differences in the Fat Contents of Salad Dressings in African-American Men

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The inherited ability to taste bitter compounds like 6-*n*-propylthiouracil (PROP) has been reported to influence fat taste detection, but this relationship has never been tested in an African-American (AA) population. This study tested the hypothesis that healthy AA PROP non-tasters have decreased ability to discriminate differences in the fat content of Italian salad dressings. An exploratory aim was to assess how gender influences this relationship. A community-based sample of 131 AAs (69 men; 62 women), with mean BMI and age of 29.7 ± 7.1 kg/m^2 and 35.9 ± 10.8 *y*, respectively, was recruited from New York City. Perceived bitterness of a 0.32 mM PROP solution was assessed via the Labeled Magnitude Scale, and continuous ratings in *mm* were used for final analyses. Ability to discriminate differences in fat content was assessed with multiple simple difference tests, where subjects compared samples ranging from 5-55% fat content by weight to a 55% fat reference sample and reported whether the dressings tasted the ''same'' or ''different.'' Scores on this test were tallied and ranged from 0-7, with higher scores meaning increased ability to detect differences in fat content. For all subjects, there was no relationship between perceived PROP bitterness and fat discrimination score (p=0.65). In men, ratings of PROP bitterness positively correlated with fat discrimination score (rho=0.25; p=0.04), while no relationship was found in women. Results suggest that ability to taste PROP influences fat taste detection of Italian salad dressings in healthy AAs, but this relationship varies by gender. It is not known whether these findings will translate to dietary habits or health, but we have assessed food preferences and anthropometrics in this sample to better clarify these relationships in future studies.

Poster Session IV: Fri. July 25

Effects of Taste Solutions on Power Frequency Content of Swallowing Submental Electromyogram (SEMG)

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It has been shown that solutions with taste induce stronger swallowing submental muscle contraction than water. This study explored the effects of five taste solution (Citric acid (sour), Sucrose (sweet), Sodium Chloride (salt), Caffeine (bitter) and Sodium Glutamate (umami)) on power frequency content of swallowing submental electromyogram (sEMG), compared with water. Fourteen healthy subjects were presented with 5 ml each of five tastants and water. Data were collected on three trials of the five tastants and water using measurements of submental surface electromyography (sEMG), which was applied for spectral analysis. Citric acid (sour) and Sodium Chloride (salt) solutions increased spectrum integrated values of the total power components. The spectrum integrated values of low frequency power (below 10 Hz) in the salt taste and of high frequency power (above 10 Hz) in the sour taste trial were significantly increased. Pleasantness and intensity of tastes had no relationship with the above observed changes. This study revealed that sour and salt taste had qualitatively different influences on the power frequency content of swallowing sEMG.

Poster Session IV: Fri. July 25

Perceptive, Psychological and Behavioural Factors as Determinants of Obesity

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Taste acuity and psychological factors are determinants of food preference and consumption, but their role in the development of weight gain has been poorly investigated. The present study evaluated the relationship of overweight and obesity with taste perception and social, relational and emotional behaviour. One-hundred-twenty subjects with excess weight (BMI = 31.6 ± 5.7 kg/m^2) and 72 normal-weight subjects (BMI= 22.2 ± 2.5 kg/m^2) underwent the following experimental protocol: i) ambulatory evaluation of nutritional status with assessment of weight excess; ii) evaluation of taste acuity (bitter, salty, sour and sweet) by means of recognition thresholds measurement (3-AFC method); iii) psychographic-behavioural evaluation by means of a structured questionnaire consisting of 4 categories of questions (anxiety status, social integration, emotional status and eating disorder). Subjects differed in taste acuity: over-weight subjects had significantly higher recognition thresholds than normal-weight subjects for bitter (0.78 ± 0.08 g/L vs 0.61 ± 0.09 g/L; $p < 0.01$), salty (9.01 ± 2.96 g/L vs 3.19 ± 2.26 g/L; $p < 0.01$) and sweet tastes (15.93 ± 3.82 g/L vs 8.84 ± 2.35 g/L; $p < 0.01$). No significant differences were found in taste acuity for sour taste.

Questionnaire and thresholds data were submitted to Principal Component Analysis to investigate how overweight and normal-weight subjects were distributed in a multidimensional space as a function of the considered variables. As compared to normal-weight subjects, overweight/obese subjects were found to have a reduced taste acuity, a poor satisfaction of their profession, a poor degree of social integration and a high degree of anxiety. Reduced taste acuity is present in overweight and obese subjects and could contribute to weight gain in association to psychological factors.

Poster Session IV: Fri. July 25

Effects of Video Game Play on Snacking Behavior and Caloric Burn: Nintendo WII vs. Microsoft X-Box

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Prior research has investigated the link comparing childhood obesity with activity participation, television viewing, and video game use. The current study compared performance, mood, cognition, physiological measures, snacking behavior, and caloric burn between the Nintendo Wii and the Microsoft X-Box gaming systems. Each participant played a boxing game on both the Wii and the X-Box and completed a control condition where no game was played. During play, participants wore an Actiwatch monitor, which measured their movement and caloric expenditure. The results showed that there was a significantly higher blood pressure and pulse with the Wii than with either the X-Box or control conditions. Furthermore, there were greater total and mean activity scores in the Wii condition which led to a greater caloric expenditure. Finally, when a snack food (M&Ms) was available during game play, those participants in the Wii condition ate the least amount of the snack. These results are particularly salient regarding the positive benefits of video game play, the reduction of snacking behavior during certain gaming conditions, and the possibility of weight loss through games requiring additional physical activity.

Poster Session IV: Fri. July 25

Decreased Ability to Discriminate Differences in Fat Content of Italian Salad Dressings is Associated with Increased Levels of Obesity in Healthy African-Americans

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Consumption of high-fat foods has been implicated in the development of obesity. The goal of this research is to determine the origins of fat intake behavior in humans. This study tested the hypotheses that variations in fat taste discrimination are related to differences in adiposity, and further, if inherited sensitivity to the bitter taste of 6-n-propylthiouracil (PROP) affects this relationship. A community sample of 131 African-Americans (69 men; 62 women) with mean BMI (29.7 ± 7.1 kg/m^2) and age (35.9 ± 10.8 *y*) completed a taste test to assess fat taste discriminability of Italian salad dressings, ranging in fat content from 5-55%. In part I, subjects rated oiliness, creaminess, and fat content of the 5%, 30%, and 55% samples on a VAS. In part II, salad dressings were compared in 7 simple difference tests. Anthropometrics (ht, wt, and waist circ.) were assessed. Simple and multiple regressions were performed using VAS ratings for each attribute from part I and total score of correct responses on part II as independent variables and waist (cm) as the dependent variable. After adjusting for age and gender, creaminess ratings for the 30% fat sample was negatively associated with waist circ. ($r = -0.27$; $p \leq 0.005$), such that subjects with greater abdominal adiposity rated the sample less creamy. There was also a trend showing that greater waist circ. was associated with poorer fat discrimination ($r = -0.16$; $p = 0.07$). When models were adjusted for PROP rating, p-values were unchanged for both relationships. These findings reveal that obesity may be associated with differences in ability to perceive fat and fat-related textural attributes in foods, and future studies are warranted. Our lab is currently testing if variation at the CD36 allele, a candidate fat taste receptor, mediates these relationships.

Poster Session IV: Fri. July 25

Evolution of *Umami* and *Kokumi* Tastes During the Aging of *Doenjang*, a Traditional Korean Soybean Paste

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Most enzymatic hydrolyzates of food proteins generally have an *umami*, and recent studies have suggested that a non-enzymatic browning reaction enhances the *kokumi* taste (a term relating to consistency and mouth-feel). *Doenjang* (DJ), a Korean traditional soybean paste, is made solely from soybeans in a two-step fermentation with mold and brine fermentation and requires years of aging to acquire its final flavor. The production of *umami* by enzymatic hydrolyzates of soy proteins and *kokumi* by non-enzymatic browning during fermentation and aging are expected. This study investigated the evolution of the *umami* and *kokumi* tastes of DJ and the possible contributions of natural protein hydrolyzates and browning during aging to its taste. We evaluated DJ aged for 6, 12, 24, 30, and 36 months for five basic tastes and the *kokumi* taste in a 3% solution containing 1% salt using the descriptive analysis method. The *umami* and *kokumi* tastes appeared after aging for 12 months and increased further following storage. The full taste evolution required additional time: 30 months for *umami* and 24 months for *kokumi*. No significant changes were found in the sweet, sour, salt, and bitter tastes until 36 months storage. Aging increased the total, free, and bound amino acids and oligopeptides, and these protein hydrolyzates were all increased significantly after 30 months storage. Aging also caused the gradual development of brown pigments, which was measured as the absorbance at OD 420 nm, and a significant increment was seen after 24 months storage. From these data, it was estimated that the evolution of *umami* and *kokumi* depend on the protein hydrolyzates and browning reaction that occurs in the fermentation and aging of DJ, respectively.

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Poster Session IV: Fri. July 25

Predictive Modelling to Design Foods with Reduced Salt, Sugar and Fat Levels

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Foods often taste better at higher than at lower levels of fat, sugar or salt. Fortunately, many related sensory attributes can also be influenced by other product properties than fat, sugar or salt level. Still, closing the sensory gap caused by fat, sugar or salt reduction often appears to be not straightforward: one reduction influences several sensory attributes in different ways and the different attributes influence each other as well. This sensory complexity relates to the way physical and chemical states of the food develop

during consumption. Integrated Sensory Response Modelling (ISRM) has been developed to test hypotheses in this area and to translate insights into design rules which help the design of better tasting low-fat, -sugar or -salt products. In ISRM, an inventory is made of in-vitro measurable physical and chemical properties which are hypothesized to characterize elements of perceivable oral food behaviour. Next, after extensive experimentation and advanced mathematical modelling and validation, those properties from the inventory are identified which, together, dominate and explain measured variation in sensory response. This selection of physical and chemical drivers, plus their weight factors, provides mechanistic insights on the relative importance of the respective elements of oral food behaviour: theory which is relevant for the sensory response under study and theory which is less relevant. For taste perception this resulted in the identification of building blocks for each of the 5 tastes, operational in real foods. The insights are also actionable: the potential scope of different technological solutions for sensory challenges follows directly from the relative impact of the driver(s) on which each solution acts upon.

Poster Session IV: Fri. July 25

Does Our Food Approach the Common Optimum Taste (COT)?

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Common sense teaches us: traditional pizza, spaghetti, and lentils differ in taste. Yet, recent innovations in food technology using fermentation, extraction, encapsulation, fat replacement, and many other techniques, leading to new food ingredients have significantly modified many traditional dishes. We were interested to study to what extent frequently purchased convenience food still maintains well-known and distinguishable taste features, or whether they lost their natural taste characteristics. Quantitative spectra of free amino acids (AA) of 6 frequently purchased convenience dishes (pizza, spaghetti, ravioli, lentil soup, chicken Cordon Bleu, cheeseburger) were performed by quantitative AA analysis (ion-exchange chromatography on an automated AA analyser). GABA concentrations were controlled (stable isotope dilution GC-MS method). The spectra of free AA revealed striking resemblance between the six dishes. When comparing the spectra with the composition of protein bound AA of the major natural ingredients, it became obvious that free AA spectra did not reflect the natural protein-bound AA. Free cysteine (CYS) was comparably rare in all probes, and the ratio ALA vs. GLY that in natural food usually ranges near 1.0, was markedly elevated in ravioli (6.2), spaghetti (4.7), and in cheeseburger (4.2). Also taste probes of these dishes when pureed and slightly coloured, appeared almost indistinguishable. Only 18/68 persons were able to distinguish between all dishes, 22/68 persons did not even identify half of the probes. CONCLUSION: Modern food technology maximizes palatability by novel combinations of all prototypical tastes, thereby converting well-known traditional dishes into ''over-delicious'' and largely indistinguishable creations with a Common Optimum Taste (COT).

Poster Session IV: Fri. July 25

''Excitatory Actions of Noradrenaline on Granule Cells in the Accessory Olfactory Bulb''

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Modulation of dendrodendritic synapses by the noradrenergic system in the accessory olfactory bulb (AOB) plays a key role in the formation of memory in olfactory mediated behaviors. We have recently shown in the AOB that noradrenaline (NA) inhibits mitral cells by increasing GABA inhibitory input, suggesting a modulatory action of NA on granule cells. Here, we show that NA (10 μM) elicited a long lasting depolarization in granule cells. This effect is mediated by activation of a_1-adrenergic receptors as the depolarization is mimicked by phenylephrine (30 μM) and completely blocked by prazosin 300 nM. The NA-induced depolarization is larger at depolarized-potentials indicating voltage dependency. In addition to this depolarization, application of NA induced the appearance of an after depolarization (ADP) following a stimulus-elicited train of action potentials. Both the depolarization and ADP were abolished by extracellular addition of the Ca^{2+}channel blockers, Ni^{2+} and Cd^{2+}, and by the inclusion of BAPTA in the recording intracellular solution indicting that the effect of NAis Ca^{2+} dependent. Furthermore, both the depolarization and the stimulus-induced ADP werecompletely abolished by flufenamic acid and SKF-96365 (30 μM, respectively). Taken together, our results suggest that the primary effect of NA in the AOB is depolarization of granule cells by a mechanism involving the activation of transient receptor potential (Trp) channels.

Poster Session IV: Fri. July 25

Striatal Neurons are a Potential Relay Between Olfaction and SVZ Neurogenesis

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Olfactory activity, seizures, and neurodegenerative disorders influence the production of newly-born olfactory bulb interneurons within the subventricular zone (SVZ). However, how olfaction influences neurogenesis is unknown.. Because the SVZ is sandwiched between the lateral ventricle and the striatum, we hypothesize that the striatum is a relay network for olfactory input and can influence SVZ neurogenesis. Here, we test whether striatal neurons signal to SVZ neural progenitor cells. We used morphological characterization, electrophysiology, and Ca^{2+} imaging to examine GABAergic striatal neurons signaling to SVZ cells. Morphological data indicate that 75% of neurons within the striatum bordering the SVZ send processes into the SVZ near both neuroblasts and astrocytes; 80% of those are medium spiny neurons (n = 130, postnatal day 15-23). Patch clamp recordings indicate that medium spiny neurons fire action potentials at 6-10 Hz with depolarization. Action potentials are sensitive to both tetrodotoxin and $GABA_A$ receptor blocker bicuculline. Depolarizations of striatal neurons during patch clamp recordings elicit Ca^{2+} responses in SVZ cells surrounding visualized neuronal processes. These Ca^{2+} responses are blocked by tetrodotoxin. Baseline Ca^{2+} activity in SVZ cells increases 20-40% after action potential induction of a nearby striatal neuron ($p < 0.01$, n = 5 slices, n = 81 cells analyzed). Striatal neurons and their processes are visualized with Alexa 568 in the patch pipette, and SVZ cells are loaded with Fluo4-AM Ca^{2+} indicator. Collectively, our results demonstrate that the striatum is in the ideal position to relay inputs from the olfactory bulb to the SVZ. Future experiments will test whether striatal spiny neurons projecting to the SVZ receive functional connections from the olfactory bulb

Poster Session IV: Fri. July 25

Noradrenergic Modulation of GABAergic Inhibition of Main Olfactory Bulb Mitral Cells

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Previous studies revealed that norepinephrine (NE) inputs from the pontine nucleus locus coeruleus (LC) to the main olfactory bulb (MOB) increase the sensitivity of mitral cells to weak olfactory input. This effect is due in part to direct a1 NE receptor-mediated excitation of mitral cells. Previous studies also indicate that NE modulates the strength of GABAergic inhibition in MOB. However, the nature of this modulation and the NE receptors involved remain controversial. The goal of the present study was to investigate the role of NE receptor subtypes in modulating the GABAergic inhibition of mitral cells using patch clamp electrophysiology in rat MOB slices. NE application bi-directionally modulated GABAergic spontaneous inhibitory postsynaptic currents (sIPSCs) in a dose-dependent and receptor specific manner. a1 receptor activation enhanced, while activation of a2 receptors inhibited, sIPSCs. Activation of b NE receptors weakly increased sIPSCs. The results indicate that NE release may bi-directionally regulate the strength of GABAergic inhibition of mitral cells depending on the NE receptor subtype activated. Functionally, this endows noradrenergic inputs with the capability to increase or decrease inhibitory processes in MOB as a function of behavioral state. NE-evoked, a1 receptor-mediated enhancement of inhibition may function to improve discrimination by increasing contrast among different odors. Consistent with this, recent behavioral findings (Mandairon et al., 2008) demonstrate that blockade of a1 receptors in MOB impairs odor discrimination.

Poster Session IV: Fri. July 25

Organization of Neuronal Stem Cell Niches in the Olfactory Midbrain of Adult Spiny Lobsters, *Panulirus Argus*

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Neurogenesis persists in the olfactory midbrain of adult spiny lobsters, *Panulirus argus*. Neuronal precursors are localized in a small proliferation zone (PZ) in each of the soma clusters of local and projection neurons (MC, LC). Close to each PZ, one putative neuronal stem cell – a neuroblast - is located and is itself surrounded by a clump of small cells constituting a putative stem cell niche (Schmidt, *J. Comp. Neurol.* 503:64-84, 2007). To characterize the cells of the clumps, we used methylene blue staining of semithin sections, immunocytochemistry, and transmission electron microscopy. These analyses revealed that the cells of the clump are unique in nuclear and cytoplasmic architecture among all cell types present in the brain. Their somata are small, have a high nuclear-cytoplasmic ratio of ca. 0.8, and form a dense mantle around a core free of nuclei. The clump cells are bipolar with a short process reaching into the core and a long process projecting outwards. Together the long processes form a duct that reaches to the PZ and contains cells in transit. The clump of cells and the duct are enveloped by several layers of processes of type-2 glial cells. Type-2 glial cells are specifically labeled by anti-phosphorylated histone H3 and anti-Gs/olf, their somata are irregularly dispersed among the neuronal somata (ratio ca. 1:40), and they have a star-shaped morphology with processes projecting in different directions. An arteriole specifically labeled by *Amaranthus caudatus* lectin is attached to the clump of cells but does not penetrate it. From these findings, we conclude that the clumps of cells surrounding the putative neuroblasts are comprised of a unique cell type and are isolated from the neuronal and vascular elements in the surround by a layer of processes of type-2 glial cells.

Poster Session IV: Fri. July 25

Molecular Guidance of Newborn RMS Neurons from the Subventricular Zone to the Olfactory Bulb

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Subventricular zone (SVZ) neural precursor cells generate neuroblasts that travel tangentially along the rostral migratory stream (RMS) toward the olfactory bulb (OB). Previous work has implicated many cellular and molecular factors emanating from the bulb that could function as chemoattractants in guiding RMS migration. Interestingly, in vivo bulbectomy does not hinder RMS neuroblast migration toward the OB. In addition, migrating neuroblasts exhibit bidirectional migration in slice culture and acute slice preparations. To better understand migrating neuroblast guidance, we sought to record and analyze the effects of specific molecular cues on this migration process. Using a transgenic mouse line (GAD65-GFP) in which RMS neuroblasts are fluorescently labeled, we developed an acute slice assay utilizing time lapse confocal imaging to individually track the migration of neurons at a population level. We analyzed the dynamics of this population based on two catagories: directionality (measured as the proportion of cells migrating toward or away from the OB) and motility (determined by displacement, total distance traveled, and velocity). Our results indicate that removal of the OB has no effect on the direction of migrating RMS neuroblasts, but does produce a significant effect on cell motility suggesting that the OB does not determine RMS guidance, but may maintain proper motility. Similarly, when we alter BDNF levels we also affect neuroblast motility but not direction. By contrast, manipulation of broader signaling molecules can effect both motility and direction. Therefore, we propose that multiple, distinct, yet possibly converging signaling mechanisms regulate directional guidance and cellular motility in migrating RMS neuroblasts.

Poster Session IV: Fri. July 25

Noradrenergic Neuromodulation in the Olfactory Bulb Regulates Odor Learning in Adult Mice

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The neuromodulator noradrenaline (NA) is supplied to the main olfactory bulb (MOB) by projection fibers arising from the locus coeruleus (LC). Neonatal rodent studies by McLean, Harley, Sullivan and colleagues have established that noradrenergic activity in the MOB underlies the associative learning of odor preferences; blockade of beta-NA receptors hinders neonates' ability to form conditioned odor preferences to a novel odor. Subsequent studies from multiple laboratories have demonstrated a number of bulbar NA effects on adult olfactory learning and on odor discrimination in motivated and spontaneous contexts; however, the complete picture remains unclear. Using surgically cannulated mice, we here show that cortical noradrenergic projections from the LC are necessary for normal olfactory habituation, and that intrabulbar infusions of NA suffice to restore this form of nonassociative learning to normal levels. We also describe the potential roles of bulbar NA in mediating aspects of olfactory associative learning and the regulation of generalization acuity.

Poster Session IV: Fri. July 25

Serotonergic Modulation of Odor Representation at the Earliest Stage of Odor Processing in Mice

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Centrifugal serotonergic fibers innervate the olfactory bulb, but the significance of these projections is unclear. Here, we imaged odor-evoked activity *in vivo* in mice expressing synaptopHluorin in olfactory sensory neurons (OSNs) under control of the olfactory marker protein promoter. Odor stimulation evoked graded fluorescence increases in glomeruli in anesthetized, freely-breathing mice. Pharmacological experiments revealed that odor-evoked glomerular activity was attenuated by increased serotonergic activity, and amplified by decreased serotonergic activity. These effects were mainly mediated by the 5-HT2C receptor. Using multiphoton microscopy, we showed that 5-HT2C receptor activation amplifies odor-evoked calcium rises in inhibitory periglomerular cells, and attenuates glutamate release from glomerular OSN terminals. We found that 5-HT2C receptors are expressed by periglomerular cells, but not by OSNs. Finally, to investigate the effect of serotonin released by remote intrinsic activation, we electrically stimulated the dorsal raphe nucleus, a major source of serotonin in the brain, and imaged glomerular activity simultaneously. Raphe nucleus stimulation attenuated glomerular activity over a wide range of stimulation parameters. The effects of raphe nucleus stimulation were absent in mice depleted of serotonergic fibers by treatment with 5,7-dihydroxytryptamine. In summary, we have shown that serotonin activates 5-HT2C receptors on inhibitory periglomerular cells, which decreases glutamate release from OSNs. Our data indicate that the serotonergic system is critical for sensory gain control in the olfactory bulb. This study also provides a framework for future investigations of the role of serotonin in olfactory perception and behavior.

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Poster Session IV: Fri. July 25

CSPGs in the Developing Mouse Olfactory Bulb

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Proteoglycans are a class of glycoproteins which carry covalently-linked glycosaminoglycan (GAG) side chains, such as chondroitin sulfate and heparan sulfate. During CNS development proteoglycans play important roles in morphogenesis and cell-cell and cell-substratum interactions. Interestingly, chondroitin sulfate proteoglycans (CSPGs) show diverse functions in the developing CNS, from forming inhibitory boundaries in many regions to promoting axon growth in others. The versatility of these proteoglycans may be reflected in the diversity of these molecules and the molecules with which they interact. The major classes of CSPGs in the developing mammalian brain are the lecticans (comprising aggrecan, versican, neurocan and brevican), phosphacan RPTPζ/β, and neuroglycan C. Each CSPG has a particular spatiotemporal expression pattern in the CNS, and interacts with different, sometimes overlapping, subsets of ligands. While some CSPGs have been localized to the developing olfactory system, no widespread screen of these inhibitory molecules has been undertaken to functionally assess their ability to modulate OSN neurite outgrowth. Using a multifaceted approach we characterized expression using PCR, in situ hybridization and immunolocalization to develop developmental profile of CSPG expression in the developing OS. Aggrecan, versican, neurocan, brevican and phosphacan show distinct spatial and temporal patterns of expression in the developing OB. In vitro analyses of OSN neurite outgrowth on a mixed CSPG substratum were performed and CSPGs were found to inhibit neurite outgrowth. Studies are currently underway to 1) determine whether the inhibitory activity resides in the GAG side chains or the core protein and 2) which CSPGs in the mix are inhibitory.

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Poster Session IV: Fri. July 25

Evidence for Regulation of Olfactory Bulb Dopamine Phenotype by Histone Deacetylases

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Olfactory bulb (OB) interneurons are derived throughout life from progenitors in the subventricular zone (SVZ) and migrate in the rostral migratory stream (RMS) to the granule and glomerular layers. In the dopaminergic (DA) subset of periglomerular (PG) cells, tyrosine hydroxylase (TH) expression is dependent on afferent synaptic activity. Previous studies in cultured neuronal cell lines demonstrated that TH expression can also be modulated by histone deacetylase (HDAC) inhibition. To investigate whether histone deacetylation is critical for TH expression in OB, neonatal forebrain slice cultures from transgenic mice containing a GFP reporter under the control of the 9 kb TH promoter (TH/GFP) were treated with HDAC inhibitors. In the absence of HDAC inhibitors, TH/GFP transgene expression was enhanced by depolarization in superficial granule and glomerular cells of the OB, but not in the rostral migratory stream (RMS). In contrast, treatment with either Trichostatin A or sodium butyrate strongly induced transgene expression in the RMS and SVZ independent of depolarization. A similar increase in the pattern of reporter gene expression in slices treated with Scriptaid, but not the inactive structurally-related control molecule, Nullscript, confirmed the specificity of HDAC inhibition. Preliminary in vivo studies with intraperitoneal administration of TSA and Scriptaid in adults did not induce GFP expression, suggesting perhaps either the drug concentrations employed to date were not adequate or that the HDAC effect is specific to neonates. The current findings suggest that histone deacetylases regulate TH expression in progenitors in the SVZ and RMS.

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Poster Session IV: Fri. July 25

Dopamine D_2 Receptor Modulation of ET Cell Bursting

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Based on their morphology and electrophysiology olfactory bulb juxtaglomerular cells are classified into three subpopulations – periglomerular (PG), external tufted (ET) and short axon (SA) cells. A subset of PG cells co-express dopamine (DA) and GABA. Since D_2 receptors are expressed on olfactory nerve terminals DA has been considered to have mainly presynaptic actions. Intraglomerular postsynaptic DA actions have not been investigated. The present experiments showed that quinpirole (100 μM), a selective D_2 receptor agonist, significantly increased the spike number, duration and frequency of spontaneous bursting in ET cells when both glutamatergic and GABAergic fast synaptic transmission were pharmacologically blocked. These effects were completely reversed by replacing quinpirole with eticlopride (10 μM), a selective D_2 receptor blocker; moreover when eticlopride was applied first quinpirole did not alter ET cell bursting parameters. Activation or blockade of D_2 receptors had little effect on the persistent Na^+- or low voltage-activated Ca^{2+} currents, two essential conductances underlying ET cell bursting. Activation of D_2 receptors did significantly reduce an outward current, the nature of which is under investigation. These

results indicate that activation of D_2 receptors enhances ET cell bursting and reduces an outward conductance. Thus ET cells receive both inhibitory GABAergic and excitatory DAergic feedback from PG cells. Due to their different time scales, these two opposing feedbacks may cooperate in modulating ET cell excitability. I_h-mediated rebound depolarization can transform multiple fast GABAergic inputs to a bursting response in ET cells (Shipley and Liu, this meeting). The slower onset of D_2Rs may allow the excitatory action of DA to amplify this rebound burst.

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Poster Session IV: Fri. July 25

Modulation Of D2, but not D1 Dopaminergic Receptors in the Olfactory Bulb Affects Odor Discrimination Performance

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Previous studies in our lab showed that when modulated through systemic injections, D1 and D2 receptors have opposing effects on odor discrimination learning (Yue et al., 2006). In the present study, twelve cannulated male Sprague-Dawley rats were used to investigate how modulation of these two types of dopaminergic receptors through direct infusion of D1/D2 agonists and antagonists in the olfactory bulb affect olfactory perception. Dopaminergic modulation was systematically altered by manipulations of D1 (agonist SKF 82958, 14.61, 43.82, 143.64 mM; antagonist SCH-23390, 13.36, 40.09, 60.14 mM) and D2 (agonists quinpirole, 78.19, 117.28, 156.37 mM; antagonist sulpiride, 0.29, 0.88, 2.93 mM) receptor activation during a simultaneous odor discrimination task. We found that modulation of D2, but not D1 receptors significantly affected rats' odor discrimination performance (ANOVA followed by Fisher posthoc tests). A significant positive correlation (Pearson's $R = 0.369$; $p < 0.01$) between blockade of D2 receptors and discrimination performance was observed. In addition, a significant negative correlation (Pearson's $R = -0.348$; $p < 0.01$) between discrimination performance and D2 receptor activation was also observed.

Poster Session IV: Fri. July 25

Cholinergic Modulation of Information Content and Temporal Aspects of Odor Responses in the Olfactory Bulb

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The activity of mitral cells in the main olfactory bulb (MOB) in rats is correlated to the phase of respiration. Using this fact, we were able to analyze the neural dynamics and odor information in the activity of mitral cells over time as they respond to an odor stimulus. We used PCA and pattern recognition methods on this time course data to investigate the role of the cholinergic system in modulating these dynamics. The data used in this analysis consisted of recordings from 23 mitral cells under control and drug conditions in anesthetized rats, which were stimulated with a sequence of five aliphatic esters in randomized order. The efficacy of cholinergic inputs into the MOB was modulated by local infusion of neostigmine (cholinesterase inhibitor) alone or in the presence of methyllycaconitine (MLA, a nicotinic ACh blocker) or scopolamine (muscarinic ACh blocker). We found that under saline control conditions, most of the information in the mitral cell responses occurred within the first three breathing cycles after the onset of odor stimulation. Increasing cholinergic activity via local infusion of neostigmine increased the information content of the responses, measured by the performance of a support vector machine and by estimates of the mutual information with odor stimuli. As a consequence, discrimination between chemically similar odorants could be achieved over a reduced number of breathing cycles.

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Poster Session IV: Fri. July 25

Cholinergic Modulation of Mitral Cell Responses to Odors *in vivo*

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Cholinergic neuromodulation in the main olfactory bulb (MOB) is hypothesized to regulate mitral cell molecular receptive ranges and behavioral discrimination of similar odorants. In vivo, extracellular, single unit activity of mitral cells in the MOB was measured in anesthetized rats in order to determine the degree of overlap in cellular receptive fields following exposure to chemically similar odorants. Increasing the efficacy of the cholinergic system in the MOB by addition of the anticholinesterase drug neostigmine (NEO) sharpened the olfactory receptive fields (ORF) of mitral cells in. In the presence of NEO, 59% of cells (n=37) exhibited significant response differences between chemically highly similar odors, compared to 30% of cells in saline conditions (n=56). Both the nicotinic antagonist MLA and the muscarinic antagonist scopolamine (SCO) attenuated NEO-dependent sharpening of ORFs. In the presence of MLA or SCO, 34% (n=32) and 36% (n=14) of cells respectively exhibited significant response differences between chemically similar odorants. These effects were statistically significant (ANOVA; effect of treatment, $F(3,135)=2.95$; $p<0.05$). Post-hoc tests showed that in the presence of NEO alone the proportion of cells that differed in response relative to chemically similar odors was significantly greater then that measured in saline or NEO+MLA conditions ($p<0.05$). There was no significant difference between NEO and NEO+SCO conditions. This finding suggests that the effects of neostigmine appear to occur via actions through the nicotinic and possibly muscarinic receptor. The findings from the electrophysiological recordings corroborate previous behavioral and computational studies.

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Poster Session IV: Fri. July 25

Sensory Input Activates Strong Intraglomerular Gabaergic Inhibition of Mitral Cell Apical Dendrites

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Olfactory signals are initially processed in the glomeruli, where olfactory nerve (ON) axons form excitatory synapses onto principal output neurons, mitral/tufted (M/T) cells and juxtaglomerular cells, including periglomerular (PG) and external tufted (ET) cells. M/T cells are regulated by inhibitory synapses onto their lateral dendrites from granule cells (GCs). It has long been presumed that PG cells inhibit M/T cells apical dendrites but evidence is limited. Classic EM studies inferred that PG cells make inhibitory synapses onto M/T cells but the ultrastructural criteria used to identify M/T cells would have included ET cells, which do receive inhibitory synaptic input from PG cells. Physiological evidence for PG synapses onto M/T cells is even less conclusive. To address this issue, mitral cells were voltage-clamped with pipettes containing high Cs^+ and held at relatively positive membrane potentials to enhance detection of IPSCs. ON stimulation produced an initial monosynaptic EPSC that was interrupted by a short latency (~7 msec) burst

of IPSCs, followed by a protracted train of intermittent IPSCs. All these IPSCs were eliminated by the $GABA_A$ receptor antagonist, gabazine. The late IPSCs were significantly reduced by APV suggesting that they derive from GCs; the early IPSC burst was relatively unaffected. Microinjection of gabazine into glomeruli blocked the early burst of IPSCs but had little effect on the late IPSCs. These results indicate that the early ON-evoked IPSCs derive from PG cells and provide intraglomerular inhibition of M/T cell apical dendrites. The later prolonged train of IPSCs derives mainly from GCs and provides infraglomerular inhibition of M/T cell lateral dendrites. The temporal dynamics of intra- *versus* infraglomerular inhibition are under investigation.

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Poster Session IV: Fri. July 25

Cholecystokinin Modulates the Activity of Tufted Cells and Granule Cells in Mouse Olfactory Bulb

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The medial and lateral halves of the olfactory bulb contain duplicate glomerular representations of functional olfactory receptors. These functional maps appear to be connected through a reciprocal circuit involving superficial tufted cells and granule cells. In rodents, tufted cells of this 'intrabulbar association system' (IAS) stain heavily for the peptide cholecystokinin (CCK), and their CCK-ergic axons make long range projections to granule cells in the mirror image hemi-bulb. We investigated the actions of CCK on superficial tufted and granule cells in mouse olfactory bulb slices using patch-clamp recording and calcium imaging. Perfusion of 10 μM CCK-8S caused a significant suppression (~ 50%) of spontaneous spike rates of tufted cells. Under voltage clamp, CCK-8S strongly enhanced spontaneous inhibitory postsynaptic currents (IPSC) in tufted cells. In slices loaded with Calcium Green-1, perfusion with 3 μM CCK-8S increased the frequency of spontaneous calcium transients recorded in a subset of granule cells in the inner plexiform layer, and also increased their spike discharge rates. Our results suggest that CCK neurotransmission in the IAS circuit works to amplify granule cell inhibition. Possible functions include cross-coordination of slow rhythmic activity in isofunctional glomerular modules, or mutual negative feedback regulation of high frequency spike output of tufted cells or mitral cells.

Poster Session IV: Fri. July 25

Oxytocin-Induced Synaptic Plasticity in the Accessory Olfactory Bulb

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When female mice are mated, they form a memory to the pheromonal signal of their male partner. Several lines of evidence indicate that the neural changes underlying this memory occur in the accessory olfactory bulb (AOB) at the first stage of the vomeronasal system. The formation of this memory depends on the mating-induced release of noradrenaline in the AOB. In addition to noradrenaline, the neuropeptide oxytocin (OT) is also released within the central nervous system during mating. Because OT has been implicated in social memory and its receptors are expressed in the AOB, we hypothesized that OT might promote the strength of synaptic transmission from mitral to granule cells in the AOB. To test this hypothesis, we analyzed the lateral olfactory tract-evoked field potential that represents the granule cell response to mitral cell activation and its plasticity in parasagittal slices of the AOB. Of the 10-, 20-, 50-, and100-Hz stimulations tested, the 100-Hz stimulation was optimal for inducing long-term potentiation (LTP). OT paired with 100-Hz stimulation that only produced short-term potentiation enhanced LTP induction in a dose-dependent manner. OT-paired LTP was blocked by both the selective OT antagonist desGly-NH_2,$d(CH_2)_5$[$Tyr(Me)^2$,Thr^4]-ornithine vasotocin and the *N*-methyl-D-aspartate (NMDA) receptor antagonist DL-2-amino-5-phosphonovaleric acid. These results indicate that OT can function as a gate to modulate the establishment of NMDA receptor-dependent LTP at the mitral-to-granule cell synapse in the AOB.

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Poster Session IV: Fri. July 25

Fetal Ethanol Experience and Olfactory Plasticity: Its Contribution to Adolescent Alcohol Abuse

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Clinical studies provide evidence for a predictive relationship between fetal ethanol exposure and adolescent abuse. Gestational exposure in humans is considered the best predictor of later ethanol abuse at this age. Little evidence exists regarding the factors contributing to this relationship. Extensive data demonstrate the general finding that olfactory experience influences olfactory function, that postnatal behaviors controlled by odor stimuli can be influenced by fetal odor exposure, and these early experiences can later modulate intake and preferences. We have been applying behavioral and neurophysiologic methods to test the hypothesis that altered olfactory system responsiveness to ethanol odor, following fetal exposure and adolescent re-exposure, act as contributing factors for postnatal drug preference. Dams receive either an *ad-lib* liquid diet that provides 35% of daily calories from ethanol on gestational days 11–20, a pair-fed non-ethanol diet, or free choice access to lab chow and water. Adolescent odor exposure is accomplished using a social transmission paradigm. Observer animals interact with a demonstrator peer that receives either a 1.5g/kg i.g. infusion of ethanol or an equal volume of tap water. Experimental rats display a tuned neural and behavioral response to ethanol odor and a predictive enhanced voluntary intake when tested in infancy. These consequences, although absent in adults, persist into adolescence. Adolescent re-exposure to ethanol odor augments the differential effect of the prenatal experience in terms of an altered olfactory response to the drug. The data demonstrate a relationship between fetal ethanol exposure and adolescent re-exposure, postnatal odor-guided responsiveness to the drug and ethanol avidity, and olfactory neural function.

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Poster Session IV: Fri. July 25

Odors and Disease: Volatile Biomarkers from Human Skin Cancer

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Dogs can detect the presence of skin cancer via olfaction, supporting the hypothesis that skin tumors produce a different profile of volatile metabolites than normal skin. To test this hypothesis, we collected volatile organic compounds (VOCs) emanating from basal cell carcinoma (BCC) tumor sites as well as VOCs from normal skin from age and gender-matched control subjects. We used solid-phase microextraction and gas chromatography/mass spectrometry (GC/MS) to collect and analyze the complex mixtures of VOCs we obtained. In addition, we also used similar techniques to compare the profile of volatile, chromatographable compounds from various types of melanoma cells and normal melanocytes, cultured *in vitro*. Samples were taken from cell culture flasks holding 5 ml of media with cells; melanoma cells or normal melanocytes that had reached high confluence (≥ 100,000 cells/ml). GC/MS data demonstrated no obvious qualitative changes between (a.) BCC sites and control sites from age and gender-matched controls and (b.) normal melanocytes and melanoma cells. In the samples derived from BCC patients and controls we examined several compounds in a quantitative fashion. These compounds were chosen because of their structure, origin and/or biogenesis and were monitored in all patients and controls. Statistical analyses of the quantitative data suggested that rather than ''new'' VOCs related to the carcinoma, we see a quantitative alteration of the normal VOC profile at the BCC site: one of the monitored compounds significantly decreases, and another significantly increases in relative concentration.

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Poster Session IV: Fri. July 25

News in Epidemiology of Olfactory Dysfunction

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Background:The prevalence of olfactory dysfunction in the general population is given in the literature differently. Brämerson et al., (2004) found olfactory dysfunction in the general population in 19% of cases in Sweden. Vennemann et al., (2007) diagnosed a smelling deficit with a total of 22% in Dortmund, a city of 590.000 habitants in Germany. Methods:From the data base of ENT department Jena, Germany, were extracted all persons who had their main residence at the time of olfactory testing in Jena between 1998 and 2004. Persons were divided into:1.''complained olfactory dysfunction'' (all persons who only reported about an subjective olfactory dysfunction), 2.''objectified olfactory dysfunction'' (all persons with an olfactory dysfunction measured in olfactory testing), 3.''really olfactory dysfunction'' (1. and 2. together) Results:Relating to the whole number of habitants of Jena a total of 0.23% of the Jena population underwent olfactory testing between 1998 and 2004. 0.08% of the Jena citizens complained an olfactory dysfunction. In 0.06% of cases an ''objectified olfactory dysfunction'' was found. Only 0.05% of the study population could be detected as ''really olfactorily disturbed''. Conclusions:0.06% of the Jena citizens have an ''objectified olfactory dysfunction''.In the Swedish population people with an olfactory dysfunction can be found about 320 times and in Dortmund 368 times more than in Jena. An explanation could be a high estimated number of unreported cases in Jena. More intensive health education programs about the prevalence, symptoms, prediction, and therapy of olfactory dysfunction are urgently necessary.

Poster Session IV: Fri. July 25

The Prognosis of Olfactory Dysfunction Depends Upon its Etiology and on Remaining Olfactory Function

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Treatment options of olfactory dysfunction are limited and patients are often not provided with a prognosis of their smell problem. We retrospectively assessed factors influencing the prognosis of olfactory function in 270 men and 381 women, aged 11 – 84 years, having twice reported to a specialized ENT center. While at the first olfactory assessment all subjects had been functionally anosmic or hyposmic, 11.3% and 8.3% of initial anosmic or hyposmic subjects, respectively, improved to normal olfactory function at the second assessment. A dependency of the final olfactory diagnosis from initial olfactory function missed statistical significance ($p=0.053$). In contrast, the final olfactory status significantly depended on the etiology of the olfactory loss ($p<0.001$). Recovery to normosmia was reached by 10.9 or 10.6% of the cases with sinunasal or infectious etiology, respectively, but only in 5.5% of the cases with traumatic etiology. Similarly, anosmia as the final outcome was found in 44.2 or 26.5% of sinunasal or viral etiology but in 64.4% of the traumatic etiology. We conclude that the prognosis of olfactory loss mainly depends on the etiology of the disorder, with trauma having the worst prognosis, and only at a secondary level on the status of the sense of smell at the first visit.

Poster Session IV: Fri. July 25

Impact of Cigarette Smoke on Olfactory Damage in Patients with Chronic Rhinosinusitis

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Our ongoing clinical research into chronic rhinosinusitius (CRS) has revealed considerable variability among subjects in the degree of remodeling in the olfactory mucosa (OM). Further, this remodeling is not clearly correlated with age or other concurrent health conditions. To explore this variability, we examined the impact of direct or indirect exposure to cigarette smoke on olfactory performance and OM pathology. Among our CRS subjects <50 years old, we identified four groups: current smokers, past smokers, nonsmokers who self-reported exposure to secondhand cigarette smoke (SHCS) in their daily lives, and nonsmokers with no reported SHCS exposure. We also examined control subjects of comparable age range with no SHCS exposure. When comparing current smokers with nonsmokers and controls, we found the olfactory performance of current smokers was similar to that of controls, but both they and CRS nonsmokers evidenced slower mucociliary clearance rates than did controls. The OM of current smokers showed signs of more severe pathological remodeling (i.e., squamous metaplasia) and cellular changes [i.e., loss of olfactory supporting cells (SC) and abnormal olfactory sensory neurons (OSN)] than did the OM of nonsmokers and controls. We also found that OM pathology was less severe in CRS patients who had quit smoking for at least 10 years as compared to those who quit <10 years ago. Lastly, we examined nonsmokers with SHCS exposure and, disturbingly, found their olfactory performance was the worst within our CRS population. Moreover, examination of their OM revealed a high prevalence of shedding of the OE and of loss of SCs and OSNs. These results suggest that both smoking and SHCS exposure accentuate the adverse effects of CRS on olfactory function and OM pathology.

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Poster Session IV: Fri. July 25

Nasal Lavage Cotinine Level as a Tool for the Assessment of Smoking Status

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Nicotine is absorbed and distributed rapidly in the body and converted to cotinine, its major metabolite, in a two-step process involving cytochrome P450 and aldehyde oxidase. Precise estimation of exposure to tobacco smoke is critical, as self reports may be inaccurate. While nicotine has a relatively short half-life of about 2 hours, cotinine has a half-life of approximately 20 hours. Therefore, measurement of cotinine levels is the preferred method for validation of self-reported smoking status. In our study of chronic rhinosinusitis (CRS) patients, we have noted that smoking status and exposure to cigarette smoke appear to represent significant risk factors for olfactory epithelial damage. We therefore sought an objective measure of smoke exposure. Cotinine levels have been measured in urine, plasma and saliva, but only nasal lavage samples were available from these patients. We sought to determine whether cotinine levels in nasal lavage fluid (NLF) would provide a reasonable estimate of nicotine exposure. To test this, we first assayed cotinine using a competitive immunoassay (Salimetrics) in saliva and NLF from 5 healthy smokers and 1 non-exposed, nonsmoker. Results were consistent, with NLF cotinine approximately 5 to 10 times higher in the smokers vs. the nonsmokers, regardless of sample type. We then assayed the NLF samples from CRS patients and compared results with self-reported smoking status and secondhand smoke exposure. Cotinine levels in exposed (n =6) vs. non-exposed (n =22) non-smokers were comparable, and lower than levels in current smokers (n =7). Data were consistent with self-reported smoking status. These data support the use of NLF cotinine as a check on smoking status.

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Poster Session IV: Fri. July 25

Factors Associated With Smell Loss in Chronic Rhinosinusitis

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Chronic rhinosinusitis (CRS) is both one of the most common chronic diseases in the U.S. and one of the most common causes of smell loss. Yet, not all sufferers of CRS experience smell problems. In order to develop effective, targeted therapies for this form of smell loss, it is critical that we identify key aspects of the disease process that impact olfactory function, and in order to better understand the impact of CRS on quality of life (QOL), it is important to better characterize its symptomology. To this end, the Monell-Jefferson Chemosensory Clinical Research Center has collected extensive data from patients with clearly defined CRS, including measures of olfactory sensitivity, nasal air flow and saccharin transit time (STT); endoscopic and computerized tomography assessments; histological assessment of olfactory biopsy specimens; measures of QOL; and measures of inflammatory factors in nasal lavage fluids. To date, 46 CRS patients have been examined, approximately half of whom have measurable olfactory loss. Those with and without smell loss differ on a number factors. For example, in our sample, patients scoring greater than 9 on the Miami CRS endoscopic staging system (Lehman et al., Am J Rhinol, 20:11-19, 2006) invariably suffer smell loss, smell loss tends to be associated with slower STT, and those with smell loss score more poorly on the SF-12 physical summary measure, as well as on several other measures of QOL, than do those without. These studies point to key factors associated with smell loss in CRS and should lead to enhanced understanding of the basis for that loss and to improved appreciation of its impact on patients' lives. Supported in part by NIH DC006760.

Poster Session IV: Fri. July 25

Cell-Autonomous Inflammation in Human Aging - Implications for Olfactory Receptor Impairment

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Olfactory impairment is a well described phenomenon in human aging. However, the underlying biological mechanisms, involvement of either peripheral or central components of the olfactory system and the early onset in related chronic diseases are not well understood. Our objective was to apply a cell systems approach to understand the basal changes in metabolism, cell signaling and cellular maintenance involved in cellular aging. We determined genome-wide steady-state messenger RNA levels in a cross-sectional study of human fibroblasts. In this initial model system we showed that cells from old donors undergo transcriptional changes adjusting metabolic processes, a phenomenon previously described in other organism as a retrograde response. The gene expression signature was further characterized by increased inflammatory levels of cytokines, chemokines, components of the complement cascade and MHC molecules, as well as changes in calcium related pathways. We investigated possible underlying mechanisms and show that the observed alterations occur as a consequence of diminished mitochondrial respiratory capacity in aged cells, which includes changes in calcium homeostasis and NF-kB activation. Our results are consistent with the view that low-grade inflammation, a hallmark of many age-associated diseases, is a cell-autonomous phenomenon and part of a cellular survival mechanism in aging cells. This supports our hypothesis that similar mechanisms may play a role in the aging of olfactory receptors. In particular changes in calcium homeostasis are known to alter sensitivity, duration and selectivity of olfaction, offering a route towards identification of mechanisms and therapeutic measures.

Poster Session IV: Fri. July 25

Olfactory Perception in Patients with Binge Eating Disorder - Is it Different from Anorexia and Bulemia?

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Olfactory perception in eating disorders is poorly investigated and the few studies conducted yielded partly contradictory results. Thus, the aims of our study was to analyze olfactory perception including intensity and hedonic estimates in patients with Binge eating disorder (BED), Anorexia (AN) and Bulemia (BU) compared to healthy controls. We employed the Sniffin' Stick test to assess olfactory function and registered intensity and hedonic estimates using visual analogue rating scales. Patients participating in the study: BED: 16 patients (mean age: 31.1 (SD: 10.8) years, body mass index (BMI): 43.9 (SD: 6.9) kg/m2), AN: 43 patients (mean age: 27.2 (SD: 11.3) years, BMI: 15.8 (SD: 2.3) kg/m2), BU: 32 patients (mean age: 25.5 (SD: 8.2) years, BMI: 20.7 (SD: 5.6). For statistical analysis we matched the three patient groups on age and gender employing the dataset HedoS-F (Hedonic

database of Smell - Franconia) as healthy controls. Following Kolmogorov-Smirnoff-testing t-tests were calculated in order to assess differences between the groups and controls. Our statistical analysis clearly revealed significant differences compared to healthy controls: BED: a decreased discrimination only, AN: increased intensity estimates, decreased discrimination and identification, BU: increased intensity estimates and a decreased discrimination. In contrast we found no significant differences in the hedonic estimates for BED, AN and BU and no significant differences in intensity estimates compared to healthy controls. Our results demonstrate that specific patterns of disturbed olfactory perception exist for BED, AN and BU.

Poster Session IV: Fri. July 25

Effects of Repetitive Stimulation with Pleasant and Unpleasant Odours

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Desensitisation in response to repetitive odorous stimuli is a common and well investigated symptom, but it is still not entirely clear whether this process relates to odour valence. Hence, our goal was to investigate changes in the perception of pleasant and unpleasant odours with multiple presentations. Thirty normosmic subjects received two pleasant and two unpleasant odours presented with air-dilution olfactometry. In the first part of the experiment, each odour was presented in four concentrations with a stimulus duration of 0.2 s. The second part was identical to the first part, except that an additional stimulus of 4 s duration was presented 45 s before each 0.2 s stimulus. After each stimulus of 0.2 s duration subjects rated the odours' intensity and hedonic tone. In addition, simultaneously with presentation of the 0.2 s stimuli EEG was recorded and analysed in the frequency domain. Results indicate that the adapting stimulus significantly decreased odour intensity and also changed the hedonic tone. With increasing concentration the pleasant odours were rated more and more intense and pleasant. However, for the two unpleasant odours changes of stimulus concentration had no substantial effect on intensity and hedonic ratings. Frequency analyses of the stimulus-linked EEG indicated that stimulation with all four odours produced a decrease in the theta band indicating an increase of arousal. During the second part of the experiment, for pleasant odours arousal increased with increasing stimulus concentration, whereas unpleasant odours did not produce such concentration-related changes. These results suggest that there are differences between the adaptation to pleasant and unpleasant odours not only with respect to intensity and hedonic tone but also regarding arousal.

Poster Session IV: Fri. July 25

Assessing Olfactory Hedonic Estimates: How Complex Does the Study Design Have To Be?

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The assessment of olfactory hedonics is poorly standardized and the reproducibility of hedonic estimates and the influence of the presentation of anchor-stimuli on hedonic estimates remains to be analyzed. Thus we investigated (1) the reproducibility of hedonic estimates and (2) the influence of repeated anchor-stimulus presentation on the hedonic evaluation employing testing series with 16 standard odors of the Sniffin' Sticks Test. In addition (3) we screened for non-linear effects of different anchor stimuli on hedonic estimates. (1) 12 vounteers (mean age: 26.17, SD: 2.82 years) participated in repeated testing sessions (n=4) over 4 weeks. (2) We tested the influence of two anchor presenting condtions (condition A) the anchor stimulus was presented at the beginning of the testing series only, condition B) the anchor stimulus was presented before each single odor) in 19 volunteers (mean age: 30, SD: 9.43 years). (3) We registered the hedonic estimates of 31 volunteers (mean age: 28.52, SD: 7.74 years) employing four different anchor-stimulus-conditions. Data were analysed by a non-parametric approach based on rank statistics and a linear mixed model. Hedonic estimates remained stable over the testing period of 4 weeks and single or repeated anchor presentation yielded similar results. The influence of different anchors – overall – was relatively small, although partly non-linear significant effects occurred.

Poster Session IV: Fri. July 25

Predicting Odor Pleasantness with an Electronic Nose

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A primary dimension of olfactory perception is odor pleasantness. How much of this particular dimension is learned and how much is innate remains a topic of debate. The innate aspect of odor pleasantness should be rigidly mappable to odorant structure. However, quantifiable structural features of odorants number in the thousands, and therefore generating such mapping is complex (Khan et al., 2007). To bypass this inherent complexity, we set out to train an electronic nose (eNose) to predict odor pleasantness. We obtained human pleasantness estimates (20 subjects, visual-analog scale), and eNose measurements (Moses II) of 70 monomolecular odorants, and built a regression algorithm from eNose to perception. We first tested this algorithm in a leave-20-out scheme on the original data, and obtained a significant prediction of odor pleasantness as a function of eNose output ($r = 0.6$, $p < 0.01$). Next, we used the eNose to smell 23 new odorants that were not part of the learning set (20 mixtures and 3 monomolecular odorants), then used the algorithm to predict their pleasantness, and finally obtained pleasantness estimates for these odorants from 20 human subjects. The correlation between the predicted and actual pleasantness estimates was $r = 0.65$ ($p < 0.01$). This result, together with our previous demonstration of predicting olfactory receptor responses with an eNose (Haddad et al., 2008), combine to demonstrate that odor aspects governing both neural and perceptual olfactory responses can be captured in part by an eNose.

Poster Session IV: Fri. July 25

"I Don't Want To Know About It". Unpleasantness Prevents Odor Identification

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Affective evaluation is one of the critical early stages in the cognitive processing of olfactory information and may involve different mechanisms for unpleasant and pleasant odorants. One hypothesis for such dissociation is that as opposed to pleasant odors, unpleasant smells would entail a "quick and dirty" pathway that may have weak links with the high-level cognitive function of language. In the present study we tested this hypothesis. Forty participants (age 19-25 years) were exposed to 9 odorants known to cover a wide range of hedonic responses (pleasant, neutral, unpleasant). The

experiment consisted in 3 sessions: 1) in a 1-second sniff, we analyzed facial mimics and spontaneous verbal responses; 2) in a 2nd task subjects were to rank odorants from the most pleasant to the most unpleasant; 3) in a 3rd delayed task, participants smelled again the odorants, and described their impressions as precisely as possible. Statistical analyses included a variable named ''identification rate'' that was based on providing a label, no matter correct or not. ''Identification rate'' was entered into an ANOVA with duration of exposure (first task / third task) and odor valence (unpleasant, neutral vs. pleasant) as within-subject variables. Results showed effects of odor valence during both the first spontaneous task ($p < .0008$) and the third delayed task ($p < .0001$): in line with our hypothesis, odor ''identification rate'' was enhanced with pleasant odors and reduced with unpleasant odors (compared to neutral odors). Taken together, these results showed that attempting to identify a smell is a spontaneous mechanism dedicated to pleasant odorants and at a very less degree to bad smells.

Poster Session IV: Fri. July 25

Shared Hedonic Responses in Rodents and Humans

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In mammals, smells evoke attractive and repulsive hedonic reactions. Although these preferences toward or against environmental stimuli are strongly shaped by experience, they are also partially innate and predetermined by the physical properties of odorants and may thus be shared by mammals. To test this hypothesis we recorded hedonic responses in humans (through sniffing and subjective ratings) and in mouse (through investigation time) using the same odorants (acetophenone, amyl acetate, diphenyl oxide, ethyl butyrate, eugenol, guaiacol, heptanal, hexanoic acid and phenyl ethanol). Results showed that mice spent significantly more time exploring certain odorants (amyl acetate, ethyl butyrate, heptanal and hexanoic acid) compared to others (acetophenone, diphenyl oxide, eugenol, guaiacol, and phenyl ethanol) ($p<.05$). Similarly, humans liked more the former set of odorants compared to the latter ($p<.05$). A control study performed in humans showed that both set of odorants did not differ in term of intensity and familiarity ($p>05$). These results strengthened the view that olfactory preferences are partly predisposed and shared between humans and rodents.

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Poster Session IV: Fri. July 25

Building a Better Intensity Scale: Which Labels are Essential?

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Intensity descriptors are used as anchors in many psychophysical scales (e.g., category, Likert, VAS) to quantify perceived sensation. Labeled scales enjoy widespread use because of their assumed simplicity, but recent work suggests that several of their key features promote inaccuracy. In particular, while the relative spacing of intensity descriptors appears to be invariant, mounting evidence indicates that the absolute intensities denoted by scale labels vary with both the experience described and the individual experiencing it. This idea presents a serious problem: Unless scale labels are proven to define equal perceived intensities to all experimental groups, comparisons among those groups are invalid. One possible way to enable such comparisons is to define the domain of interest as all sensory experience, as the General Labeled Magnitude Scale (gLMS) does with its top anchor of ''strongest imaginable sensation of any kind''. However, we believe that the word ''imaginable'' detracts from the universality of the gLMS's top anchor; the intensities of imagined and experienced sensations are correlated, yet individual differences in imagery may confer different meanings to the label. Moreover, the gLMS contains intermediate labels spaced empirically, but these labels may lack sufficient context to be helpful. To explore these ideas, we compared ratings of 17 remembered sensations rated on the gLMS vs. a line with endpoints labeled ''no sensation'' and ''strongest sensation of any kind ever experienced'' vs. a numerical scale (0-100) labeled similarly. All three scales produced equivalent ratings, supporting our views. In short, the simplest labeled scale allowing group comparisons may be linear or numerical, with endpoint labels expressed in terms of all sensations experienced.

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Poster Session IV: Fri. July 25

Olfactory Stimulation in Major Depression: A Robust Technique to Elicit Pleasure?

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Anhedonia, the loss of joy and pleasure in life, is a major symptom of depression. Although anhedonia represents a severe symptom of the diesase, pilot studies and clinical reports indicate that the perception of olfactory pleasure could be undisturbed during the clinical time course of Major Depression. In order to test this hypothesis we registered olfcatory hedonics of 37 patients (16 male, 21 female) with a DSM-IV diagnosis of Major Depression. Using the 16 standard odors of the Sniffin' Sticks Test we registered the hedonic estimates for each participant on a bipolar analogue rating scale. Depression severity was assessed with the German version of the Beck Depression Inventory, anhedonia with the German version of the Snaith-Hamilton-Pleasure-Scale. Our analysis of variance revealed no interaction between hedonic estimates and severity of depression, neither expressed in BDI scores nor via the SHAPS. Our study clearly demonstrates that the ability to perceive olfactory pleasure is not significantly influenced by episodes of Major Depression. This further supports the therapeutic use of odors in pleasure retraining treatments bringing back hedonia in the patients' lifes. At an anatomical level we interpret our data in the way that the symptom of anhedonia in depression is mediated by a brain structure different from structures involved in the evaluation of olfactory hedonics.

Poster Session IV: Fri. July 25

Thalamic Role in Olfactory Hedonics

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Olfactory information projects from primary olfactory cortex to orbitofrontal cortex by two routes; a direct and an indirect route via the mediodorsal (MD) nucleus of the thalamus. The role of this thalamic path in olfactory processing remains unknown. One case study reported that bilateral thalamic infraction in MD nucleus was related to alteration in hedonic tone, whereby odors were perceived as less pleasant than before the lesion. To systematically test for a thalamic role in hedonic perception of odors, we tested patients with focal thalamic lesions and healthy matched controls on odor detection and identification, as well as both auditory and olfactory pleasantness and intensity scoring and recognition memory. Because the lesions were mostly unilateral, each subject was tested in both nostrils/ears separately. To date we tested 6 patients and 4 healthy controls. Initial analysis

revealed no difference in detection, identification and memory performance across nostrils in patients, nor between patients and controls (all $t < 0.49$ all $p > 0.65$). However, there was a trend for patients to rate odors, but not sounds, as less pleasant than controls in both nostrils (4 out of 6 patients, normalized mean rating patient = -0.55, control = -0.34, $t = 1.69$, $p < 0.1$). Furthermore, there was a trend for patients to rate odors as less intense in the ipsilesional compared to the contralesional nostril (4 out of 6 patients, normalized mean rating ipsilesional = 0.24, contralesional = 0.39, $t = 1.46$, $p < 0.15$), and as less intense when compared to controls (normalized mean rating patient = 0.24, control = 0.53, $t = 1.9$, $p < 0.06$). Our preliminary results suggest that the thalamus doesn't take part in basic olfactory processes such as detection and identification, but may influence olfactory hedonic tone.

Poster Session IV: Fri. July 25

Effect of Aroma on Empathy

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Objectives: Empathy has been identified as the primary factor in the success of the therapeutic relationship. Yet interventions to enhance this have been limited. Odorants have been identified to have an effect on generosity and may have an influence on empathy.

Methods: One hundred subjects (84 F, 16M) average age 32 (18-64), blinded to the experimental hypothesis underwent the empathy Helpful Response Questionnaire (HRQ), a series of six vignettes. In a double-blinded fashion, in a counter-balanced order, with proboscis was covered alternately with an unscented tissue, and tissue odorized with the scent of Vicks® (a mixture of eucalyptus, camphor, and menthol). While exposed to the odorant subjects completed the first half of the HRQ followed by a fifteen minute washout period, and then the second group of vignettes exposed to the non-odor control or visa versa. Following this, odor hedonics and presence of olfactory evoked nostalgia (to the scent of Vicks®), and olfactory ability based on the Alcohol Sniff Test was obtained. The HRQ score was blindly rated based on the Miller four point scale. Statistical significance was determined independently using paired difference t test.

Results: Eighty-three were normosmic. Total HRQ score ranged from 0-26. No order effect was seen. Increase in empathy with odorant (compared to the unodorized tissue) ($p<0.05$): Entire group (n=100), 19% increase; Of those with positive hedonics to Vicks (n=71), there was a 22% increase; those with positive hedonics and normosmia (n=63), there was a 26% increase; those with positive nostalgia to Vicks (n=42), there was a 27% increase.

Conclusion: This warrants investigation in those with empathy impairment such as autism or Asperger's syndrome.

Poster Session IV: Fri. July 25

The Function of the Gustducin in the Soft Palate Taste Buds Differs from that in the Tongue

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Gustducin is a taste-specific G-protein mediating bitter, sweet and umami tastes. Based on the expression patterns of gustducin, the function of gustducin has been implicated primarily in bitter taste in the circumvallate (CV) papillae and in sweet taste in the fungiform (FF) papillae. We have recently examined the expression patterns of gustducin and IP3R3 in taste buds of the soft palate (SP), CV and FF in the rat by double-color whole-mount immunohistochemistry. Gustducin was expressed in almost all (96.7%) IP3R3-expressing cells in taste buds of the SP, whereas gustducin-positive cells were 42.4% and 60.1% of IP3R3-expressing cells in FF and CV, respectively. These data suggest that gustducin may be involved in signal transduction of all tastes of sweet, umami, and bitter in the SP, in contrast to its limited function in the tongue taste bud. To confirm the broad role of gustducin in the taste transduction on the SP, responses from three major gustatory nerves in gustducin-KO mice were recorded electrophysiologically and the response properties were compared among the nerves. In consistent with the immunohistochemical results in the rat, nerve responses to both sweet and bitter stimuli were markedly reduced in the greater superficial petrosal nerve (GSP) of gustducin-KO mice. In contrast to the GSP, the chorda tympani nerve (CT) and the glossopharngeal nerve (GL) showed reduced responses to sweet and bitter stimuli, respectively. Immunohistochemistry of gustducin and IP3R3 in mice showed that 91.1% of IP3R3-expressing cells in the SP was gustducin positive. These results demonstrate that gustducin is involved in the different lines of the taste-signaling pathway depending on the taste cell differentiation.

Poster Session IV: Fri. July 25

A Novel Role For Gα-Gustducin: Regulating the Responsivity of Taste Cells

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The Gα-protein, α-gustducin, is present in mammalian taste buds and activates phosphodiesterases to depress levels of cAMP. However, gustducin's role in taste transduction remains unclear. Hence, we asked whether baseline levels of cAMP are altered in the absence of α-gustducin. We found that cAMP in unstimulated taste buds is 3.8 fold higher in gus-knockout mice than in wild-type mice (t-test; $p = 0.0005$; $n = 6$). We then tested whether the tonically elevated cAMP activates Protein Kinase A (PKA) to influence responses to tastants. Using calcium-imaging, we stimulated gus-GFP-labeled circumvallate taste cells with the bitter tastant denatonium (10 mM). As expected, denatonium elicited little or no increase in intracellular Ca^{2+} in the GFP labeled cells from knockout mice. However, when PKA was inhibited with 10μM H-89, responses to denatonium were dramatically unmasked in some cells, and enhanced 6.5-fold on average (t-test; $p < 0.0001$; $n = 21$). Interestingly, H-89 also significantly increased Ca^{2+} responses to denatonium 2.9-fold in gustducin expressing wild-type cells (t-test; $p < 0.0001$; $n = 26$). Thus, we suggest that cAMP, through PKA, dampens Ca^{2+} responses to tastants, and that an important role of gustducin is to maintain cAMP levels tonically low to ensure adequate Ca^{2+} signaling.

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Poster Session IV: Fri. July 25

Expression of the GαQ Family in Taste Buds of Posterior Tongue: Possible Role in Detection of Sweet and Umami

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Taste cells (''Type II'' or ''TRC'') express G protein-coupled taste receptors for sweet/umami (T1Rs) or bitter (T2Rs) and proteins involved in signaling cascades. Transduction downstream of T1Rs and T2Rs relies on PLCβ2-mediated elevation of Ca^{2+}. Gustducin, a G protein α subunit characteristic of taste buds, is co-expressed with these receptors in only some TRC. The pattern of expression and role of other Gα-proteins in TRC is less understood. We utilized RT-PCR and immunocytochemistry to examine expression of the Gαq family (αq, α11, α14, α15) in mouse taste epithelium. An antibody directed against Gαq/11/14 stains substantial numbers of TRC in vallate and foliate papillae, but few in the palate or fungiform papillae. This antibody stains few TRC in a Gαq null mouse suggesting that much of the reactivity in taste tissue is attributable to Gαq. Analyses of mRNA expression in taste buds show that Gαq expression far exceeds expression of Gα11, although substantial expression of Gα14 also is detected in a taste-selective manner. RT-PCR on isolated GFP-labeled TRC shows that Gαq and Gα14 are expressed predominantly in Type II cells. Taste tissues from WT, TRPM5-GFP and T1R3-GFP transgenic mice were used in immunocytochemistry to further evaluate whether Gαq expression is cell-type specific. Nearly all of the PLCβ2 /TRPM5+ TRC of the foliate and vallate papillae are immunoreactive for either Gαq/11/14 or gustducin, but not both. In T1R3-GFP mice, foliate and vallate TRC that strongly express GFP stain with the Gαq/11/14 antibody. In summary, sweet- and umami-responsive TRC in foliate and vallate papillae likely express Gαq/14 but not gustducin.

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Poster Session IV: Fri. July 25

Identification of Camp Transduction Pathway in the Sugar Receptor Cell of the Fly: Study by the Patch Clamp Techniques

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In the sugar receptor cell of the fly, location of signal transduction pathway activated by cAMP has not been indicated, though transduction pathways mediated by second messengers, such as cGMP and IP_3/Ca^{+2} have previously been suggested. We examined those 3 locations of transduction pathways. Evidence of cGMP transduction pathway was obtained by the observation of an inward current from the sugar receptor cell when cGMP solution (pseudo inner solution plus 2,7 μM) was injected to the sugar receptor cell via a patch pipette in the whole cell clamp. Function of IP_3, as a second messenger, was recognized when the IP_3 solution (the pseudo inner solution plus 2,7 μM IP_3) was injected to the sugar receptor cell and an inward current flow was observed. When Ca^{2+} solutions (3 mM and 5 mM) plus EGTA (2 mM) were examined to the sugar receptor cell in the whole cell mode, an inward current flow was observed. Evidence of cAMP transduction pathway was obtained by the observation of an inward current flow stimulated by cAMP solution (pseudo inner solution plus 2,7 μM). Inhibitors and activators for the second messengers (GDPßS, ruthenium red and forskolin) were also examined. The evidences obtained showed that cAMP may contribute to transduction of the sugar receptor cell of the fly to the great extent.

Poster Session IV: Fri. July 25

Camp Triggers Ca^{2+} Influx in Mouse Presynaptic/ Type III Taste Cells

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The second messenger, cAMP, is modulated during taste transduction. Yet, the significance of cAMP changes and the taste cell types in which they occur (Type I glial-like; Type II Receptor; Type III cells with synapses) remain unclear. We explored the effect of elevating cAMP on Ca^{2+} levels, using Fura-2 imaging of isolated mouse vallate taste buds. Stimulating taste buds with forskolin + IBMX to elevate $[cAMP]_i$ evoked Ca^{2+} responses in 38% (49 out of 128) of Presynaptic/Type III taste cells (defined by their Ca^{2+} response to KCl depolarization). GFP-labeled Receptor cells from PLCβ2-GFP mice did not show Ca^{2+} responses following forskolin and IBMX. About 70% of Presynaptic cells express Gad1. Using Gad1-GFP mice, we found that only Presynaptic taste cells *lacking* Gad1 responded to cAMP elevation. cAMP-induced responses were generated by Ca^{2+} influx and blocked by H89, an inhibitor of cAMP-dependent protein kinase A (PKA). cAMP-evoked Ca^{2+} influx was blocked by nifedipine, an inhibitor of L-type voltage-gated Ca channels. In contrast, inhibitors of P/Q- type (ω-agatoxin IVA) or N-type (ω-conotoxin GVIA) Ca channels had no effect on cAMP-evoked Ca^{2+} responses. Interestingly, ω-agatoxin did block depolarization-induced Ca^{2+} responses in all Presynaptic cells. Thus, Ca^{2+} influx upon depolarization is primarily through P/Q-type channels whereas influx triggered by cAMP is through L-type Ca channels. Consistent with these data, single cell RT-PCR showed that the L-type subunit (α1C) was expressed primarily in GAD-negative Presynaptic cells, while the P/Q-type (α1A) was expressed in all Presynaptic cells. Thus, cAMP may modulate the function of synapses in some taste cells.

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Poster Session IV: Fri. July 25

Taste Profiling Utilizing Multi-Photon Fluorescence Lifetime Imaging (FLIM) in Intact Taste Tissue

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Recent advances in microscopy and fluorescence imaging techniques have led to multiple applications of non-invasive, non-destructive and quantitative methods for monitoring intracellular processes in biological systems. Here we demonstrate the set-up and capabilities of a novel technique in profiling the response of individual taste cells in intact taste papillae to a range of stimuli. Our current studies highlight experiments performed using a multi-photon system that permits optical sectioning of intact fungiform taste papillae obtained by biopsy from micro pigs (*Sus Scrofa Domestica*). The papillae ranged from 500-1000 mm in diameter and each papilla contained up to 20 taste buds. Iontophoresis was used to load calcium green-1 dextran into taste cells from the apical surface. Papillae were mounted in a

two-compartment chamber that permitted independent perfusion of the apical and basolateral surfaces of the tissue. Taste cells remained viable for up to 4 hrs, which allowed responses to a variety of taste compounds. Viability of cells was characterized by high intracellular calcium values. Calcium concentrations in taste cells were determined using fluorescence lifetime measurements (FLIM) obtained by time-correlated single-photon counting. These measurements, which are not affected by dye concentration, were used to assess the resting and peak calcium levels in taste cells. Temporal changes in calcium in response to taste stimuli were measured as relative changes in fluorescence intensity. The combination of intensity and lifetime measurements provides complementary information about calcium responses by establishing which cells are maintaining low intracellular calcium levels at rest. Additionally we discuss some in-house developed imaging data quantification and analysis tools.

Poster Session IV: Fri. July 25

Identification of Genes that Define Specific Taste Cell Populations

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A comprehensive genome-wide survey of gene expression in fungiform (FG) and circumvallate (CV) taste buds was conducted to identify novel taste-specific genes. Taste buds and lingual epithelium (non-taste tissue) were collected by laser capture microdissection and genome-wide microarray analyses were performed to generate a taste bud expression database (see abstract by Hevezi et al.). Bioinformatics analyses of the taste bud database identified ~200 taste-specific genes predicted to encode transmembrane proteins with no known function in taste. Double label in situ hybridization analysis identified eight new genes expressed in sweet, bitter, and umami cells (TRPM5-positive) and one new gene expressed in sour cells (PKD2L1-positive). In addition, three genes defined additional taste cell populations. GPR113, a class B orphan GPCR, is expressed in a novel subset of TRPM5 cells that expresses T1R3 but not T1R1, T1R2, or gustducin. GPR113 may complex with T1R3 to generate a novel taste receptor, or, alternatively, this population may represent a precursor to other TRPM5 lineages. TMEM44, a protein with seven predicted transmembrane domains, is expressed in cells that may represent taste cell precursors. Lastly, we identified another gene, SNMX-29 (Senomyx taste-specific gene 29), which is expressed in a unique taste cell population distinct from other taste cell populations. These and other data identifiy SNMX-29 as a candidate salty taste receptor. In conclusion, a genome-wide survey of taste bud gene expression has identified numerous taste-specific genes that define unique taste cell populations. The discrete expression patterns observed support a model whereby each taste receptor cell population is tuned to a specific taste quality. Bryan D. Moyer and Peter Hevezi are co-first authors.

Poster Session IV: Fri. July 25

Barriers in Mouse Taste Buds: Dye Penetration Studies

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Tight junctions (TJs) are ultrastructural specializations that join adjacent cells in epithelial tissues. TJs provide a semipermeable barrier that separates the environment of the apical, or mucosal surface of an epithelium from its basolateral, or serosal spaces. In taste buds, TJs between apical tips of taste cells in the taste pore prevent sapid stimuli from penetrating into the intragemmal spaces surrounding taste cells. Thus, TJs confine most gustatory stimuli to the chemosensitive tips of taste receptor cells. Immunostaining for TJ proteins and dye penetration studies confirm this notion (Michlig et al., 2007). Michlig et al., (2007) also showed that one component of TJs (claudin 7) coats the entire basolateral surfaces of taste cells. This raises the possibility that there may be additional barriers controlling access to taste cells, perhaps even access from the circulation. To test this hypothesis we assayed the ability of several dyes (including Lucifer yellow, FITC-Dextran, Alexa Fluor 594, Texas red-dextran) to penetrate into taste buds when thin slices (100-200 μm) of lingual epithelium were completely bathed in the tracer. We found that dyes applied in this manner readily diffused into non-taste epithelium surrounding taste buds but remarkably, were completely excluded from taste buds. Our findings suggest that there is a formidable barrier within taste buds that restricts dyes and other compounds from reaching taste cells, even if the compounds bathe the basolateral surface of the lingual epithelium. This raises the intriguing possibility that taste buds represent a privileged, confined environment protected by a "blood/taste bud" barrier, and over which TJ proteins may exert powerful and selective control.

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Poster Session IV: Fri. July 25

Characterization of Human Fungiform Taste Bud Cells in Primary Culture

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Previously we developed conditions for primary culture of rat taste cells that yielded cells exhibiting molecular and functional properties similar to their in vivo counterparts (Ozdener et al., 2006). Humans differ from rats in their sensitivity to a number of taste stimuli, and in the organization of taste buds within papillae. Accordingly, we aimed to develop a reproducible protocol for isolating and maintaining long-term cultures of human taste bud cells. Cells from human fungiform papillae obtained by biopsy were successfully maintained in culture for more than six passages (6 months) without loss of viability. Cells within these cultures displayed many molecular and physiological features characteristic of mature taste cells. Gustducin, phospholipase Cβ2, (PLC-β2), T1R3, T2R5 and TRPM5 mRNA were detected by reverse transcriptase-polymerase chain reaction and products confirmed by sequencing. Immunoprecipitation and Western blot analysis demonstrated gustducin and PLC-β2 expression in the same samples, and expression of these markers was detected immunocytochemically in 60% and 30% of cells, respectively. Cultured cells also exhibited robust increases in intracellular calcium in response to appropriate concentrations of several taste stimuli. Electrophysiological studies indicated that some cells developed voltage-activated currents, as well as depolarizing receptor currents upon application of taste stimuli. These results indicate that isolated taste cells from adult humans can be cultured and maintained for at least six passages.

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Poster Session IV: Fri. July 25

Response Properties of Taste Cells in Intact Fungiform Taste Buds

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Vertebrate taste buds are complex structures containing several functional cell types. Considerable progress has been made in relating response properties to particular cell types, with sweet, bitter and umami responses being generated in subsets of type II cells. The cells mediating salt and sour responses are less well defined. In addition, lateral interactions between taste bud cells have been described and responses to stimuli of more than one taste quality appear to be generated in type III cells, subsequent to activation of adjacent, quality-specific type II cells. The substrates for interactions between taste cells are lost when cells are isolated and compromised to some extend in taste bud slices. To address this problem we have used two-photon fluorescence intensity and lifetime imaging of calcium green to measure stimulus induced changes in intracellular calcium in taste cells in *intact* fungiform papillae from micro-pigs. Although acquisition of lifetime measurements using time-correlated single-photon counting was too slow to follow temporal changes in calcium, this technique could be used to obtain actual calcium concentrations at rest and at the peak of the taste response. Temporal changes in calcium were measured as relative changes in fluorescence intensity. Taste stimuli evoked both increases and decreases in intracellular calcium. Oscillatory calcium responses lasting many minutes were observed in some cells in response to sweet and bitter stimuli. Many cells responded to stimuli of a single taste quality, consistent with previous imaging results. Responses to taste stimuli of different taste qualities were also observed in some cells, often after a brief delay. This approach allows direct visualization of cell-to-cell interactions in taste buds *in situ.*

Poster Session IV: Fri. July 25

Transient Receptor Potential Channel M5 and Phospholipasec-β2 CO-Localizing in Zebrafish Taste Receptor Cells

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To understand the vertebrate gustatory systems, we analyzed taste signaling molecules of model fishes and showed that fish *plc-β2* is expressed in the taste bud cells which also express taste receptors, fish *t1rs* or *t2rs*. In mammals, transient receptor potential channel M5 (*Trpm5*) is co-expressed with *Plc-β2* in the taste receptor cells, and both PLC-β2 and TRPM5 are essential elements in the signal transduction of sweet, bitter and umami stimuli. It is still unknown whether TRPM5 is expressed in taste buds in zebrafish (*Danio rerio*) as well as in mammals. In this study, we searched the zebrafish genomic DNA database with the TBLASTN program using the amino acid sequences of mouse and human TRPM5, and identified the zebrafish homologue of TRPM5 (*zfTrpm5*). We performed an amino acid homology search of human, mouse, Japanese puffer fish (*Takifugu rubripes*) and zebrafish TRPM5, and found TRPM5 sequences that were highly conserved between zebrafish and puffer fish (77%). *zfTrpm5* also showed 58% amino acid sequence identity to the mouse and human homologues. We examined its expression in the gustatory system by RT-PCR and *in situ* hybridization. *zftrpm5* was found to be expressed in the taste buds of the lip, mouth cavity, gill rakers, pharynx, barbel and head skin of zebrafish. Using a transgenic zebrafish line that expressed GFP under the control of the *plc-β2* promoter, we showed that *zftrpm5* is expressed in GFP-labeled taste bud cells in this transgenic line also expressing zebrafish *plc-β2*. These results suggest that *zftrpm5* and *plc-β2* co-localize in zebrafish taste receptor cells. Thus, there may be common signaling pathways of taste transduction in a wide variety of vertebrates from fishes to mammals.

Poster Session IV: Fri. July 25

T1R3 Knockout Mice Prefer Polycose but not Sucrose Taste

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In addition to their well-known preference for sweet taste, rats and mice are attracted to the taste of glucose polymers derived from starch (e.g., Polycose). The present study determined if the T1R3 taste receptor, essential for sugar and artificial sweetener preferences, contributes to Polycose preferences. Two-bottle 60-sec licking and 24-h intake tests compared the Polycose and sucrose preferences of T1R3 knockout (KO) and C57BL/6J wildtype (WT) mice. In licking tests, T1R3 KO mice preferred 4-32% Polycose to water although their overall preference was less than WT mice (82% vs. 94%). In contrast, these KO mice failed to prefer 4-32% sucrose; overall KO and WT sugar preferences were 57% and 95%. In 24-h Polycose vs. water tests (0.5 – 32%), KO mice preferred 2% - 32% and WT mice preferred 1% - 32% Polycose. Overall, 24-h Polycose preference was somewhat less in KO than WT mice (80% vs. 88%). WT mice preferred 0.5 – 32% sucrose to water while KO mice were indifferent to 0.5 – 8% sugar but preferred 16-32% sucrose, which may be due to post-oral effects. Overall 24-h sucrose preference was substantially lower in KO than WT mice (56% vs. 91%). Across the concentration range, KO mice had higher intakes and preferences for Polycose than sucrose (10.7 vs. 5.1 g/30 g BW; 80% vs. 56%). These results indicate that the T1R3 sweet receptor has only a minor role in the preference for glucose polymers. This supports behavioral and electrophysiological data indicating that Polycose and sucrose have different taste qualities in rodents.

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Poster Session IV: Fri. July 25

Allelic Variations Upstream of the *T1R3* Gene Correlate with Sucrose Sensitivities in Humans

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Members of the human TAS1R class of taste-specific G protein-coupled receptors have been proposed to function in combination as heterodimeric sweet taste receptors. TAS1R2/TAS1R3 heterodimers recognize sweet taste stimuli. We hypothesized that allelic variation of the *TAS1R3* receptor gene can be one of the mechanisms determining differences in individual sensitivities to sweet compounds. To test this hypothesis, we analyzed sucrose and sucralose sensitivities of 130 unrelated human volunteers aged 20 - 55 years, using a sorting test and subsequent cumulative R-index score. Then, we examined the genomic sequence of the *T1R3* gene in these individuals including exons, introns, and upstream and downstream regions, to survey all polymorphisms that could affect amino acid composition, RNA splicing, or potential regulatory regions. Quantitative trait analysis revealed significant association (adjusted $p<0.01$) between phenotypes and SNPs located in the genomic region 2000 bp upstream of *T1R3* coding sequence. Together the associated alleles account for approximately 8% of the total variation in sweet sensitivity in our subject population. Bioinformatic analysis predicts these polymorphism are located in the regions of transcription factor binding sites. Finally, we investigated putative functional effect of these polymorphisms using GFP reporter expression analysis. Taken together, our data suggest that sequence variations in the promoter region have effect on transcription of *T1R3* gene and thus can modulate sweet sensitivity *in vivo*.

Poster Session IV: Fri. July 25

Functional Analysis of Naturally Occurring Human Sweet Receptor Variants

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Members of the human TAS1R class of taste-specific G protein-coupled receptors have been shown to function in combination as heterodimeric sweet taste receptors. *TAS1R2*/*TAS1R3* heterodimers recognize multiple sweet taste stimuli. The human *TAS1R2* gene encodes a 7 transmembrane domain G protein-coupled receptor that acts as the carbohydrate-binding component of this receptor complex. Haplotype diversity studies indicate that this gene demonstrates substantial variability in the worldwide human population, and evolutionary analyses suggest that this variation is likely to have functional effects on the receptor protein it encodes. In order to investigate whether natural SNP variations in human *TAS1R2* result in functional alterations, we constructed 18 expression constructs corresponding to all of the natural occurring variants of the T1R2 protein. These constructs were co-transfected with T1R3 and evaluated in a cell-based assay for their responses to sucralose, perillartine and cyclamate, which were chosen based on their presumed distinct binding sites within the sweet heterodimer. These studies revealed that one haplotype of African origin shows diminished responses to all three sweeteners. This variant has three amino acid changes from the consensus T1R2 sequence and one of these changes is a Lys -> Gln replacement in helix 4 of the transmembrane domain. Since this receptor variant exhibits diminished responses to all sweeteners tested, we have hypothesized that introduction of a glutamine at this position is imparting a global change in receptor activation, structure, and/or G-protein coupling. Our results suggest that sequence variations in *TAS1R2* are likely linked to alterations in the sweet taste sensitivity and/or preferences within subsets of the human population.

Poster Session IV: Fri. July 25

Temperature- and Gurmarin-Sensitivity of the Chorda Tympani Nerve Responses to Sweeteners in the Wild-Type, T1R3-, Ggust-, TRPM5-KO Mice

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Sweet taste responses occur through one major pathway involving T1R2/T1R3 receptors, Gα subunit, gustducin (Ggust) and temperature-sensitive TRPM5 channels. In mice, sweet responses of the chorda tympani (CT) nerve are classified into two components; one is inhibited by gurmarin (Gur) [Gur-sensitive (GS)] and the other is not [Gur-insensitive (GI)]. To examine additional pathways for sweet taste responses, we investigated GS of CT responses to sweeteners at 15, 25 and 35°C in mice lacking T1R3, Ggust or TRPM5 (KO mice) and wild type (WT) mice. In WT mice, CT responses to sucrose (Suc), glucose (Glc), saccharin (Sac) and SC45647 (SC) were suppressed to 30-70% of control by Gur. Responses to these sweet stimuli exhibited temperature-dependent increase (TDI). In all KO mice, CT responses to Suc, Glc and Sac were greatly reduced, and responses to SC were totally abolished. In T1R3-KO mice, residual responses to Suc and Glc exhibited TDI and GS. In Ggust-KO mice, Suc and Glc responses exhibited TDI but no GS. In TRPM5-KO mice, Glc responses exhibited both TDI and GS. In all KO mice, Sac responses exhibited neither TDI nor GS. Moreover, the lingual application of a proteolytic enzyme, pronase, almost fully abolished the residual responses to Suc and Glc but did not affect the responses to Sac in all KO mice. These results suggest that (1) responses to sweeteners in both of GS and GI components may occur through the major pathway involving T1R3, Ggust and TRPM5, and (2) existence of T1R3-independent-GS pathway for responses to Suc and Glc, and (3) existence of TRPM5-independent and temperature-sensitive GS pathway for responses to Glc, and (4) an indispensable role of Ggust on GS sweet taste responses in mice, and finally (5) existence of the sweet-independent reception pathway for responses to Sac.

Poster Session IV: Fri. July 25

The Taste Receptor Gene, *TAS1R3*, is Involved in Taste Responses to Ethanol in Mice

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When alcoholic beverages are consumed, they first activate chemosensory receptors in the oral and nasal cavities, and then exert postingestive effects. Individual differences in chemosensory perception of ethanol flavor may influence ethanol consumption in humans and laboratory animals. In our studies with mice, we are genetically dissecting quantitative trait loci (QTLs) that affect ethanol consumption through oral sensory and non-sensory mechanisms. In crosses between ethanol-preferring C57BL/6ByJ and ethanol-avoiding 129P3/J inbred strains, we have mapped the *Ap3q* (alcohol preference 3 QTL) locus, which overlaps with the *Sac* (saccharin preference) locus on distal chromosome 4. The *Sac* locus has been positionally identified as the *Tas1r3* gene. This gene encodes a G protein-coupled receptor, T1R3, which is expressed in taste buds and is a component of a sweet taste receptor. Data from inbred, hybrid, congenic and knockout mice demonstrate that the *Ap3q* and *Sac* loci are identical and correspond to the *Tas1r3* gene. Allelic variation of the *Tas1r3* gene has pleiotropic effects on ingestive responses to sweeteners and ethanol in long-term and brief-access tests, and influences taste quality perception of ethanol. This finding is important for our understanding of the mechanisms influencing alcohol consumption in humans and laboratory animals.

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Poster session IV: Fri. July 25

Multiple Chemosensory Modalities of Ethanol in Hamsters

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Blood ethanol levels associated with inebriation in humans do not occur in golden hamsters, *Mesocricetus auratus*, no matter how much they drink. Yet, hamsters highly prefer ethanol to water, but it is not known whether it tastes sucrose-like. Using conditioned taste aversions (CTA), we tested generalizations from 10% ethanol to sucrose and other stimuli that may be ethanol-like to humans. The experimental group (n=6) was given ethanol and the control group (n=8) was given water to drink for 1 hr before ip injection of 0.15 M LiCl (2mL/100g bw). After 2 recovery days, 2 replicates of 10 test stimuli (TS): 100 mM sucrose; 5%, 10%, 20% ethanol; 10% isopropyl alcohol; 10 ppm capsaicin; 10 mM vanillin; 10 mM caffeine, 1 mM quinine·HCl and water, were presented for 1 hr in counterbalanced order. The ethanol series was included to test effects of TS concentration. Isopropyl alcohol was included for its odor and sting, capsaicin for its sting, vanillin for its sweet smell, quinine for its bitter taste, and caffeine for its effect on

alertness. Water was the control TS. Average data for the 2 replicates of each stimulus were analyzed by within-subjects ANOVA, using TS intake (mL) ratios for each ethanol-conditioned animal to mean control intake. A significant overall effect of TS ($p<.000001$) was based on differences ($p<.001$) between the TS ratio of 90±10% for water and TS ratios of ~30% for the alcohols (33±10% for 5%; 31±5% for 10%; 20±5% for 20% ethanol; and 33±9 % for 10% isopropyl alcohol), 41±9 % for sucrose and 37±12% for capsaicin. Thus, ethanol has multiple chemosensory modalities in hamsters. Like mice (Blizard 2007), hamsters find ethanol both alcohol-like and sucrose-like. However, hamsters detect a "hot pepper" quality in ethanol, which is quinine-like to mice.

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Poster Session IV: Fri. July 25

Sweetness of Lysozymes in Mammalian Milk

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Lysozyme is a bacteriolytic enzyme that catalyzes the hydrolysis of β-1,4 glycosidic bonds of the peptidoglycan of bacterial cell walls. This accounts for its main biological function of protecting the host from bacterial infections. Recent studies have identified chicken lysozyme as a sweet protein. We purified various avian and reptile lysozymes from egg white and found that not only chicken lysozyme but also that found in turkey, quail, guinea fowl, and soft-shelled turtle egg whites elicits sweetness at a threshold value of around 20 μM. In mammals, lysozyme is present in breast milk as well as other mucosal body fluids and plays an important role in innate immunity. It is present abundantly in human milk and reduces the risk of microbial infections in the gastrointestinal tract of breast-fed infants. To determine whether human lysozyme is sweet, we cloned lysozyme cDNA from a human placental cDNA preparation and expressed it using the *Pichia pastoris* protein expression system. The human lysozyme secreted in the culture was purified by cation-exchange chromatography; it elicited sweetness at a threshold value of 10 μM, which is similar to chicken lysozyme but less astringent. The other four mammalian lysozymes were prepared from their genes and examined for their tastes by sensory test, using the same method. Mouse, dog, and bovine milk lysozymes were found to elicit the same sweetness as that observed in human lysozyme; however, bovine stomach lysozyme was substantially tasteless at less than 1 mM. Bovine stomach lysozyme differs from other milk lysozymes in physicochemical properties. The finding that all the tested mammalian milk lysozymes are sweet would provide us a new insight into the biological functions of the sweet taste.

Poster Session IV: Fri. July 25

Modulation of Taste Sensitivity by GLP-1 Signaling in Taste Buds

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The sensitivity of many sensory systems is dynamically modulated through mechanisms of peripheral adaptation, efferent input, or hormonal action. In this way, responses to sensory stimuli can be optimized in the context of both the environment and the physiological state of the animal. Though the gustatory system critically influences food preference, food intake and metabolic homeostasis, the mechanisms for modulating taste sensitivity are poorly understood. Using immunohistochemical, biochemical and behavioral approaches in mice, we found that glucagon-like peptide-1 (GLP-1) signaling in taste buds modulates taste sensitivity. GLP-1 is produced in two distinct subsets of mammalian taste cells, while the GLP-1 receptor is expressed on adjacent intragemmal afferent nerve fibers. GLP-1 receptor knockout mice show dramatically reduced taste responses to sweeteners in behavioral assays, indicating that GLP-1 signaling normally acts to maintain or enhance sweet taste sensitivity. A modest increase in citric acid taste sensitivity in these knockout mice suggests GLP-1signaling may modulate sour taste, as well. Together, these findings suggest a novel paracrine mechanism for the regulation of taste function.

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Poster Session IV: Fri. July 25

The Grueneberg Ganglion – A Chemosensory Organ?

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The detection of odors and pheromones in mammals is mediated by chemosensory neurons of the main olfactory epithelium (MOE) and the vomeronasal organ (VNO), which generally express the olfactory marker protein OMP. We have found that OMP is also expressed in cells of the so-called Grueneberg ganglion (GG), a cluster of neuronal cells in the vestibule of the anterior nasal cavity. Chemosensory responsiveness of olfactory neurons is based on the expression of distinct receptors: odorant receptors in the MOE or pheromone receptors in the VNO, respectively. To scrutinize whether neurons in the GG may indeed be chemosensory cells, they were subjected to molecular phenotyping. It was found that a distinct vomeronasal receptor type was expressed in the majority of GG neurons which were concomitantly endowed with the G proteins G_o and G_i; both are also present in sensory neurons of the VNO. Expression of odorant receptors was only observed in very few cells during perinatal stages; a similar number of cells expressed adenylyl cyclase type III and $G_{olf/s}$. These findings demonstrate that the GG mainly comprises cells with a VNO-like phenotype. The GG neurons extend axonal processes which fasciculate to form nerve bundles that project caudally along the roof of the nasal cavity and through the cribriform plate, finally terminating in the olfactory bulb of the brain. In summary, the expression of olfactory signaling proteins as well as the axonal projection to the olfactory bulb, strongly support the notion that the GG may indeed have a chemosensory function.

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Poster Session IV: Fri. July 25

Characterization of Cultured Porcine Olfactory Epithelial Cells

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Despite the increasing importance of the pig as a large animal model, little is known about porcine olfactory epithelial cells and their progenitors. The

aim of this study is to investigate the potential of porcine olfactory epithelium to provide a stable culture, to determine viable populations of progenitor cells, and to evaluate the proteins and gene products expressed by the cells. Samples were obtained from porcine olfactory tissue. Neuroepithelial cells were isolated and expanded over a period of at least 7 weeks. Differentiation was assessed by immunocytochemistry and RT-PCR. Proliferative capability and telomere length were determined by flow cytometry. A mixed population of epithelial and neural cells could be isolated and expanded over a period of at least 7 weeks, while expressing either markers for early neurons (doublecortin, nestin) in neurosphere-like clusters, developed neurons (β-III tubulin), epithelial cells (cytokeratin) or glia-like cells (GFAP). Propidium iodide-FACS analyses showed slow proliferation capability of adherent cells. FACS analyses for ALDH expression confirmed the presence of a progenitor cell population (more than 10%) over a period of 7 weeks. PCR assessments showed the presence of OMP, β-III tubulin, CK5, NCAM, GFAP, Musashi-1, GAP43, Galectin-1, Galectin-3 genes. The replicative potential of cells showed no decrease of telomere length (1.75% at day 7 versus 1.20 at day 28). Our data indicate that porcine olfactory epithelium contains a subpopulation of progenitors, which can be cultured in vitro, while maintaining their progenitor characteristics. This may provide strategical knowledge for the use of the pig as a large animal model for progenitor olfactory cell transplantation studies prior to application of these strategies in humans.

Poster Session IV: Fri. July 25

Immunocytochemical Features of Microvillous Cells in the Olfactory Epithelium of Mice

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The olfactory epithelium of rodents contains ciliated olfactory neurons, supporting cells and basal cells. Furthermore, microvillous cells are present between the ciliated olfactory cells. Only some of the microvillous cells seem to project an axon to the olfactory bulb. The function of microvillous cells without an axon remains enigmatic. Our recent studies revealed several different types of microvillous cells in the olfactory epithelium of mice. These cells do not have an axon that penetrates the basal lamina. In addition to a mouse where tau-GFP replaced the IP_3R_3 coding region, we utilized immunocytochemical and electron microscopic methods to further distinguish between the different types of microvillous cells. Apart from cells that expressed TrpM5 (Lin et al., Chem. Senses 2007) and the TrpC6-expressing microvillous cells described by Elsaesser et al., (Eur. J. Neurosci., 2005), we detected microvillous cells that are TrpM5- and TrpC6-negative but immunoreacted with antisera against IP_3R_3. Another component of a possible transduction pathway seen in these cells is the G-protein α-subunit Gq/11. These microvillous cells span the height of the olfactory epithelium but their cell bodies are located in the layer of the supporting cells. Beneath the nucleus the cells taper to a broad basal appendix that ends between the basal cells but does not penetrate the basal lamina. Experiments to elucidate a possible function of these cells are under way.

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Poster Session IV: Fri. July 25

Structural and Ultrastructural Characterization of a Novel Class of Cells Expressing OBP-1F in the Rat Olfactory Epithelium

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Olfaction is based on the reception of odorant molecules reaching the olfactory receptors through a thin layer of mucus, whose composition is tightly regulated. Odorant binding proteins are one of the major proteins of mucus and participate in perireceptors events of the olfactory message. Among the three OBP described in rat, the OBP-1F is mainly synthesized and secreted by the lateral nasal glands (LNG) and Bowman's glands (Pevsner et al., 1988). Interestingly, the expression of OBP-1F was demonstrated by both qPCR and western blot in the rat olfactory mucosa (OM) itself, and was modulated by a 48 hr food starvation (Badonnel et al., 2007). In the course of in situ hybridization and immunohistochemistry investigations to find possible sites of OBP-1F production in the OM itself, we highlighted a novel class of cells. These cells were identified in discrete zones of the olfactory epithelium (OE), located in the posterior area of the nose. Dispersed along the thickness of the OE, these cells revealed a globular shape of about 20μm and histological features similar to mucopolysaccharides-secreting cells commonly described in both intestinal and respiratory mucosa as goblet cells. Observations by both transmission and scanning electron microscopy completed the characterization of these cells, by showing numerous droplets with a homogenous matrix structure together with an eccentric nucleus.Our study demonstrate the presence of a novel class of secretory cells expressing OBP-1F in the rat OE.

Poster Session IV: Fri. July 25

"A Novel Olfactory Receptor Gene Family in Teleost Fish: Phylogenomics and Cell Type Expression"

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For two of three mammalian olfactory receptor families (OR & V2R) ortholog teleost families have been identified, the third family (V1R) has been thought to be represented by a single, closely linked gene pair. We identified four further V1R-like genes in five teleost species analyzed. In the phylogenetic analysis these *ora* genes (olfactory receptor class A-related) form a single clade, which includes the entire mammalian V1R superfamily. Homologies are much lower in paralogs than in orthologs, indicating that all six family members precede the speciation events in the teleost lineage analyzed. These *ora* genes are under strong negative selection, as evidenced by low dN/dS values in comparisons between orthologs. A pairwise configuration in the phylogenetic tree suggests the existence of three ancestral Ora subclades, one of which has been lost in amphibia, and a further one in mammals. Unexpectedly, two *ora* genes exhibit a highly conserved multi-exonic structure and four *ora* genes are organized in closely linked gene pairs across all fish species studied. The *ora* gene repertoire is highly conserved across teleosts, in striking contrast to the frequent species-specific expansions observed in tetrapod, especially mammalian V1Rs, possibly reflecting a major shift in gene regulation as well as gene function upon the transition to tetrapods. All *ora* genes are expressed specifically in the olfactory epithelium of zebrafish, in sparse cells within the sensory surface, consistent with the expectation for olfactory receptors. The olfactory epithelium contains

three subtypes of olfactory receptor neurons, ciliated, microvillous and crypt cells. For the latter cell type no receptors are known so far. We are currently investigating whether these orphan crypt cells express ora genes.

Poster Session IV: Fri. July 25

Analysis of Putative Olfactory G-Protein Coupled Receptors in *Danio Rerio*

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G-protein-coupled receptors (GPCRs) are transmembrane receptors that transduce an extracellular signal into an intracellular signal via interaction with heterotrimeric G proteins. Rhodopsin type or class A GPCRs constitute the largest among four classes of GPCRs. Although this class has been extensively studied, there are still a considerable number of orphan receptors present. All the chemosensory receptor genes detected so far, such as odorant, taste and vomeronasal receptors belong to the GPCR family, with many of them being class A or class A-related genes. Hence, it would be desirable to find out if some of these orphan receptors might be involved in olfactory perception. We used bioinformatic approaches to identify such candidate genes. Next, we analysed the expression pattern of candidate GPCRs by means of in situ hybridization. The results of these studies will be reported.

Poster Session IV: Fri. July 25

Functional Asymmetry in the Olfactory System of a Flatfish (*Solea Senegalensis*)

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Increasing evidence shows that sole have different sensitivities in their two olfactory epithelia suggesting a functional asymmetry in the olfactory system. We propose that the upper (right) epithelium is more involved in chemical communication whilst the lower (left) is specialised in food detection; such a functional asymmetry of the olfactory system has not been described in a vertebrate. The aim of the current study was to test whether this asymmetry extends to the transduction pathways used by the receptor neurones in the two epithelia. Generally, olfactory transduction occurs *via* G proteins, adenylate cyclase or phospholipase C. The olfactory sensitivity to a range of stimuli was tested in both epithelia before and during exposure to SQ-22536 (adenylate cyclase inhibitor) or U73122 (phospholipase C inhibitor). The odorants used were L-cysteine (detected equally by both epithelia), L-phenylalanine (putative food related odorant; lower epithelium) and taurocholic acid (putative conspecific communication; upper epithelium). Our results suggest that the main pathway involved in detection of L-cysteine and L-phenylalanine is *via* phospholipase C. The olfactory sensitivity to taurocholic acid seems to involve both adenylate cyclase and phospholipase C. Combining pharmacological data with cross-adaptation suggests that the greater sensitivity of the lower epithelium to L-phenylalanine is due to specific receptors on this epithelium that act *via* phospholipase C. Furthermore, the greater sensitivity of the upper epithelium to taurocholic acid seems to be due to specific receptors which act *via* adenylate cyclase.

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Poster session IV: Fri. July 25

Changes in Olfactory Sensitivity During the European Eel (*Anguilla Anguilla*) Life Cycle

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The aim of this study was to test whether physiological changes are paralleled by changes in olfactory function during different stages of the European eel's life-cycle. Sensitivity to diverse odorants (amino acids, bile acids, bile fluids, Na^+ and Ca^{2+}) was assessed by extra-cellular recording from the olfactory nerve of seawater or freshwater and immature or mature males. Sensitivity to amino acids did not differ markedly, whether fresh- or seawater-adapted, immature or mature. Sensitivity to bile acids and bile fluid, however, depended on the physiological status; in general, freshwater eels were more sensitive to bile acids than seawater fish. However, sensitivity to taurochenodeoxicolic acid was similar, independent of physiological status, and mature males had higher sensitivity to cyprinol sulphate than immature males. Furthermore, seawater males had higher sensitivity to conspecific bile fluid than freshwater males, this sensitivity being highest in mature eels. All eels, whether freshwater or seawater adapted, mature or immature, responded to increases in external $[Na^+]$. Conversely, freshwater eels respond to increases in external $[Ca^{2+}]$ whereas seawater-adapted fish responded to reductions of external $[Ca^{2+}]$ in a concentration-dependent manner. Moreover, mature males had a lower sensitivity than immature males for Ca^{2+}. Together, these results suggest that olfactory sensitivity in the eel is modulated according to the environment (seawater or freshwater) and/or reproductive status (immature or mature). We suggest that this may reflect changes in diets between seawater and freshwater eels and/or the changing importance of chemical communication during different life-stages (e.g. maturation and migration).

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Poster Session IV: Fri. July 25

Factors Influencing Peripheral Olfactory Responses of Female Round Gobies (*Neogobius Melanostomus)*

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Electro-olfactogram (EOG) responses recorded from ovulated females, in response to male odours, are stronger than responses recorded from non-ovulated females, in the round goby (*Neogobius melanostomus*), a fish species invasive to the Great Lakes. We are using a variety of techniques to investigate factors contributing to these peripheral olfactory responses in ovulated versus non-ovulated females. Throughout the year, wild-caught female gobies are tested for the following: (1) olfactory sensitivity to putative steroidal pheromones and male odours (i.e. conditioned water extracts) (2) levels of hormonal steroids (3) signs of olfactory neuroprotection against apoptosis in the olfactory epithelium and (4) gonadal development. EOG recordings from females during the breeding season have demonstrated that extracts from male-conditioned water elicit robust EOG responses in female gobies. The response magnitude was particularly strong upon the application of extracts containing elevated levels of free and conjugated 11-oxo-etiocholanolone, novel steroids synthesized and released by the male goby. During the winter (outside of the breeding season), the EOG response magnitude and sensitivity to all odours were reduced. Analyses of EOG

responses, steroid levels, olfactory epithelial apoptosis and gonadal development during the breeding and non-breeding seasons are on-going.

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Poster Session IV: Fri. July 25

The Effects of Androgen on Olfactory Response to Prostaglandins and Olfactory Sensory Neuron Proliferation in Fish

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In vertebrates, steroid hormones, acting on central neural structures, can influence responses to ethologically-relevant odours, such as pheromones. However, little is known about hormonal effects on olfactory sensory neuron (OSN) physiology and cell proliferation. In this study we treated juvenile tinfoil barbs (*Barbonymus schwanenfeldii*) and redtail sharkminnows (*Epalzeorhynchus bicolor*) for 21 days with 17α-methyltestosterone (MT) to determine the effect on both OSN proliferation and physiological (electro-olfactogram, EOG) responses to putative hormonally-derived pheromones. We found that MT treatment increased both the magnitude and sensitivity of EOG response to prostaglandins, but did not affect responses to amino acid and steroid odours; thus, MT-treated and control juveniles had EOG responses of adult males and females, respectively. Steroids and reproductive maturity have been observed to induce morphological change and affect cellular organization in the peripheral olfactory organ. To determine if OSN proliferation might be responsible for the androgen effect on olfactory response to prostaglandins, we examined cell division in the olfactory epithelium using 5-bromo-2'-deoxyuridine (BrdU). BrdU is incorporated into the DNA of new cells during DNA synthesis. Fish were injected with BrdU (10 μl/g) every two days during MT treatment and subsequently sacrificed one hour after injection. We performed immunocytochemistry to compare the density of labelled OSNs in control and MT-treated fish. Results of this study will lead to a clearer understanding of how hormonal changes associated with sexual maturity might affect adult responses to sex pheromones by altering the proliferation and physiology of OSNs.

Poster Session IV: Fri. July 25

Is there Functionality to the Spatial Distribution of Olfactory Receptors within Dorsal Zone of Olfactory Epithelium?

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Olfactory stimuli are represented not only by their odorant/ligand affinity. *A chromatographic process in olfaction*–a separation of odorants based on their chemical properties and flow dynamics across the nasal cavity–has been initially proposed and demonstrated by Mozell et al. Our objective is to correlate the expression pattern of olfactory receptors (ORs) of the dorsal zone of mouse olfactory epithelium with the odotopic properties of their cognate ligands, i.e. volatility, hydrophobicity and water solubility. Our hypothesis is that olfactory receptors for polar, hydrophilic odorants are present in extreme dorsal regions of olfactory epithelium where the airflow is high, while ORs for non-polar, hydrophobic odorants are absent. To test this hypothesis we combined microarray analysis of RNA expression and microtransplantation of plasma sheets containing native olfactory receptors into Xenopus oocytes for electrophysiological characterization of ligand selectivity. Left and right hemisections of the dorsal olfactory epithelium are separated into parallel subsections along the anterior-posterior axis. Left hemisections containing native ORs are processed using a cilliary membrane preparation and injected into oocytes. Microtransplanted native ORs from each subsection are tested against 30 odorants using two-electrode voltage clamp and a robotic electrophysiology system (Opus 6000A, Molecular Devices). The pattern of expression of ORs is characterized using microarray analysis of RNA expression in parallel subsections. Correlating the chemical properties of each odorant together with the topographical location of its cognate OR will shed light on the spatial distribution of odorant responses within the olfactory epithelium, thus demonstrating the functional organization of ORs at the periphery.

Poster Session IV: Fri. July 25

The Septal Organ Expresses Broadly Tuned Olfactory Receptors

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The septal organ (SO) is a small island of olfactory epithelium located at the ventral base of the nasal septum. This distinct chemosensory subsystem expresses identified odorant receptors, but its function remains unknown. Using perforated patch clamp recordings, we investigated the response properties of septal organ neurons in the intact epithelium of mice to a panel of 45 odorants or mixtures. Odor stimulation was delivered by pressure ejection through seven-barrel pipettes. Out of 328 neurons tested, approximately 70% responded to odor stimulation. Among the responsive neurons, 72.5% responded to multiple odorants. The olfactory receptor SR1 (MOR256-3) is expressed in ~50% of SO cells and also in the ventral zone of the main olfactory epithelium (MOE). To analyze the origin of the broad tuning of the SO cells, we recorded from SR1-expressing cells in a novel genetically engineered mouse strain, SR1-IRES-tauGFP. We observed that all SR1-expressing cells in the SO and in the MOE respond to diverse odorants (n = 8 and 10 respectively). Furthermore, all SR1 cells in both SO and MOE responded to a selected odorant (amyl acetate) with a nanomolar threshold and a broad dynamic range covering three to four log units. Finally, we recorded the responses from the labeled cells in a mouse strain in which the SR1 coding region has been deleted and replaced with RFP, using the same set of odorants. The response properties of RFP-positive cells were radically different from SR1-expressing cells: only 22% were broadly-tuned (n = 8). This study suggests that some olfactory receptors are relatively broadly tuned and may serve as general odor detectors. The septal organ, by concentrating some of the broadly-tuned receptors in the air path, may play a role in alerting the organism.

Poster Session IV: Fri. July 25

The Electroolfactogram Correlates with the Effect of Odor on Antidromic Spikes in Olfactory Sensory Neurons

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Antidromic spikes were evoked in olfactory sensory neuron populations by electrical stimulation of the olfactory bulb nerve layer in pentobarbital anesthetized rats. The stimulation and recording sites correspond to the olfactory nerve projection paths. The latencies of these spikes varied depending upon distance from the stimulus electrode. Dual simultaneous recordings indicated conduction velocities in the C-fiber range, around 0.5 m/sec. These

spikes are concluded to arise from antidromically activated olfactory sensory neurons. Low frequency electrical stimulation was used to track changes in the size and latency of the antidromic population spike during the odor response. Strong odorant stimuli suppressed the size of the spike and prolonged its latency relative to spikes evoked without odor stimulation. We interpret this result as collision between the antidromic volley and the orthodromically evoked action potentials in olfactory nerve. The degree of suppression of the spike was measured by representing the spike during odor presentation as a fraction of the corresponding spike during a blank. Stimulus intensity was varied across stimulus flow rate and concentration The amount of spike suppression was strongly correlated with the EOG evoked at the same site across odorants and across intensity. We conclude that antidromic spike suppression represents spiking activity in olfactory sensory neuron axons driven by odorants and that its correlation with the EOG shows the accuracy of the EOG as an estimate of intracellular potential in the population of olfactory sensory neurons.

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Poster Session IV: Fri. July 25

Odorant and Concentration-Specific Electro-Olfactograms Recorded at the Human Olfactory Epithelium

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We set out to ask whether electro-olfactograms (EOG) recorded directly from the human olfactory epithelium are odorant and concentration-specific. Each of 22 subjects (12 women, mean age = 23.3Y) was tested with two odorants, either Valeric Acid and Linalool (VA-Lin: n=12) or IsoValeric Acid and L-Carvone (IVA-LC: n=10), each delivered at 0%, 25%, 50%, 75% and 100% dilution with $8_{l/min}$ heated (37C) and humidified (80%) clean air (ISI = 30 s, Stim. Dur. = 500 ms, 4 events per condition, Sampling rate = 0.0625Hz). Extracting N1 and P2 latencies and amplitudes, as well as frequency domain analysis, together suggested that whereas odorant identity could not be determined based on N1 and P2 amplitudes or response-frequency distributions, it could be determined by fitting polynomial curves to each of the subject's responses ($R^2 \geq 0.955$; VA-Lin: $P(C_0) = 0.0275$, IVA-LC: $P(C_1) = 0.0101$, $P(C_2) = 0.0019$). In contrast, odorant concentration was clearly reflected in N1 amplitude, that increased with increased concentration ($P_{(VA)} = 0.027$, $P_{(IVA)} = 0.023$, $P_{(LC)} = 0.0013$, $P_{(Lin)} = <0.0001$). Of the 40 pairwise concentration comparisons, 27 significantly differed in N1 amplitude, and 24 significantly differed in frequency power at 0.3Hz. Together these results suggest that odorant concentration is reflected in EOG amplitude and odorant identity is reflected in the overall shape of the EOG response. These findings substantiate original observations made by Kobal, and suggest that EOG is a promising tool for probing olfactory coding directly at the level of olfactory neurons in humans.

Poster Session IV: Fri. July 25

Coding Intermittency in Odor Signals with Ensembles of Bursting Olfactory Receptor Neurons

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The structure of water- and air-borne odor plumes suggests that spatio-temporal information is inherent in the chemical signal and allows that animals may be capable of extracting that information from the odor signal. If so, there is little information on what structural and functional algorithms the olfactory circuitry might utilize to capture intermittency inherent in odor signals. We addressed this question in both the vertebrate (mouse, rat) and invertebrate (lobster) olfactory systems. In both organisms we identified a rhythmically active subset of primary olfactory receptor neurons (ORNs). Patch clamp and calcium imaging of neural activity showed that the rhythmic discharge of the ORNs can be entrained by odors in phase-dependent manner. The spontaneous and evoked activity of the bursting ORNs, together with that of the more typical tonically-active ORNs with which they co-localize, were incorporated into a computer simulation of an integrated assemblage of rhythmically- and tonically-active ORNs. We 'stimulated' the assembly with defined temporal patterns of odors with different intensity profiles, including those simulating the structure of odor fields measured experimentally by planar laser induced fluorescence. We analyzed the output to test the extent to which incorporating rhythmic properties into primary sensory detectors can yield significant gains in functionality. We found that synchronization of the bursting ORN ensembles improved the detection of weak signals. Additionally, we found that those bursting ORNs whose range of inherent bursting frequency most closely matched the frequency of the stimulus were selectively synchronized by the stimulus, potentially providing a novel means to extract useful information about the relative spatial distribution of the odor source.

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Poster Session IV: Fri. July 25

Olfactory Coding in *Anopheles Gambiae*

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Blood-feeding mosquito species act as vectors for the transmission of malaria, which is a leading cause of death worldwide. The malaria burden is heaviest in sub-Saharan Africa, where the *Anopheles gambiae* mosquito is the major vector. Olfactory cues are imperative for the identification and localization of blood-meal hosts by *A. gambiae* and other mosquitoes. Odors are detected by olfactory receptor neurons (ORNs) which express one or more odor receptor (*Or*) genes that confer a unique odor sensitivity to the neuron. A family of 79 odor receptors has been identified in *A. gambiae* (Fox, 2001; Hill, 2002). Two of these receptors have been shown to respond to specific olfactory stimuli in an *in vivo* expression system in *Drosophila* (Hallem, et al., 2004). We are now using the same expression platform to systematically, functionally characterize the *A. gambiae* odorant receptor family. We are testing each receptor against a panel of odorants selected for ecological and behavioral relevance as well as chemical diversity and volatility. We find that each receptor possesses a distinct odor response profile and tuning width. By characterizing the complete AgOr family, we can conduct a global, functional analysis of *Anopheles* odor coding. Such information may prove useful in the control of malaria mosquitoes.

Poster Session IV: Fri. July 25

Coding of Odor Mixtures in *Drosophila* Olfactory Receptor Neurons

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Most natural odors are complex mixtures consisting of multiple volatile compounds. However, in contrast to the coding of pure odors, the logic by which complex odors are encoded in the olfactory system remains largely

unknown. In this study, we focus on the coding of odor mixtures in the periphery, the first station of olfactory information processing. *Drosophila* is particularly an ideal model system to address odor mixture coding because the molecular identity of the cognate receptors in most olfactory receptor neurons (ORNs) is known and their response profiles to individual pure odors available. Using extracellular single-unit recording, we obtained responses of *Drosophila* ORNs to a series of binary odor mixtures, with odorants of different properties (excitatory, inhibitory or neutral) systematically paired across a wide concentration range to examine the modality and degree of signal integration. We observed four integration modes: addition, suppression, potentiation, and masking, depending on the identity and concentration of the individual odorants in the mixture. Furthermore, dose-response analyses revealed possible mechanisms underlying the signal integration modes in the ORNs. These findings provide insights into how fly ORNs integrate information from odor mixtures and may suggest new avenues for the development of specific compounds that mask pest-attracting odors.

Poster Session IV: Fri. July 25

Receptor Guanylyl Cyclase-Mediated Odor Recognition in the Olfactory Epithelium

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The mammalian olfactory system consists of several spatially segregated subpopulations of sensory neurons, each projecting to different areas of the brain and likely communicating different chemosensory information. Some of these subpopulations use different signaling cascades for transducing information from chemosensory molecules into electrical membrane signals. One subset of ciliated olfactory neurons within the main olfactory epithelium expresses the orphan receptor guanylyl cyclase GC-D and the cyclic nucleotide-gated channel subunit CNGA3, suggesting that these cells utilize a cGMP-dependent transduction mechanism for chemodetection. By combining gene-targeting of *Gucy2d*, which encodes GC-D, with patch clamp recording and confocal Ca2+ imaging from single dendritic knobs *in situ*, we find that GC-D cells recognize the peptide hormones uroguanylin and guanylin. These molecules stimulate an excitatory, cGMP-dependent signaling cascade in GC-D cells that increases intracellular Ca2+ and action potential firing. Responses are eliminated in both *Gucy2d* and *Cnga3* null mice, demonstrating the essential role of GC-D and CNGA3 in the transduction of these stimuli. The mechanisms used for olfactory coding by the GC-D cells differ sharply from those employed by canonical OSNs or VSNs. Most notably, a mixture consisting of only two peptide ligands stimulates virtually all GC-D cells. Despite this remarkably high degree of functional uniformity, on a finer scale we observed some heterogeneity among GC-D cells: they can be divided into three functional classes, each exhibiting a somewhat different peptide recognition profile. The implications of functional heterogeneity for coding of chemosensory signals by GC-D neurons are under investigation.

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Poster Session IV: Fri. July 25

Olfactory Necklace Glomeruli Form a Molecularly and Functionally Unique Domain of the Mouse Olfactory Bulb

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The necklace domain of the mouse olfactory bulb consists of a collection of glomeruli that are morphologically and spatially distinct from those involved in processing traditional odors and pheromones. These necklace glomeruli are known to be innervated by two input olfactory subsystems: the GC-D neurons and the Grueneberg Ganglion (GG). While the GC-D neurons uniquely employ cGMP as a stimulant-evoked second messenger and have carbon dioxide-sensing capabilities, the molecular pathways and function of the adult mouse GG remain largely uncharacterized. Given the common innervation target of the GC-D neurons and the GG, we explored the relationship between these two systems at the molecular level. Using immunohistochemistry, we assayed the expression in the GG of proteins crucial to the function of the GC-D neurons. Similar to the GC-D neurons, the GG cell bodies richly express the cGMP-stimulated phosphodiesterase 2a, suggesting the GG may also employ a cGMP second messenger pathway. The GG also expresses the carbon dioxide-acting carbonic anhydrase II. In contrast to the GC-D neurons, the GG does not express the particulate guanylyl cyclase D (pGC-D) isoform. This motivated us to investigate if another pGC isoform is expressed in the GG. Instead, we found that most GG cells express pGC-G, and a few cells express the natriuretic peptide-stimulated pGC-A. These results demonstrate that both inputs to the necklace domain use cGMP second messenger pathways and that both inputs potentially have carbon dioxide-sensing functionalities. These results suggest that the necklace domain, in addition to being spatially-segregated, is also a molecularly and functionally unique olfactory processing region in the adult olfactory bulb.

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Poster Session IV: Fri. July 25

Molecular Mechanisms Underlying Formation of the Odor Map in the Mouse Brain

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Neurons expressing a given odorant receptor (OR) project axons to a pair of fixed glomeruli on the olfactory bulb (OB), leading to formation of a topographic map (odor map). In our study, *in situ* hybridization of olfactory epithelium (OE) sections with the OR probes indicated that the OR genes whose mRNAs were detected in the proximal glomeruli on the OB appeared to be expressed in subareas of zone 4 along the dorsomedial (DM) /ventrolateral (VL) axis. Furthermore, the subareas of zone 4 along the axis in the OE seemed to be specified by the expression of neuropilin-2 in a DM-low and VL-high manner, corresponding to the glomerular arrangement along the dorsal (D)/ventral (V) axis in the OB. On the other hand, in the most ventro-lateral region of the OE, there are another subset of guanylate cyclase-D (GC-D)-expressing neurons that detect CO_2 and project axons to necklace glomeruli in the OB. We have found that a member of bone morphogenic proteins (BMPs) is specifically expressed in the GC-D neurons and discussed the role of BMP signaling in the neural development.

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Poster Session IV: Fri. July 25

BIG-2 Mediates Olfactory Axon Convergence to Target Glomeruli

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Olfactory sensory neurons expressing a given odorant receptor converge axons onto a few topographically fixed glomeruli, leading to establishment of the odor map in the olfactory bulb. Here we report that BIG-2/contactin-4, an axonal glycoprotein belonging to the immunoglobulin superfamily, is expressed in a subpopulation of mouse olfactory sensory neurons. A mosaic pattern of glomerular arrangement is observed with strongly BIG-2-positive, weakly positive, and negative axon terminals in the olfactory bulb, which is overlapping but not identical with those of Kirrel2 and ephrin-A5. There is a close correlation between the BIG-2 expression level and the odorant receptor choice in individual sensory neurons. In BIG-2-deficient mice, olfactory sensory neurons expressing a given odorant receptor frequently innervate multiple glomeruli at ectopic locations. Furthermore, the presence of a putative heterophilic adhesion receptor for BIG-2 is implicated from a BIG-2/AP overly experiment. These results suggest that BIG-2 is one of the axon guidance molecules crucial for the formation and maintenance of functional odor map in the olfactory bulb.

Poster Session IV: Fri. July 25

Olfactory Sensory Axons Expressing Different Receptors Converge on Individual Glomeruli in Mice Lacking Olfactory Marker Protein

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Olfactory bulb glomeruli are widely thought to be functional units receiving inputs from olfactory sensory neurons (OSNs) expressing only one olfactory receptor gene. This canonical model has been supported by histological investigation of a few genetically labeled glomeruli, and by imaging studies interrogating glomeruli with small number of odors. Here, we used multiphoton microscopy and a large set of odors (~100) to probe glomerular odor responses in adult mice that express synaptopHluorin (spH), a reporter of synaptic activity, in mature OSNs. In these mice, spH is knocked into the olfactory marker protein (OMP) locus. We found that OMP-spH heterozygous mice have uniform glomerular responses to different odor stimuli, in agreement with the canonical model. However, in OMP-spH homozygous mice that lack OMP completely, individual glomeruli can have heterogeneous responses to odorants – a single glomerulus can circumscribe up to four functionally distinct and spatially contiguous subregions. This intraglomerular heterogeneity was not a result of postsynaptic processing since it persisted after pharmacological blockade of postsynaptic activity. The observed functional heterogeneity is parsimoniously explained by convergence of OSNs expressing multiple odorant receptors within a glomerulus. At least 20% of the active glomeruli were heterogeneous, and 'mixed' glomeruli could be identified reproducibly across animals. In addition, wide-field fluorescence imaging revealed that the same set of odors activated ~40% more glomeruli in OMP -/- than in OMP +/- mice. We are currently investigating how the postsynaptic circuitry samples inputs from these mixed glomeruli. Our findings suggest a role for OMP in axon targeting, and offer new insight on how OSN axons and bulbar targets compete for synaptic space.

Poster Session IV: Fri. July 25

Functional Imaging of Odor Representations After Methyl Bromide-Induced Lesion and Regeneration of Receptor Neuron Inputs to the Olfactory Bulb

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The olfactory system exhibits robust regenerative capacity in the face of peripheral damage. After lesion of the olfactory epithelium, olfactory receptor neurons (ORNs) regenerate and re-innervate the olfactory bulb (OB).This process has only been characterized anatomically and has been limited to a single receptor-tagged population. Here, we characterized ORN re-innervation of the OB using functional measures. We used chronic imaging in mice expressing synaptopHluorin in ORNs to characterize odorant response maps in the dorsal OB before exposure to the selective olfactotoxin methyl bromide (MeBr) and 12 weeks after exposure. In some animals, one naris was closed reversibly to protect that side from exposure. In OBs on the unlesioned side, the positions of glomeruli in odorant response maps were unchanged across the recovery period, confirming that odor representations are stable over months. On the lesioned side, recovery was more variable, but response maps consistently included glomeruli located in approximately the same position as before lesion. In addition, lesion-recovered maps often included signals that were diffusely distributed or apparent as foci significantly smaller than a glomerulus, even when imaged at high magnification. These results are consistent with previous observations of lesion-recovered P2 receptor-expressing ORNs, in which many homotypic axons converge onto a single glomerulus while a smaller subset of fibers terminate in other glomeruli. We conclude that the olfactory system is capable of largely reconstituting spatial maps of glomerular activation after peripheral lesion. The data also suggest that some ORNs make functional connections in inappropriate glomeruli. Ongoing experiments are addressing the determinants of this functional mistargeting.

Poster Session IV: Fri. July 25

Increased Sniffing is Associated with a Behaviorally Relevant Suppression of Dorsal Glomerular Responses Observed for a Binary Mixture of Unrelated Odorants

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By mapping glomerular responses to 365 odorants using 2-deoxyglucose uptake, we found that odorant chemistry is systematically represented in domains of the rat olfactory bulb. Because natural odors involve mixtures rather than isolated odorants, interactions are possible between responses to the components. We now have mapped responses to methyl benzoate and decanal alone and in a mixture. While ventral responses to decanal occurred for the mixture at the same high level as for the isolated chemical, equally strong dorsal responses to pure methyl benzoate were greatly suppressed in the mixture. A go/no-go alternative choice task in which rats were trained to respond to the mixture showed generalization to decanal but not to methyl benzoate, suggesting that the observed similarity in activity patterns evoked by the mixture and decanal is associated with a similarly perceived odor. Others have shown decreased dorsal glomerular responses during high-frequency sniffing in optical imaging studies, leading us to ask if our rats sniffed the mixture more intensely than the components. Indeed, we detected more sniffs in response to the mixture than to either component presented alone,

raising the interesting possibility that the sniffing was an active response to overall stimulus complexity. However, the continued strong ventral response to decanal indicates that sniffing does not act as a general filter to temper responses to all major odorant components. Our results show that important mixture interactions may involve increased processing that can be seen by imaging the entire glomerular layer in unanesthetized, unrestrained animals.

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Poster Session IV: Fri. July 25

The Recovery of the Intrabulbar Map Following Unilateral Naris Closure

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Intrabulbar connections are mediated by external tufted cells (ETCs) that receive input from glomeruli on one side of the olfactory bulb and send their axons to discrete loci on the opposite side of the same bulb (Schoenfeld et al., 1985). The specificity of these connections gives rise to an intrabulbar map that precisely and reciprocally links isofunctional glomeruli (Belluscio et al., 2002; Lodovichi et al., 2003). Anatomical studies examining the development of these projections revealed that they target broad areas of the bulb on the opposite side during the first postnatal week and refine to their adult precision by 7 weeks of age (Marks et al., 2006). These studies further revealed that map refinement is strictly dependent upon afferent activity with no apparent critical window such that a decrease in odorant-induced activity produces a broadening of the intrabulbar projections. In this study we sought to determine if the intrabulbar map is capable of recovering its precise adult organization after a period of olfactory deprivation. We performed reversible naris closure experiments in mice from either 4-7 or 7-10 weeks of age, then removed the blocks for survival periods of up to 9 weeks. Our results clearly show that returning normal olfactory experience allows the intrabulbar projections to re-refine themselves, suggesting that the process of activity dependent refinement does not stop once the map is mature. Instead, intrabulbar projections appear to remain in a constant state of refinement throughout life.

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Poster Session IV: Fri. July 25

Precise Circuitry Links Bilaterally Symmetric Olfactory Maps

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In the mouse olfactory epithelium, each olfactory sensory neuron (OSN) typically expresses one odorant receptor out of a repertoire of ~1000. OSNs expressing common receptors converge into one or a few glomeruli in the olfactory bulb, forming bilaterally symmetric olfactory maps. By injecting neuronal tracer into single identified glomeruli, we found that the bilaterally symmetric olfactory maps in the olfactory bulbs are precisely linked by an olfactory cortical area called anterior olfactory nucleus pars externa (AONpE). c-Fos mapping and physiological recordings further revealed that the activity within one olfactory bulb can be topographically transferred to the contralateral olfactory bulb, and this contralateral activation requires the AONpE. Using a behavior essay, we found that contralateral transfer of olfactory memory depends on the AONpE. Our data strongly suggest that the AONpE precisely links bilateral olfactory maps and plays an important role in bilateral exchange of olfactory information. Our study also suggests that bilateral linking of the bilateral olfactory bulbs by the AONpE may provide a genetically tractable model for studying interhemispheric connections in the forebrain.

Poster Session IV: Fri. July 25

Intrinsic Connections of the Anterior Olfactory Nucleus

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The anterior olfactory nucleus (AON) is a central olfactory cortical structure reciprocally connected with the olfactory bulb and piriform cortex. The main portion of the AON (*pars principalis*) is a ring of cells encircling the anterior portion of the anterior commissure. While *pars principalis* is often divided simply by "compass" points (yielding *pars dorsalis, pars medialis, pars lateralis,* and *pars ventroposterior*), there is little agreement regarding the location of borders. Functional differences may exist between these zones since cells in the varying regions differ in their morphology and neurochemical phenotypes. The potential for intrinsic processing within *pars principalis* remains to be elucidated. In this study, we used small injections of the anterograde tracer *Phaseolus vulgaris* leucoagglutinin (PHA-L) to explore the topography of the interconnections between subdivisions. Focal injections in *pars principalis* revealed widespread connections throughout the structure. Nevertheless, distinct zonal patterns were observed, including substantial projections from *pars dorsalis* and *medialis* to *pars lateralis*, a projection from *pars ventroposterior* to *dorsalis* and *lateralis*, and fibers connecting *pars lateralis* to *dorsalis*. Projections also differentially targeted superficial or deep zones within Layer II, the compact cell body layer. These results are a further indication that AON subdivisions may play differential roles in olfactory information processing. Taken together with previous findings, these results suggest that *pars dorsalis* and *lateralis* are the targets of a feedforward associative network within *pars principalis*, which may serve to aid in odor identification and discrimination.

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Poster Session IV: Fri. July 25

Novel Subdomains within the External Plexiform Layer of the Developing Mouse Olfactory Bulb

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The objective of this study is to identify and characterize molecules that guide olfactory sensory neurons towards their targets in the mouse main olfactory bulb. We hypothesized that one class of axon guidance molecules would exhibit differential expression within the developing external plexiform layer of the olfactory bulb. The external plexiform layer was chosen because it contains numerous uncharacterized cell types that interact with the axons of olfactory sensory neurons. We performed a microarray screen to identify differentially expressed genes within the developing external plexifom layer. External plexiform cells from different regions of E17.5 olfactory bulb were extracted via laser microdissection. RNA from these cells was isolated, amplified, labeled and applied to microarrays. We have identified *connective tissue growth factor*, *melanoma cell adhesion molecule*, *jagged 1*, *protocadherin 7*, and *protocadherin 17* as exhibiting differential expression within the developing external plexiform layer of the olfactory bulb. The expression of these genes separates the olfactory bulb into previously uncharacterized subdomains. To further characterize the involvement of these genes in olfactory bulb mediated axon guidance we employed a nasal ablation paradigm. We observe a change in the expression of *connective tissue growth factor*, *protocadherin 7*, and *protocadherin 17* in the mouse olfactory bulb following olfactory sensory neuron ablation indicating that these genes can be regulated trans-synaptically. *Protocadherin 7* and *protocadherin*

17 are homophilic cell adhesion molecules that belong to the same subfamily of the delta protocadherins. We show that both of these delta protocadherins are expressed within nonoverlapping subsets of olfactory sensory neurons within the olfactory epithelium.

Poster Session IV: Fri. July 25

Glomerular Mapping in the Sea Lamprey - Evidence for Two Spatially Distinct Neural Inputs

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The peripheral olfactory organ ofthe sea lamprey contains several lamellar folds lined by olfactory epithelium, as well as a tubular diverticulum, named the accessory olfactory organ (AOO), located in the caudo-ventral portion of the peripheral olfactory organ. In this study, we investigated neural connectivity between the peripheral olfactory organ and eight radial olfactory bulb locations containing olfactory glomeruli. Labelled cells in the olfactory epithelium and the AOO were examined following retrograde application of postmortem (carbocynanine) and in vivo (biocytin) neuronatomical tracers. The labelled olfactory epithelial cells exhibited the cell morphology previously associated with olfactory sensory neurons. The labelled AOO cellswere short and flask-shaped, with abundant apical cilia. The neural projections from the AOO were confined to the medial region of the olfactory bulb. In turn, the projections from the main olfactory epithelium were distributed equally to all glomerular territories, including the medial region. These results suggest that the sea lamprey olfactory bulb has two neurally distinct glomerular regions. Functionally, this could mean that the output pathways of the medial region differ from the output pathways from the remaining olfactory glomeruli.

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Poster Session IV: Fri. July 25

Neural Pathways and Mechanisms Underlying Olfactory-Locomotor Transformations in Lampreys

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It is widely recognized that olfactory inputs elicit various motor behaviors - yet the underlying neural pathways and mechanisms have not been identified in any vertebrate species. In this study, we have used an *in vitro* lamprey preparation of the isolated brain, rostral spinal cord and olfactory epithelium (OE) for investigating olfactory-locomotor transformation. Application of odorants or pheromones onto the surface of the OE elicited long-lasting depolarizations recorded from reticulospinal (RS) cells, the brainstem motor command neurons in lampreys. We next investigated underlying neural pathways and mechanisms. Stimulation of the olfactory nerve (ON) induced excitatory post-synaptic potentials in RS cells, and stimulation of the medial part of the olfactory bulb (MOB) elicited broad excitation of RS cells, when observed using electrophysiology and calcium imaging. Injections of glutamate (3 mM) into the MOB induced sustained depolarizations in RS cells, accompanied by fictive locomotion recorded from ventral spinal roots. Anatomical tract-tracing experiments revealed a prominent projection from the MOB to a diencephalic structure, the posterior tuberculum (PT), the stimulation of which evoked synaptic responses in RS cells as well as swimming activity in the semi-intact preparation. Injections of glutamate receptor antagonists into the PT, or the Mesencephalic Locomotor Region (MLR), which controls locomotion, blocked the RS response to ON stimulation. In conclusion, we show that olfactory sensory inputs can activate locomotor command neurons and that the olfactory inputs transit through medial territories of the OB, the PT and the MLR before reaching RS neurons. This study is the first description of an olfactory–locomotor pathway in vertebrates.

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Poster Session IV: Fri. July 25

Context Dependent Olfactory Enhancement of Optomotor Flight Control in *Drosophila*

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Following a chemical plume of food odors is a challenge faced by many organisms. For flying insects the task is complicated by wind that distorts the plume and buffets the fly. To maintain an upwind heading, and thus stabilize their orientation in a plume, insects such as flies and moths make use of strong context specific visual equilibrium reflexes. For example, flying straight requires the regulation of image rotation across the eye, whereas minimizing side-slip and avoiding a collision requires regulation of image expansion. In flies, visual feedback stabilizes plume tracking, and visual rotation and expansion optomotor responses are controlled separately. Are olfactory signals integrated with optomotor responses in a manner dependent upon visual context? We addressed this question by investigating the effect of an attractive food odor on active optomotor flight control in a 'virtual-reality' flight simulator. In this paradigm, a fly is tethered in the center of a cylindrical arena of LEDs, and a diode casts a shadow of the beating wings onto an optical sensor, which encodes amplitude and frequency for each individual wing stroke. An odor port and vacuum delivered a continuous odor plume to the suspended fly. Odorant caused flies to both increase aerodynamic power output and steer straighter (paired t-test, expansion $p<0.001$, rotation $p<0.05$). However, when challenged with wide-field optic flow, odor resulted in enhanced sensitivity to rotation but reduced sensitivity to expansion (paired t-test, expansion $p<0.01$, rotation $p<0.05$). For both visual conditions, flies tracked motion signals more closely in odor. These results suggest a simple search algorithm by which olfactory signals enhance the salience of visual stimuli and modify optomotor control in a context dependent manner, thereby enabling an animal to fly straight up a plume and approach odiferous objects.

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Poster Session IV: Fri. July 25

Towards a Mechanistic Understanding of Food Odor Driven Motion Using Zebrafish (*Danio Rerio*)

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An understanding of the olfactory neural pathways which link olfactory responses to food odors with locomotion would be invaluable to a range of basic and applied research questions. In this study, both neural and behavioural responses were characterized over a 10,000-fold concentration range of a food odorant (the amino acid L-alanine). Neural activity was characterized using c-fos immunoreactivity and behavioural responses were characterized by quantifying swimming activity in flow-through tanks. Both neural (c-fos) and behavioural responses were first validated using a positive

control, the convulsant pentylenetetrazole. This exposure caused concentration-dependent increases in swimming activity and abundance of c-fos immunoreactive cells. With amino acid exposure, we observed behavioural attraction as well as concentration dependent c-fos expression. In odorant exposed fish, particular olfactory bulb (OB) regions with increased c-fos immunoreactivity included nuclei in the lateral region of the OB, previously associated with amino acid responses. Our research aims to characterize and correlate brain responses with behavioural responses toelucidate the network linking of responses. In this case, we determined the extent to which OB responses related to a behavioural feeding response. Future applications of our findings include the mechanistic determination of contaminant neurotoxicity and the neural basis for other ecologically relevant behaviours such as con-specific recognition.

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Poster Session IV: Fri. July 25

Floral CO_2 as a Cue in Moth Foraging

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It has been proposed that CO_2 cues from flowers play a role in the foraging behavior of moths. However, the value of floral CO_2 cues for moth behavior is not fully understood. By combining ecological studies with morphological, physiological and behavioral research on moths, we are unveiling the informational value of CO_2 in a natural context. Our study system consists of the sphingid moth *Manduca sexta* and its hostplant *Datura wrightii.* By measuring CO_2 levels and nectar volumes from unvisited flowers we found that floral CO_2 levels have informational value about nectar resources in flowers from plants living in hot and dry conditions, but not in those living in a cooler, more humid environment. Thus, under certain conditions, moths may make use of the CO_2 emitted by flowers to improve their foraging strategy. Moth visits, involving generation of air turbulence next to and within flowers failed to alter CO_2 emission. Moreover, experimental depletion of air within *Datura* flowers failed to reduce CO_2 emissions from average when measured 10 min. after depletion. These data suggest that at the onset of foraging, floral CO_2 levels could be used by moths as 'honest signals' for nectar. However, during foraging the two variables would increasingly decouple, so that previously visited flowers may still attract (and 'deceive') moths. Moth CO_2 receptor cells are found in a specialized organ. A comparison of this organ in non-foraging sphingid moths is helping us to understand possible roles of CO_2 in moth foraging. Moreover, recordings of the responses of neurons in the antennal lobe of moths indicate that CO_2 information is integrated with information about floral odors. Our behavioral and neurophysiological data suggests that CO_2 may play an important role in the context of nectar foraging.

Poster Session IV: Fri. July 25

Quantification of Selected Host-Seeking Behavior in Mosquitoes

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Although target recognition of mosquitoes has been examined in various manners for an extended period, the contribution each specific attractant makes toward host recognition remains unknown, largely due to a lack of studies focusing at the level between electorophysiological recordings and field experiments. In addition, olfactometers merely quantify transfer of the mosquito from one side of the cage to the other, while field experiments using hosts such as human are too complex to determine contributions of single attractants due to the presence of amyriad set of chemical cues. In light of the advantages and disadvantages of previous studies, a new assaying device able to quantify the selected host-seeking behavior was designed. At first, we focused on CO_2 and heat and set up a thermo-controlled target combined CO_2 releaser as an artificial host target. The device that also includes infrared sensors can quantify touch down behavior to target, food, and background simultaneously. Interestingly, use of both CO_2 and heat in order to recognize the target appears to be important to the mosquitoes. In the presence of CO_2 only mosquitoes became active but did not show target recognition behavior as observed by videorecording suggesting that CO_2 is an initiator of mosquito target recognition that combines both CO_2 and heat. Thus, we define this blood sucking behavior monitored by this device as CO_2 –activated thermo sensing (CATS) behavior. In order to identify organs required for CATS behavior we removed maxillary palps from the female head and found the behavior to be lost. Therefore we found that maxillary palps were essential for CATS behavior and suggested that the maxillary palps contained CATS behavior-related candidate neurons and genes.

Poster Session IV: Fri. July 25

The Molecular and Cellular Basis of Olfactory-Driven Behavior in Larval-Stage Disease Vector Mosquitoes

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The mosquitoes *Anopheles gambiae* and *Aedes aegypti* are the principal afrotropical vectors for human malaria and dengue/yellow fever, respectively. A central component of these mosquitoes vectorial capacity is the ability to maintain sufficient populations of blood feeding adults. This, in turn, depends on the ability to recognize and respond to chemical cues that mediate feeding and survival during pre-adult (larval) stages. Here we employ a behavioral assay to detail the response profiles of *An. gambiae* and *Ae.aegypti* larvae against a range of chemical stimuli that are dependent upon the integrity of the larval antennae. Parallel molecular analyses have identified a subset of the *An. gambiae* and *Ae.aegypti* odorant receptors (Ag/AaORs) that are localized to discrete neurons within the larval antennae and which facilitate odor-evoked responses in *Xenopus* oocytes that are consistent with the larval behavioral spectrum. These studies introduce new paradigms for mosquito behavior as well as represent the first molecular characterization of olfactory processes in mosquito larvae. These advances may enhance the development of vector control strategies targeting olfactory pathways in larval-stage mosquitoes to reduce the catastrophic effects of malaria and other insect-borne diseases.

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Poster Session IV: Fri. July 25

Sniffing Behavior and Odor Representations Measured in the Behaving Mouse

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Sniffing is a complex behavior thought to play a critical role in odor information processing and perception. While the mouse has become a prominent model for studying olfaction, little is known about sniffing behavior in mice. In this study we monitored sniffing behavior in C57Bl/6 mice throughout several behavioral paradigms. Sniffing was recorded from an intranasal cannula during unstructured exploratory behavior and during performance in three commonly-used olfactory paradigms: a habituation/dishabituation task, a sand-digging based discrimination task, and a nose-poke based discrimination task. We found that sniff frequencies in quiescent mice ranged from 3 to 5 Hz – higher than that reported for rats and hamsters. During active exploration, sniffing reached maximal frequencies of ~12 Hz for brief (1 – 2 sec) periods. Sniffing behavior varied between tasks as well as for different behavioral epochs of each task. For example, mice performing the digging-based task showed no increase in sniff frequency prior to digging, while mice performing a nose-poke based task showed reliable increases. Mice showed robust increases in sniff frequency prior to reward delivery in all tasks and when nose-poking in a non-olfactory task. In a separate set of experiments, we imaged receptor input to the olfactory bulb of awake, head-fixed mice as they performed odor discriminations. We found that sniff frequency strongly shaped both the temporal structure and spatial organization of receptor neuron input to the olfactory bulb in awake mice. Together, these findings provide basic data on sniffing behavior in mice and demonstrate that such behavior may allow a mechanism by which odor representations are contextually modulated as early as the level of the primary sensory neurons.

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Poster Session IV: Fri. July 25

Olfactory Information Processing in Behaving Mice

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The vast majority of our knowledge about the function of the mammalian olfactory system has been gleaned from anesthetized preparations. Recordings from mitral cells, the first recipients of olfactory information after the odor receptors, have been made in awake behaving animals. It was shown that these neurons respond very differently in the awake state compared to the anesthetized state. The critical features of the neuronal code carrying information about olfactory stimuli are still unknown. Single and multicellular recordings combined with olfactory psychophysics experiments yield some clues about the nature of the olfactory code. In behavioral experiments we demonstrated that the accuracy of an odor discrimination task increased with the longer rising odor exposure - even beyond one sniff. Electrophysiological measurements in behaving mice showed that the firing rate odor response saturated at approximately the same time as when behavioral accuracy reached its maximum. That suggests that sensory integration happens at the mitral cell level or earlier. This observation sets restrictions on the possible models of olfactory information processing.

Poster Session IV: Fri. July 25

Olfactory Discrimination of Aliphatic Odorants at 1 PPM – Too Easy for Mice to Show Odor Structure-Activity Relationships?

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Functional studies suggest that the neural representations of odorants vary systematically as a function of molecular structural features such as carbon chain length or functional group. Psychophysical studies in both humans and animal models have reported some correlations between perceived odor quality and these molecular properties but the generality of such correlations is unknown. Using an operant conditioning paradigm we therefore tested the ability of CD-1 mice to discriminate between 25 odorants comprising members of five homologous series of aliphatic odorants (C4-C8) presented at a gas phase concentration of 1 ppm. We found a) that all mice significantly discriminated between all 50 stimulus pairs that involved odorants sharing the same functional group, but differing in carbon chain length, as well as between all 50 stimulus pairs that involved odorants sharing the same carbon chain length but differing in functional group, b) a significant negative correlation between discrimination performance and structural similarity of odorants in terms of differences in carbon chain length with the acetic esters and the 2-ketones, but not with the 1-alcohols, n-aldehydes, and n-carboxylic acids tested, c) a lack of systematic differences in discrimination performance as a function of type of functional group, and d) that presentation of stimuli at 0.1 ppm did not impair discrimination performance. These findings demonstrate that CD-1 mice have an excellent discrimination ability for structurally related aliphatic odorants. Given that olfactory discrimination performance critically depends on stimulus concentration, it may be that presentation of odorants at 1 ppm was too easy (that is: too high above detection threshold) for the mice to show consistent odor structure-activity relationships.

Poster Session IV: Fri. July 25

Olfactory Discrimination of ''Odorless'' Mineral Oils by Behaviorally-Trained Mice

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Mineral oil (MO), a common diluent for oil-based odorants, is typically labeled as ''odorless.'' In olfactory research, an understanding of the nature of the diluent is as critical to stimulus control as is the odorant. For example, use of a diluent containing volatiles turns even a simple odorant into a complex stimulus capable of producing complex, or unintended physiological responses quite unlike that to the simple odorant alone. Unpublished behavioral findings from our laboratories suggest that MOs obtained from different sources are perceived as distinct odorants. To explicitly test this notion, we trained mice to discriminate pairwise comparisons of four MOs obtained from different vendors (Fisher, Sigma, CVS and Walmart). Five mice (C57BL/6J) were trained to perform a two-odor discrimination task in a liquid-dilution olfactometer. All of the mice easily acquired the discrimination at levels of 85% or higher for all MO pairwise comparisons. To determine if the different MOs were discriminable when used as diluents with a single odorant, the mice were then trained to discriminate the different MOs in the presence of two suprathreshold levels of cineole (10^{-4} and 10^{-5}% v/v). Even in the presence of suprathreshold levels of cineole, all of the mice were able to easily discriminate between each MO with 85% or higher accuracy. These results suggest that MOs from different sources possess unique odor profiles and that these diluents may affect the perception of the intended odorant. The data also suggest that investigators should consider possible diluent-odorant interactions when using MO as a diluent in olfactory studies.

Poster Session V

Woundmonitor: Monitoring Volatiles to Detect Infection

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Array based gas sensor technology now offers the potential of a robust analytical approach to odour measurement for medical use. Wounds become infected when microorganisms from the environment or from the patient's body enter the open wound and multiply. We are developing a rapid and reliable method for detection of microbial infection by monitoring the headspace from the infected wounds funded via an IST-027859 EU project WOUNDMONITOR. We present results obtained by analysing the headspace volatiles emitted from Staphylococcus *aureus*, Streptococcus *pyogenes*, and Pseudomonas *aeruginosa* in order to identify volatile markers of infection. The results from GC-MS analysis are enabling us to build a system for non invasive wound monitoring using an array of gas and odour sensors, for point of care monitoring of patients. Sensors based on metal oxide and conductive polymer films were produced and modified and refined to detect the key markers for the bacteria types frequently found in clinical conditions.The criteria for selection of the sensors was determined by the sensitivity and selectivity of the sensors to a limited number of the volatile compounds (VOC) produced by bacteria defined as the most frequently found during treatment of certain wounds. For sampling from swabs or dressings from patients a solid phase microextraction approach was used for preconcentration of the low concentrations of volatile compounds emitted. An instrument was constructed that incorporated an automated solid phase microextraction desorption system, a hybrid sensor array, electronics, and data processing to enable the system to be used for clinical validation. The instrument is being validated over the next year in two hospitals where patients with serious burns are treated.

Poster Session V

Afferent and Efferent Connections of the Parabrachial Nucleus in the C57BL/6J Mouse

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Although the mouse is an experimental model with an increasing importance in various fields of neuroscience, the characteristics of its central gustatory pathways has not yet been well documented. In the present study, we investigated the afferent and efferent connection patterns of the mouse parabrachial nucleus (PbN), a key interface in the pons between brainstem and forebrain gustatory areas. In Experiment 1, we iontophoretically injected the retrograde tracer Fluorogold into the PbN. In general, the PbN was found to receive projections from the medullary reticular formation, the nucleus of the solitary tract, the periaqueductal gray, the lateral hypothalamus (LH), the paraventricular nucleus, the central nucleus of the amygdala (CeA), the bed nucleus of the stria terminalis, the insular cortex, the infralimbic cortex, and the lateral prefrontal cortex. In Experiment 2, fluorescent latex microspheres (red and green) were pressure microinjected into pairs of forebrain structures including the gustatory thalamus (VPMpc), LH, or CeA in order to reveal both the distribution, and the degree of collateralization, of retrogradely-labeled afferents in the PbN. Rostrally, there was dense labeling of CeA- projection neurons, and sparser labeling of VPMpc-projection neurons in the external lateral subnuleus. Only a few of these were double-labeled, projecting to both areas. LH-projecting cells comprised a very discrete population in the central lateral subnucleus where no double-labeled neurons were observed. More caudally, in the waist area of the PbN, where taste responses are most often recorded, VPMpc-, CeA-, and LH-projecting cells were found intermingled.

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Poster Session V

GPR Expression in the Rat Taste Bud Relating to Fatty Acid Sensing

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Fat is an attractive food, and we tend to find fatty foods more palatable than low-calorie, low-fat foods. It was recently reported that rodents and humans recognize the presence of fat infoods not only by texture but also chemically in the mouth. We previously reported that fatty acid translocase (FAT/CD36) is expressed in taste bud cells and is related to fatty acid sensing in the mouth. In this study, we investigated the expression of G protein-coupled receptor GPR40 and GPR120,known as a fatty acid receptor, in the tongue. Using RT-PCR, we were not able to detect GPR40 mRNA in the tongue. In contrast,GPR120 mRNA was detected in the epithelium containing taste buds in the circumvallate papillae but not in the nonsensory epithelium. Western blotting analysis using anti-GPR120 antibody showed a protein band, the molecular weight which corresponds to that of GPR120, indicating that this antibody could recognize rat-derived GPR120 in homogenate of colon and circumvallate papillae. Immunohistochemistry using anti-GPR120 antibody revealed GPR120-positive cells were located in the enteroendocrine cells. Furthermore, some cells in each taste bud were stained positively with more intense labeling in the apical part of the cells. Doubleimmunostaining of GPR120 and CD36 revealed that majority of GPR120 immunoreactive taste cells did not express CD36. These results raise the possibility that GPR120 is expressed in the taste cells, possibly the gustatory cells, in the circumvallate papillae, sensing dietary fat as well as CD36 that expressed in the taste bud cells.

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Poster Session V

Long-Chain Fatty Acids Induce Intracellular Ca^{2+} Via G-Protein Coupled Receptor 120 (GPR120) and Positive Licking Behavior in Mice

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CD36 on the tongue was reported to be a putative fatty acid (FA) receptor that detects fat. Recently, G-protein coupled receptor 120 (GPR120), which was originally reported in the colon as a long-chain FA recognition receptor, was also found in the epithelium of circumvallate papillae, however, the pivotal role of GPR120 on the tongue remains unclear. The structure of GPR120 is a seven-transmembrane receptor, which differs from the two-transmembrane receptor CD36, but is similar to the bitter, sweet and umami receptors. Considering the structure, GPR120 might be important as fat receptor on the tongue similar to other taste receptors. In this study, to understand the role of GPR120 on the tongue, we first screened the potent ligands for GPR120 using human GPR120 overexpression HEK293 cells. Intracellular Ca^{2+} ($[Ca^{2+}]_i$) induction in human GPR120-overexpressed cells was monitored by measuring fluo-3 fluorescence using spectrophotometer. Stimulatory activities were detected for unsaturated free FAs with a chain length of C_{14} to C_{22}. Saturated FAs, and several trans-form of unsaturated

FAs were not strong ligands for GPR120. Moreover methyl olelate and methyl linoleate, which lack a carboxy group also did not induce the $[Ca^{2+}]_i$. Secondly, we investigated the palatability of various kinds of long-chain FAs by licking test in BALB/c mice, finding that the palatability of FAs in mice is very similar to the selectivity of ligand activity for GPR120. These data suggest that long-chain unsaturated FAs are good ligands for GPR120, and these substances also induced high licking behaviors in mice, which is suggestive of the importance of GPR120 as well as CD36 on the tongue for fat recognition.

This study was supported by the Program for the Promotion of Basic Research Activities for Innovative Bioscience.

Poster Session V

RNA Interference of GPR120 Inhibits Responses to Fatty Acids in the Enteroendocrine Cell Line, STC-1: Implications for Fatty Acid Transduction

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Nutrient-induced stimulation of enteroendocrine cells (EECs) leads to release of the hormones GLP-1 and CCK that contribute to satiety. Our research has been focusing on the mechanisms that underlie the ability of fat to stimulate enteroendocrine cells during food intake. Recently, we have identified potential fatty acid (FA)-activated proteins in the enteroendocrine cell line STC-1 including FA-sensitive potassium channels and several FA-activated G protein coupled receptors (GPCRs) including GPR120, GPR40 and GPR41/43. To explore functional responses to FAs in STC-1 cells, we have used patch clamping and Ca^{2+} imaging. Long chain unsaturated FAs (LCFAs) cause depolarization and elicit concentration-dependent increases in intracellular Ca^{2+} in STC-1 cells. Using heterologous expression, we have shown that the magnitude of FA responses is dependent upon the subtypes of potassium channels expressed in STC-1 cells. We have used pharmacological approaches to explore the route for FA-induced Ca^{2+} changes and depolarization; our data shows that FA induced Ca^{2+} changes but not FA-induced depolarization is dependent on extracellular calcium. Removal of extracellular Na^+ also reduces the magnitude of the FA response suggesting that TRPM5/4 channels may contribute to the depolarization that occurs in the FA transduction pathway. LCFAs also elicited rapid, Na^+ dependent TRPM5/4 like currents. LCFA-induced TRPM5/4 like currents were significantly reduced when expression of GPR120 was knocked down using RNA interference suggesting that GPR120 is upstream of TRPM5/4 channels, where it may represent the primary FA receptor in EECs.

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Poster Session V

Fatty Acids Elicit Membrane Depolarization and a Rise in Intracellular Calcium in Rodent Taste Cells

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In recent years, a number of studies have demonstrated the ability of components in fats, specifically free fatty acids (FA), to activate taste receptor cells (TRCs) and elicit behavioral responses consistent with there being a taste of fat. We have used both molecular and cell-based assays to explore the mechanism of FA transduction in TRCs in more detail. TRCs express several types of putative FA-responsive proteins including the FA-binding protein CD36, FA-sensitive K^+ channels and FA-activated G protein coupled receptors (GPCRs) that appear differentially expressed in the various taste papillae. Using ratiometric calcium imaging, fatty acids (1-30 μM) elicit a rapid increase in intracellular calcium in approximately one-third of TRCs. This response is partially dependent upon extracellular Na^+, suggesting an involvement of cation channels, like TRPM5 in the FA response. Removal of extracellular Ca^{2+} also inhibits the response pointing to a role of voltage-gated Ca channels or other Ca^{2+} transporters. Patch clamp recording in rodent TRCs shows that FA application elicits a rapid depolarization with pharmacological properties consistent with those found by functional calcium imaging. A model for the transduction of FAs in TRCs consistent with these data will be presented.

Supported by NIH DK59611, UAES Project 00630 and International Flavors & Fragrances.

Poster Session V

Fatty Acid-Induced Changes in Intracellular Calcium in Somatosensory Cells: Mechanisms Underlying the Textural Perception of Fat

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Sensory recognition of dietary fat has become increasingly important given the epidemic of obesity which is driven partially by high dietary fat intake. Besides the recent work of taste of fat, the textural properties of fat have been well documented to occur via the activation of trigeminal ganglionic neurons (TGN). Molecular data from our laboratory have demonstrated that there are a variety of putative FA-responsive proteins expressed in TGNs including FA-sensitive potassium channels, the FA binding protein CD36 and several FA-activated G protein coupled receptors. We have used fura-2 based calcium imaging to explore the ability of FAs to elicit increases in intracellular calcium ($[Ca^{2+}]_i$) in rat TGNs. FAs (1-100 μM) elicit robust changes in $[Ca^{2+}]_i$ in approximately one-half of TGNs in a concentration-dependent fashion. In general, responses to poly/mono-unsaturated FAs occur in cells independent of those that respond to saturated FAs. In TGNs, cells exhibit FA responses that are independent of extracellular Na^+ but are either dependent or independent of extracellular Ca^{2+} possibly indicative of multiple functional cell types. Store depletion by thapsigargin significantly reduces but does not abolish the FA-induced Ca^{2+} response. We also tested FA induced membrane depolarization in TGNs by patch clamp recording. Linoleic acid elicits membrane depolarization in TGNs with time course similar to that seen for the rise in $[Ca^{2+}]_i$. Moreover, GDP-βs and U73122 block 75% and 50% of linoleic acid induced TGN depolarization, respectively. We will present a model based upon available data linking GPCRs, CD36, store-operated cation channels, and FA-sensitive potassium channels in the responses of TGNs to FAs.

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Poster Session V

Removal of the Submaxillary and Subligual Salivary Glands Impairs Linoleic Acid Taste Discrimination

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We previously found that bilateral transection of the gustatory chorda tympani nerve (CTX) significantly impairs the ability of rats to detect linoleic acid (LA; an essential free fatty acid that is the main component of corn oil). Surprisingly, the CT nerve was unresponsive to a broad range of LA concentrations in whole nerve electrophysiological experiments. LA may require a background of saliva to activate taste cells. This would explain, in part, the discrepancy between our behavioral data (in which saliva is present) and CT electrophysiological data (in which saliva is rinsed off with water). Moreover, CTX also partially desalivates the animal, due to denervation of the submaxillay and sublingual salivary glands. Therefore, impairment of LA taste discrimination after CTX may result from transection of the chorda tympani nerve itself, a secondary decrease in saliva, or both. To examine this issue, the present study measured LA taste discrimination thresholds in animals without the submaxillary and sublingual salivary glands. Seven days after surgery, animals were given a conditioned taste aversion (CTA) to 88 μM. The CTA to 88 μM LA was confirmed before and after generalization testing to more dilute LA concentrations in two-bottle tests with water. We found that partial desalivation of animals resulted in a slight increase in LA discrimination thresholds (i.e. from ~11 μM to ~ 22 μM), suggesting the saliva is important for LA taste responses. However, this effect was not as pronounced as in CTX animals (i.e. from ~ 11 μM to ~ 44 μM). Thus, CTX impairs LA taste discrimination by removal of sensory input to fungiform taste buds as well as by decreased saliva.

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Poster Session V

Human Detection of Free Fatty Acids

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There is increasing evidence for a taste component for free fatty acids (FFA). Human work has taken two approaches: psychophysical studies and modified sham feeding trials. The former have used masking to isolate the taste component and reveal humans can detect low concentrations of 18 carbon FFAs varying in saturation. This study employed the same approach to determine whether humans can detect FFA varying in chain length. Thresholds were determined in 35 adults for caproic (C6), lauric (C12), linoleic (C18:2) and stearic (C18:0) acids in a vehicle containing 5% mineral oil, 5% gum acacia and 0.01%EDTA after capsaicin desensitization and with nares closed and under red light. Thresholds were 0.017±0.006%w/v-caproic, 0.190±0.07%w/v-lauric, 0.100±0.05%w/v-linoleic and 0.117±0.03%w/v-stearic. A modified sham-feeding trial was also conducted and the change of plasma triacylglycerol (TG) concentration was monitored as a biomarker for FFA detection. Most evidence for a cephalic phase fat response(CPFR) is based on multiple exposures over a 2h period. To assess the ecologic validity of this response, to-date, 12 healthy adults have modified sham fed full-fat and fat-free cream cheese for single 10s exposures and then either replicated the trials or increased exposure times if they failed exhibit a TG rise of at least 10mg/dl within 30m of full-fat exposure. Approximately 70% of participants have responded, indicating 10s, as would occur with any fat ingestion, is a sufficient for CPFR. Most individuals also responded to the fat-free stimulus, but the TG rise was lower. These findings further support a taste component for FFAs and extend knowledge to a wider array of FFA and shorter exposure times.

Poster Session V

Mapping Input from T1R3 Sweet Taste Receptors to Central Gustatory Neurons in Mice

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The T1r3 taste receptor mediates behavioral preference for many sweets. Here, the tuning properties of central taste neurons influenced by T1r3 were mapped to define connections between T1r3 and the brain. Taste responses were electrophysiologically recorded from single nucleus tractus solitarius neurons in anesthetized T1r3 knockout (KO; Damak et al., 2003) and C57BL/6 wild-type (WT) mice. Cells were tested with a battery of stimuli, many across multiple concentrations. Sweet stimuli included glycine, sucrose, proline, fructose, glucose, sorbitol, saccharin, and acesulfame-K. Also tested were NaCl, $NaNO_3$, Na-acetate, MSG, KCl, HCl, citric acid, quinine, denatonium and papaverine. 24 WT and 19 KO neurons tested with a uniform set of stimulus concentrations were recorded. All neurons were partitioned into groups by their responses to multiple sweet stimuli using *k*-means clustering. Groups were found that harbored only WT cells: such neurons (n=11) showed sweet responses not found in KO cells, logically implicating them as dependent on T1r3. The sweet selectivity of each T1r3-dependent WT cell was evaluated by receiver operating characteristic (ROC) analysis of all available sweet and non-sweet responses. Across cells an average (±SE) of 27±1 sweet and 21±1 non-sweet stimulus trials were analyzed. ROC indexed the probability (P) that sweet and non-sweet responses could be correctly discriminated by assuming those to sweets are larger. High discrimination performance (P near 1) would result for cells showing selective tuning towards sweets and the majority (64%) of T1r3-dependent cells displayed $P \geq .9$. Yet others (36%) showed lesser or poor P values, reflecting cells broadly tuned. Input from T1r3 is received by a heterogeneous pool of central taste neurons in C57BL/6 mice.

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Poster Session V

High Energy High Fat Diet Alters Pontine Taste Coding in Rat

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Prolonged ingestion of high fat (HF) diet is associated with overconsumption and obesity, but the underlying mechanisms are unknown. One possibility is that HF diets alter integration of orosensory and homeostatic processes regulating meal size. To investigate this, we used acute and chronic extracellular recording in the pontine parabrachial nucleus (PBN) while stimulating the tongue with various concentrations of sucrose (0.03-1.5M) in male Sprague Dawley rats. Three groups were used, one received ad libitum high fat diet (HCHF, 60%kcal), one received regular chow (ND) and one was pair-fed with a restricted amount of HF diet calorically equal to the ND group (NCHF). After 6 weeks, this regimen resulted in significant weight gain in both HF groups compared to ND (HCHF: +26%, $p<0.01$; NCHF: +11%, $p<0.05$), with no statistical difference between HF groups ($p=0.07$) despite a higher daily caloric intake in the HCHF rats (21%, $p<0.02$). Oral glucose tolerance did not differ across groups. Sucrose-responsive PBN neurons (N=127) in HCHF rats demonstrated significantly higher spontaneous firing rates compared to NCHF (+105%; $p<0.01$). In addition, sucrose concentration-response functions differed between experimental groups ($p<0.01$). Neurons in HCHF rats had decreased threshold concentrations compared to the NCHF (0.14±0.05M vs. 0.28±0.05M, $p<0.01$) and maximal neuronal responses occurred at significantly lower sucrose concentrations (0.36±0.04M) compared to NCHF and ND

(0.56±0.06M, p<0.01, 0.53±0.06M, p<0.05, respectively). These findings demonstrate that dietary history may influence taste processing in the hindbrain and suggest that increased energy intake, more so than dietary fat itself or factors secondary to obesity, is contributory.

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Poster Session V

Temporal Coding of Taste in the Parabrachial Nucleus of the Pons in the Rat

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Previous studies of taste-evoked spike trains in the nucleus of the solitary tract (NTS) of the rat have shown that spike timing can distinguish among tastants of different qualities (sweet, sour, salty and bitter). The aim of the present study was to determine if spike timing is also informative at the level of the pontine parabrachial nucleus (PbN), the main target of taste-related output form the NTS in the rat gustatory system. Rats were anesthetized with urethane and prepared surgically for electrophysiological recording from the PbN. Taste stimuli representing the four basic taste qualities were presented in separate trials and the evoked responses from single PbN cells were recorded. At least 10 trials of each tastant were presented. To assess the contribution of the temporal characteristics of the response to the discrimination among tastants, a family of metrics that quantifies the similarity of two spike trains in terms of spike count and spike timing was used. Temporal characteristics of taste responses were analyzed for the first two sec of response. Results demonstrate that spike timing in PbN cells can convey a significant amount of information about taste quality, beyond what can be conveyed by spike count alone. These data extend previous findings in the NTS and support the idea that temporal coding mechanisms are widespread in the gustatory neuraxis.

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Poster Session V

Benzodiazepine Modulation of Gustatory Coding in the Parabrachial Nucleus

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Benzodiazepine agonists delivered systemically or to the parabrachial nucleus (PBN) increase consumption and behavioral measures of gustatory evaluation. However, electrophysiological PBN gustatory responses after benzodiazepines have not been characterized. We evaluated PBN gustatory neuron responses before and after injections of chlordiazepoxide (CDP). Gustatory responsive cells in the PBN were profiled for responses to 1.0M sucrose, 0.1M NaCl, 0.03M citric acid, and 0.003M QHCl before and/or after systemic CDP (20 mg/kg) or saline delivery. Of the 129 cells recorded, 16 cells were tested both before and after CDP injection and 7 cells were tested both before and after saline. In this CDP subgroup, spontaneous activity and the responses to QHCl were significantly suppressed. Responses to sucrose, NaCl and citric acid were not changed, however, more cells responded best to sucrose and fewer responded best to citric acid and QHCl after CDP. Breadth of tuning (entropy) was reduced after CDP in cells that were broadly tuned initially. No such changes occurred after saline injection. In the ''between'' groups, after CDP the magnitude of responses to citric acid and QHCl were reduced. The proportion of cells responding best to sucrose also more than doubled. There were no shifts after saline injection. Overall, CDP reduced the response magnitude and/or proportion of cells responding best to citric acid and QHCl, and it increased the proportion of cells responding best to sucrose. Thus, CDP may modify taste evaluation through taste quality-specific rate-coding effects in the PBN.

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Poster Session V

Gustatory-Responsive Neurons in the Parabrachial Nuclei Receive Convergent Afferent Input from the Area Postrema in the Hamster

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Department of Anatomy, Southern Illinois University School of Medicine Carbondale Afferent fibers of vagus nerve carry visceral sensory information from various organs including the gastrointestinal lumen. The sensory afferents enter the brainstem and terminate within the caudal nucleus of the solitary tract (NST) in an overlapping topographic manner. In addition, vagal afferent fibers project heavily to the bilateral area postrema (AP). It was reported that taste neurons in the parabrachial nuclei (PbN) were coactivated by gastric distension, indicating that the PbN is one of the sites that the integration of taste and viscerosensory information takes place. Here, we examined whether electrical stimulation of the AP activates taste neurons in the PbN in urethane anesthetized hamsters. Taste solutions were 0.032 M sucrose, NaCl, QHCl, and 0.0032 M citric acid. When a taste cell was isolated in the PbN, its taste response profile was examined and rectangular pulses (0.5 ms, 0.1 mA, 1/3 Hz) were delivered to the bilateral AP. Stimulation of ipsi- and contralateral AP activated 30 of 40 (75%) or 23 of 40 (57.5%) PbN taste cells, respectively. The response latencies of the PbN cells after the ipsi- and contralateral AP stimulation varied from 6 to 20 ms (mean = 10.44 ms) and 11 to 36 ms (mean = 24.91 ms), respectively. The responses following the ipsilateral AP were exclusively excitatory while 3 of 23 cells activated following the contralateral AP were inhibitory. These results indicate that taste neurons in the PbN receive extensive convergent input from the AP.

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Poster Session V

The Distribution of Gustatory-Activated FOS Expression in PBN Neurons that Project to the Central Nucleus of the Amygdala

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Taste information in the CNS follows both thalamocortical and limbic forebrain paths from the parabrachial nucleus (PBN) in the pons. Limbic targets such as the central nucleus of the amygdala (CeA) are thought to play a role in stimulus palatability. We first examined the distribution of gustatory-activated c-Fos expression in PBN of neurons retrogradely labeled from CeA. We also examined the distribution of c-Fos in viscerosensory and gustatory regions of NST. A retrograde tracer, fluorogold (FG), was injected bilaterally into CeA of adult male rats. Five days later, rats were stimulated with sucrose, quinine or NaCl via an intraoral cannula for 15 minutes, and then perfused. The distribution of FG and c-Fos in PBN and NST was examined using immunohistochemical methods. In PBN, the highest density of FG-labeled neurons was found in the external lateral and external medial subnuclei. A lower density of FG-labeled neurons was found in the ventral lateral, central medial, and waist areas. In addition, retrograde FG labeling was observed in caudal NST, but not the gustatory rostral region. Quinine induced c-Fos expression throughout PBN, especially the external

subnuclei, whereas sucrose and NaCl-elicited c-Fos labeling was predominantly found in lateral and central medial subnuclei. Double-labeling indicated a substantial amount of PBN neurons activated by each quality that project to the CeA. We also used a DBH antibody to identify noradrenergic axons and their presynaptic terminals on FG-labeled neurons in the PBN. Co-localization of FG and ionotropic glutamate receptor (GluR2/3) labeling was observed in neurons in the external lateral subnucleus. Collectively, these studies characterize the nature of the gustatory projection from PBN to CeA.

Poster Session V

Retrograde Fluorescent Tracer Injections into Brainstem Gustatory-Responsive Regions Suggest that Descending Forebrain Projections Originate Largely from Separate Neuronal Populations in Rat

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Stimulation or inactivation of forebrain structures like the gustatory cortex (GC), bed nucleus of the stria terminalis (BNST), central nucleus of the amygdala (CeA), and lateral hypothalamus (LH) differently regulates taste responsive neurons in the nucleus of solitary tract (NST) and the parabrachial nucleus (PBN). The present study investigated whether this descending influence originates from a shared or distinct population of forebrain neurons. The retrograde tracers Fast Blue (FB) and Fluorogold (FG) or green (GRB) and red (RRB) fluorescent retrobeads (LumaFluor, Inc.) were injected iontophoretically or by using pressure pulses (10ms at 20psi) into the taste-responsive regions of the NST and the ipsilateral PBN under electrophysiological guidance in three rats. Seven days later, the animals are euthanized and tissue sections containing the LH, CeA, BNST, and GC were processed for co-localization of FB and FG or GRB and RRB. The results showed that the CeA is the major source of input to the NST (84.1 ± 12.8 cells/ section) and the PBN (81.8 ± 12.1), compared to the BNST (36.9 ± 7.8; 39.3 ± 9.1), the LH (37.8 ± 5.8; 34.0 ± 5.0), and the GC (22.4 ± 3.2; 23.7 ± 1.2). Of the total number of retrogradely labeled cells, the incidence of tracer co-localization was 25% (±5%) in the GC, 20% (±5%) in the CeA, 21% (±5%) in the BNST, and 16% (±3%) in the LH demonstrating that some forebrain neurons send projections both to the NST and PBN taste areas. Nevertheless, it appears that the majority of descending input to the gustatory NST and PBN originates from distinct neuronal populations. This arrangement provides an anatomical substrate for differential modulation of taste processing in the first and second central synapses of the ascending gustatory system.

Poster Session V

Expression of C-FOS in Parabrachial Nucleus Following Bitter Stimulation to Denervated Taste Buds of the Rat

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Previous studies demonstrated that taste stimulation induces the increase in number of c-fos-immunoreactive (-IR) neurons in parabrachial nucleus (PBN) in normal adult rats. It is known that injury to gustatory nerve causes the degeneration of taste buds. To date, however, it is unclear whether gustatory nerve injury causes the changes in expression pattern of c-fos-IR neurons in PBN following taste stimulation. The present study examined the expression of c-fos-IR neurons in PBN in denervated rats. Moreover, expression of mRNA and protein for α-gustducin, a taste specific G protein related to bitter transduction, in denervated circumvallate papilla (CVP) was examined. Bilateral crush injury of glossopharyngeal nerve was performed in 8-week-old male rats. Bitter stimulation (0.001M quinine) was applied to the posterior portion of tongue 6, 9, 12 and 15 days following injury (PO), and c-fos immunohistochemistry was applied to PBN. The expression of α-gustducin was also examined in taste buds by RT-PCR and immunohistochemistry. In normal animal, bitter stimulation evoked approximately twofold number of c-fos-IR neurons in dorso-lateral portion of PBN compared to that following application of distilled water (DW). On PO6 when very few taste buds and α-gustducin-IR taste cells were detected in the trench wall, number of c-fos-IR neurons in PBN evoked by bitter stimulation was almost identical to that following DW application. On PO12 when there was no taste buds in the trench wall, number of c-fos-IR neurons increased to approximately twofold compared to that after DW application. Interestingly, mRNA for α-gustducin was detected constantly during entire experimental periods. These results suggest that bitter stimuli may transmit centrally even there was no apparent mature taste bud.

Poster Session V

Sweet Expectations: Greater Response in the Anterior Insula and Midbrain to Unexpected Compared to Expected Sweet Taste

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We used fMRI to test whether whole brain response to a sweet taste varies as a function of whether it is expected or unexpected. A 2x2 factorial design was employed with expectation (valid or invalid cues consisting of the spoken word ''sweet'' or ''tasteless'') and stimulus (tasteless or 0.56M sucrose solution) as within-subject factors. This gave rise to a measure of brain response during four different conditions: 1) hearing ''sweet'' followed by receipt of sweet (expected sweet); 2) hearing ''sweet'' followed by receipt of tasteless (unexpected tasteless); 3) hearing ''tasteless'' followed by receipt of sweet (unexpected sweet); and 4) hearing ''tasteless'' followed by receipt of tasteless (expected tasteless). 70% of the trials were valid (condition 1 and 4). As predicted, we found a main effect of expectation such that attentional, gustatory and limbic regions responded significantly more to tasteless and to sweet solutions when unexpected. We also observed a stimulus by expectation interaction with greater response in the midbrain and the bilateral anterior insula/ frontal operculum during receipt of sweet when it was not expected. These findings are consistent with prior work showing that midbrain dopamine neurons and their target regions respond preferentially to unexpected food reward. Our results extend prior knowledge by showing that the encoding of sweet taste in primary sensory cortex is influenced by expectation. These findings highlight the impact of reward context effects on early encoding of gustatory stimuli in the human brain.

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Poster Session V

Employing DNA-Functionalized Carbon Nanotubes to Detect Biologically-Derived Odorants

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DNA-functionalized carbon nanotubes can detect biologically-derived odorants. Single-stranded DNA (ss-DNA) is the chemical recognition site and single-walled carbon nanotube field effect transistors (swCN-FETs) are the read-out component. Nonanal, C_5-C_8 organic acids and dimethylsulfone were selected as target odorants since they emanate from a variety of mammals, including humans. Compounds were dissolved in odorless (and VOC-free) light-white mineral oil and introduced to the nanotubes. The ss-DNA, SwCN-FETs selectively detect one of the odorants, hexanoic acid. This may be due to the odorants' water solubility or the DNA base sequences. A change of the DNA base sequence may alter the response to hexanoic acid as well as other odorants. Our results suggest that the chemical nature of odorants and DNA base sequences affect the selectivity of odorant detection. These sensors are promising for electronic olfaction systems consisting of coupled sensor arrays and an odor recognition algorithm: required for "electronic-nose" applications in medicine and homeland security.

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Poster Session V

Visualization, Manipulation and Recording of Nanotube Olfactory Cilia

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Olfactory signal transduction is conducted at very fine cell compartment expressing nanotube structure (100 nm diameter). Up to this point, physiological experiments treating such fine structure are very limited, obviously because of technical limitations. Problems were mainly situated in (a) visualization of this thin structure without fixations, (b) manipulation of substances in the highlighted area and (c) simultaneous recoding from the living cilia. To overcome such difficulties, we employed a combined technique of the patch clamp and photolysis of caged compound under fine visualization of nano-scale structure with the laser-scanning confocal microscope. To understand the nature of cytoplasmic messengers and the transduction channels (CNG, Cl(Ca)) on the single cilium, cilia were loaded with both caged compounds (either cAMP or Ca) for photolysis and lucifer yellow for fluorescent visualization. When the local area (ca. 1 μm length) of cilium loaded with caged cAMP was illuminated, the cell showed an inward current response exceeding a hundred pA of current, presumably generated by the high density CNG & Cl(Ca) channels, expressing a high signal amplification to the local ciliary excitation. At the same time, linear summation of small currents was observed with local weak illuminations. With the mapping, it was confirmed that transduction channels are present along entire cilium. Also, responses induced by two different parts within the single cilium were independent, when monitored with adaptation. Based on these observations, we discuss about the real-time biochemical behavior of enzymes, second (& third) messengers and ion channels within the nanotube olfactory cilia in relation to the signal amplification, adaptation, masking and olfactory manipulation.

Poster Session V

A Cell-Based High-Throughput Screen for New Insect Repellents

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Preventing mosquito bites is a key component of strategies to control the spread of infectious diseases such as malaria, yellow fever, and dengue fever. We recently revealed that DEET (N, N-diethyl-meta-toluamide), which has been used as the most effective insect repellent for more than 50 years, masks host odors by inhibiting subsets of insect odorant receptors (ORs). We therefore developed a cell-based high-throughput screening assay to search for new compounds, structurally unrelated to DEET, that inhibit insect ORs. Using heterologous HEK293T cells stably expressing malaria mosquito (*Anopheles gambiae*) odorant receptors, GPROR2 and GPROR7, we observe odor-evoked Ca^{2+}-increase when the cognate ligand, 2-methyl phenol, was applied. From a high-throughput screen of 91,520 compounds, 161 compounds (0.17%) showed more than 80% inhibitory effect on the GPROR2+GPROR7-evoked Ca^{2+} response, compared to the response with no compounds. The effect of selected compounds was further examined in single cells transiently transfected with different ORs using real-time Ca^{2+} imaging. Among our best hits, we identified 5 compounds that inhibit diverse insect ORs with different ligand specificites. These candidate compounds show at least 100-fold greater potency compared to the effect of DEET in our assay, but do not show the off-target effects on mammalian ion channels recently observed for DEET. These results provide a proof of principle that high-throughput screening for insect OR antagonists can provide a starting point for the design of safer and more effective insect repellents.

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Poster Session V

On a Chip Demonstration of a Functional Role for Odorant Binding Protein in the Preservation of Olfactory Receptor Activity at High Odorant Concentration

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The molecular mechanisms underlying odorant detection have been investigated using the chip based Surface Plasmon Resonance technique by focusing on the dynamic interactions between transmembrane Olfactory Receptor OR1740, odorant ligands and soluble Odorant-Binding Protein (OBP-1F). Purified OBP1-F specifically and quantitatively bound OR1740 present in the lipid bilayer of nanosomes derived from transformed yeasts, in the absence of odorants. A double level of specificity was demonstrated : on the one hand, OBP-1F differentially bound ORs compared to unrelated G Protein Coupled Receptors, and on the other hand, OBP-1F was more efficient than other members of the lipocalin family at binding ORs. The receptor preferential odorant ligand (helional) released bound OBP-1F from the OR-OBP complex, while unrelated odorants failed to do so. OBP-1F modified the functional OR1740 dose–response to helional, from a bell-shaped to a saturation curve, thus preserving OR activity at high ligand concentration. This unravels an active role for OBPs in olfaction, in addition to passive transport or a scavenger role. This sensorchip technology was applied to assessing native OBP-1F in a biological sample. Rat olfactory mucus also displayed significant binding to OR1740 nanosomes, and the addition of helional yielded the dissociation of mucus OBP from the receptor. This new concept of SPR bioelectronic sensors provides tools to understand the molecular mechanisms of peripheral odorant detection, with the direct evaluation of competitive OR-OBP, OR-odorant and OR-OBP-

odorant interactions, without labeling. It can indeed be employed to investigate biologically relevant questions, such as in the field of olfaction/nutrition crosstalk, with samples from animals in various nutritional or physiological states.

Poster Session V

Odorant Receptor Signaling in Human Prostate Cancer Cells

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Olfactory receptors (ORs) are expressed not only in the sensory neurons of the olfactory epithelium, where they detect volatile substances, but also in various other tissues where their potential functions are largely unknown. Here, we report the physiological characterization of human OR51E2, also named prostate-specific G-protein coupled receptor (PSGR) due to its reported expression in prostate cells. We identified androstenone derivatives as ligands for the recombinant receptor. PSGR can also be activated with the odorant β-ionone. Activation of the endogenous receptor in prostate cells by the identified ligands evoked an intracellular Ca^{2+} increase by a mechanism different from that involved in OR signaling in olfactory neurons. Exposure to β-ionone resulted in the activation of members of the MAPK family, inhibition of cell proliferation and induction of apoptosis. Our data give support to the hypothesis, that some ectopically expressed ORs have additional functions.

Poster Session V

Identifying the Male Mouse-Derived Pheromone(S) that Mediate Estrous Induction

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We are interested in identifying the neural circuit in the female mouse that mediates the effects of pheromones on female reproduction. Mature male urinary pheromones both advance female puberty and induce accelerated cyclicity in mature, group-housed females. The identity, however, of the estrous inducing pheromone remains a matter of controversy. Without reproducible estrous induction by male pheromones, the activated chemosensory neurons and the central neuroendocrine mechanisms of these phenomena remain unknown. A Balb/cByJ bioassay for accelerated pubertal uterine growth (the Vandenbergh effect) was used to evaluate previously identified puberty-accelerating pheromones (including HMH, SBT, farnesene, MUPs, hexapeptide, and isobutyl- and isoamylamine), all of which failed in the Balb/cByJ Vandenbergh bioassay. In order to isolate the puberty-accelerating pheromone, the bioassay was used to detect the unknown pheromone from crude urine and track its bioactivity through sequential fractionation techniques. We have now isolated total bioactivity in a fraction characterized by low-molecular weight, nonvolatile, polar molecules; the nature of this fraction suggests a novel pheromone for Balb/cByJ estrous induction. XCMS analyses against castrated urine, fractionated in parallel, reveals several putative candidates for the pheromone. Additional fractionation techniques and subsequent MS analyses are underway in order to further purify and molecularly identify the Balb/cByJ puberty-accelerating pheromone. These studies are necessary steps towards characterizing the neural circuitry that underlies social regulation of reproductive fitness.

Poster Session V

The Isolation and Characterisation of Candidate Odorant and Pheromone Receptors from the Light Brown Apple Moth, *Epiphyas Postvittana*

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Most of the world's major crop pests are members of the Lepidoptera. Understanding how these insects are attracted to their target crops and con-specific mates may enable the development of new pest control strategies. An obvious starting point for such a strategy is the isolation and characterisation of the pest's odorant and pheromone receptors (ORs and PRs). However, to date, ORs and PRs have only been isolated from two lepidopterans, the tobacco budworm (*Heliothis virescens*) and the silkworm (*Bombyx mori*). The light brown apple moth, *Epiphyas postvittana* (*Epos*), is a major pest for horticultural industries. From an EST sequencing database comprising 5,739 sequences, three candidate OR genes were identified through similarity to known ORs. While one of these receptors (EposOR2) is orthologous to the non-canonical receptor Or83b, functional expression in Sf9 cells revealed that the other two receptors recognise plant volatiles, including methyl salicylate and citral (EposOR1 and EposOR3, respectively). Fifty-nine percent of the EST sequences do not contain an identifiable open reading frame, suggesting that these sequences may lie in the long 3′ UTR of the gene. In order to screen these ESTs for further OR and PR genes, a microarray-based approach was taken. Since ORs are antennal-specific and PRs are sex-specific in their expression, differential screening of the microarray with body and antennae RNA and male and female antennae RNA has been employed. Analysis of the data to date has identified a number of new male-biased genes, some of which may encode pheromone receptors.

Poster Session V

Molecular Basis for Pheromone Reception by Antennal Neurons of *Heliothis Virescens*

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The remarkable ability of male moths to detect female-released sex-pheromone with high sensitivity and selectivity is mediated by specific sensory neurons housed in long sensilla trichodea on the antenna. Females of the tobacco budworm *Heliothis virescens* use a multicomponent blend to attract males and in males electrophysiological studies have assigned identified pheromonal compounds to three different types of sensilla trichodea. This specific responsiveness implies that sensory neurons in the sensilla types express distinct receptors. We have identified candidate pheromone receptors of *Heliothis virescens,* which form a relatively conserved group of moth olfactory receptors. By *in situ* hybridisation the receptor types could be allocated to sensory neurons housed in long trichoid sensilla surrounded by cells expressing pheromone binding proteins (PBPs). Immunohistochemical approaches visualized the receptor protein in the dendritic processes of the antennal neurons. Functional analysis of heterologously expressed receptors stimulated with pheromonal compounds solubilized by means of DMSO revealed that distinct receptor types responded to several compounds. Substituting the organic solvent with pheromone binding proteins to solubilize the hydrophobic pheromone compounds revealed an increase in sensitivity and specificity; it was found that cells expressing HR13 responded in the presence of HvirPBP2 specifically to the main component of the sex

pheromone blend. These data provide further evidence that the combination of a distinct receptor type and binding protein forms the basis for the specific responsiveness of moth antennae to distinct pheromone components.

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Poster Session V

Identification of Sex Pheromone Receptors from Four Moth Species

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Male moths detect the conspecific female-emitted sex pheromone components and their blend ratios. The receptors for these components have so far been identified in only two moth species, *Bombyx mori* and *Heliothis virescens*, yet it remains unknown how male moths detect the blend ratios with these receptors. Here we report on the identification of the receptors for the main sex pheromone components in four moth species, *Plutella xylostella*, *Mythimna separata*, *Nokona pernix* and *Diaphania indica*. We cloned putative sex pheromone receptor genes, *PxOR1*, *MsOR1*, *NpOR1* and *DiOR1* from *P. xylostella*, *M. separata*, *N. pernix* and *D. indica*, respectively. Each gene was exclusively co-expressed with an Or83b orthologous gene in male olfactory receptor neurons (ORNs) that are surrounded by pheromone binding proteins (PBP). By oocyte voltage clamping, we tested the ligand specificity of PxOR1, MsOR1, NpOR1 or DiOR1 co-expressed with an Or83b family protein. In these experiments, dose-dependent responses could only be recorded for the main sex pheromone component of each corresponding moth species. We conclude that the cloned genes encode sex pheromone receptors that are narrowly tuned to their respective sex pheromone components. Furthermore by two-color *in situ* hybridization using probes against sex pheromone receptor and *Or83b* orthologous gene mRNAs, we found that the proportions of ORNs expressing each sex pheromone receptor are correlated with the ratios of the components they detect in the pheromone blend. This correlation suggests an optimal adaptation of population ratios of ORNs to the blend ratios of the conspecific sex pheromone in the antennae of male moths.

Poster Session V

Activation of Bombykol Receptor Neurons by Ectopically Expressed Olfactory Receptor Triggers Pheromone Searching Behavior in Male Silkmoths

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Insects utilize sex pheromones to identify and orient towards conspecific females. In the silkmoth, *Bombyx mori*, female moths emit two pheromone components, bombykol and bombykal. Bombykol, which is detected by the sex pheromone receptor BmOR1, is sufficient to elicit full sexual behavior in male moths. Here, using transgenic silkmoths ectopically expressing a pheromone receptor from another moth species in bombykol receptor neurons, we show that receptor selectivity determines the chemical response specificity for the initiation of pheromone searching behaviors. We first generated a transgenic moth line that expresses GAL4 under the control of a putative promoter sequence of BmOR1. Using the driver line, we expressed PxOR1 encoding a pheromone receptor of the diamondback moth, *Plutella xylostella*, specifically tuned to the major pheromone component (*Z*)-11-hexadecenal (Z11-16:Ald) in bombykol receptor neurons of male silkmoths. Bombykol receptor neurons expressing PxOR1 showed electrophysiological responses to Z11-16:Ald stimulation in a dose-dependent manner, but not to the other two pheromone components of *P. xylostella*, (*Z*)-11-hexadecenol and (*Z*)-11-hexadecenyl acetate. Male moths expressing PxOR1 exhibited a typical pheromone searching behavior in response to Z11-16:Ald and also to females of *P. xylostella*, indicating that PxOR1 expressed in bombykol receptor neurons conferred the ability to respond Z:11-16Ald at electrophysiological and behavioral levels. These results show that only the ligand specificity of the olfactory receptor in bombykol receptor neurons is responsible for the expression of pheromone searching behavior in silkmoth.

Poster Session V

Sulfated Steroids as Natural Ligands of Mouse Vomeronasal Neurons

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Among mice, pheromones and other social odor cues convey information about sex, social status, and identity; however, the molecular nature of these cues is largely unknown. To identify these cues, we screened chromatographic fractions of female mouse urine for their ability to cause reproducible firing rate increases in the pheromone-detecting vomeronasal sensory neurons (VSNs) using multielectrode array (MEA) recording. Active compounds were found to be remarkably homogenous in their basic properties, with most being of low molecular weight, moderate hydrophobicity, low volatility, and possessing a negative electric charge. Purification and structural analysis of active compounds revealed multiple sulfated steroids, of which two were identified as sulfated glucocorticoids, including corticosterone 21-sulfate. Sulfatase-treated urine extracts lost more than 80% of their activity, indicating that sulfated compounds are the predominant VSN ligands in female mouse urine. As measured by MEA recording, a collection of 31 synthetic sulfated steroids triggered responses 30-fold more frequently than did a similarly-sized stimulus set containing the majority of all previously-reported VSN ligands. Collectively, VSNs detected all major classes of sulfated steroids, but individual neurons were sensitive to small variations in chemical structure. VSNs from knockouts for the sensory transduction channel TRPC2 did not detect these compounds. Urine concentrations of the two sulfated glucocorticoids increased many-fold in stressed animals, indicating that information about physiological status is encoded by the urine concentration of particular sulfated steroids. These results provide an unprecedented characterization of the signals available for chemical communication among mice.

Poster Session V

High-Throughput Microarray Detection of Vomeronasal Receptor Gene Expression in Rodents

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We did a comprehensive data mining to explore the vomeronasal receptor (V1R & V2R) repertoires in mouse and rat using the mm5 and rn3 genome

respectively, followed by designing a high-density oligonucleotide array containing all of these receptors and other selected genes of interest. This array enables us to detect the expression of specific expression of vomeronasal receptors in vomeronasal organ (VNO). 172 mouse V1Rs and 98 V2Rs were detected to be highly enriched in VNO, while only 108 rat V1Rs and 87 V2Rs have elevated expression level in VNO. The array also enables us to monitor the temporal expression pattern which indicates a functional change over time course for these so-called pheromone receptors. Expression analysis of other non-receptor genes, half of which are homo-domain containing transcription factors, reveals possible regulatory functions of them during the development of VNO.

Poster Session V

Nitric Oxide in Sensory Neurons of the Murine Olfactory System

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The small gaseous signalling molecule nitric oxide (NO) is involved in various physiological processes including regulation of blood pressure, immunocytotoxicity and neurotransmission. In the peripheral olfactory system of rodents, NO seems to have a function in the embryonic development of the olfactory epithelium (OE) and its regeneration after injuries. However, an implication of NO in olfactory signal transduction has not been demonstrated yet. In the present study we show for the first time the expression of the endothelial isoform of NO synthase (eNOS) in mature olfactory sensory neurons (OSNs) of adult mice on mRNA and protein level. Furthermore, using NO-sensitive micro electrodes, we were able to demonstrate that NO is released from individual OSNs in a stimulus dependent manner. The release of NO is dependent on the concentration of the stimulus as well as the presence of extracellular calcium ions. It can be blocked by inhibitors of NO synthase and NO-release was not detectable in OSNs derived from eNOS deficient mice. Searching for a role of NO in the mature olfactory epithelium, we could not find a significant difference between wild-type and eNOS deficient mice in basal cell proliferation. In contrast, analyzing EOG recordings from these animals revealed a significant role for NO in modulation of temporal aspects of olfactory signal processing and adaptation of odorant-induced signals. The findings presented here provide evidence for the presence and function of eNOS in mammalian olfactory sensory neurons. NO as a diffusible messenger could act in an autocrinal way, influencing the OSN directly and/or in a paracrinal way, providing a fast mediator of interaction between cells of the OE. This work was funded by the International Graduate School of Neuroscience.

Poster Session V

The Impact of Pentoxifylline and Theophylline on the Electro-Olfactogram of the Mouse – A Pilot Study

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Background: Until now there is no convincing therapy for non-inflammatory smell loss that would provide a long-lasting effect. It has been hypothesized that pentoxifylline and theophylline can improve olfactory function in humans. It is unclear whether this possible effect is due to an impact on the peripheral olfactory receptor neuron (ORN) or the central nervous system. Aim of this pilot study was to investigate the effect of local administration of pentoxifylline and theophylline on the electro-olfactogram (EOG) of mice. Changes in the EOG due to drug administration would strengthen the idea that the drugs act at a peripheral level.

Material and methods: EOG was recorded in 22 fresh mice cadavers. An olfactometer was used to apply phenyl ethyl alcohol as an olfactory stimulus. In a blinded fashion either pentoxifylline 20 mg/ml, theophylline 20 mg/ml or NaCl 0.9 mg/ml were administered to the olfactory epithelium always followed by the administration of lidocaine. The EOG was obtained before and after drug application.

Results: An increase of the EOG amplitude was observed after administration of pentoxifylline and theophylline while it decreased after the application of NaCl. The application of lidocaine resulted in a decrease of the EOG amplitude.

Summary: The observed drug effect on the EOG supports the hypotheses that pentoxifylline and theophylline act at the level of the ORN.

Poster Session V

Correlation Between Human Masking and Odorant Suppression of Cell Responses and of Voltage-Dependent Currents

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Despite the wide use of odorants as malodor masking agents, little is known about the cellular events by which odors suppress malodors. The present study was undertaken to investigate the cellular mechanisms of olfactory masking, and to survey the possibility for its industrial applications. Based on the information that odor molecules attenuate malodor-induced transduction current in the olfactory receptor cells (ORCs), we first investigated the relationship between perceived malodor-masking and ORC responses. The qualitative ability of twenty odorants to suppress the smell of isovaleric acid was evaluated by sensory panelists. In parallel, the efficacies of three representative odorants in attenuating the inward current induced by isovaleric acid in isolated newt ORCs were examined by the whole-cell patch clamp method. From the comparison, it was confirmed that the odorants attenuated the malodor-induced current in the same relative order of malodor suppression, thereby indicating a possible relationship between cellular events and sensory perceptions. In addition, it has been shown that voltage-gated currents are also suppressed by odorant molecules, presumably representing the molecular homologies expressing odorant suppression at the molecular interactions. We examined the effects of twenty odorants on voltage-gated current in newt ORCs. The suppression of malodor showed a positive correlation with odorant suppression of the voltage-activated inward current. Our results suggest that the olfactory masking involves inhibition of ionic channel activity in ORCs. Furthermore, the present work provides a novel idea that the high-throughput screening of masking agents can be achieved by evaluating the effects of agents on particular types of voltage-gated channels.

Poster Session V

Mechanism of Olfactory Masking

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In the human history, the flavor and fragrance have been broadly employed not only for inducing the sense of scent, but also for masking the unpleasant smells. Such dual effects of odorants are explained by the fact that human olfaction receives two opposing effects of excitation and inhibition from odorant molecules. Especially, a unique property of wide-spectrum and low-selective odorant inhibition of the olfactory signal has been employed in the smell-masking industries, such as the usages of spices, the development of perfumes or aromatherapy treatments. This wide-spectrum olfactory inhibition has been shown to be at the sensory receptor cell level, but its molecular mechanism has remained open. We report that inhibitory effects of odorants to the membrane ionic channel are directly responsible for the olfactory masking. The cyclic nucleotide-gated (CNG) channel that is a key element that converts odorant stimuli into electrical signals is sensitive to odorant inhibitions, consistent with the expression of wide-spectrum olfactory inhibition. In addition, we show that the spectra for human olfactory masking have a positive correlation with those of the CNG channel blockage. The present work suggests that CNG channels switch on/off the olfactory signaling pathway, and that the on/off signals are both non-linearly amplified by the subsequent opening and closing of $Cl_{(Ca)}$ channels. Furthermore, the olfactory cilia where CNG channels are densely distributed are directly exposed to the body-external environments covered by the mucus layer. The olfaction could thus be gain-controlled with volatile chemicals from the outside of the body.

Poster Session V

Role of PACS-1 in the Ciliary Localization of the Olfactory Cyclic Nucleotide-Gated Channel

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Ciliopathies are an emerging class of human disorders that involve defects in ciliary protein trafficking or assembly. Our laboratory has shown that impaired ciliary protein transport in olfactory sensory neurons (OSNs) leads to anosmia in animal models and human patients. Surprisingly, while compartmentalization of signaling molecules in the cilium is required for normal olfactory function, very little is known regarding the mechanisms controlling protein delivery into olfactory cilia. Here, we show a role for phosphofurin acidic cluster sorting protein 1 (PACS-1) in the ciliary localization of the olfactory CNG channel. PACS-1 is an intracellular sorting protein that mediates its effects through the binding of acidic clusters on the cargo protein. This interaction is dependent on CK2 phosphorylation of both PACS-1 and its cargo. Amino acid sequence analysis reveals that CNGB1b, but not CNGA2 or CNGA4, contains multiple putative PACS-1 binding sites, while *in vitro* kinase reactions confirm that CNGB1b is a substrate for CK2. Additionally, we show that PACS-1 is expressed in OSNs and that the CNG channel and PACS-1 can interact *in vivo*. Using confocal microscopy and ciliated MDCK cells, we demonstrate that alterations in PACS-1 using site-directed mutagenesis or shRNA silencing results in deficits in CNG channel ciliary trafficking. Similarly, pharmacological inhibition of CK2 causes a loss of CNG channel from the cilia and accumulation at the basal body. Since mislocalization of the CNG channel from cilia leads to anosmia in mice, we hypothesize that alterations in PACS-1 function in the OSN will lead to mistargeting of the CNG channel and subsequent olfactory dysfunction. This hypothesis is currently being investigated in native olfactory epithelium.

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Poster Session V

Functional Characterization of the Multiple PDZ Scaffolding Protein MUPP1 in Olfactory Receptor Signal Transduction

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The unique ability of mammals to detect and discriminate between thousands of different odorant molecules is governed by the diverse array of olfactory receptors (ORs) found on the dendrites of olfactory sensory neurons (OSNs), in the nasal epithelium. Little is known to date about interaction partners of ORs and their role in the signal transduction process. Certain OR subtypes possess classical PDZ domain binding motifs in their C-terminal regions, established sites for protein-protein interactions. Interaction with PDZ domain containing proteins plays a central role in organizing diverse cell signalling assemblies. We found the Multi-PDZ Domain Protein 1, MUPP1, expressed in the cilia and dendritic knobs of olfactory neurons. The scaffolding protein MUPP1 is composed of 13 PDZ domains and represents a possible nucleator or regulator of the olfactory response by acting as first building block of a putative "olfactosome". We found that ORs and MUPP1 interact and characterized the interaction *in vitro* and in a recombinant expression system. The physiological function of this interaction in the olfactory signal transduction cascade, as well as the identification of the other binding partners of MUPP1, are currently elucidated.

Poster Session V

β-Arrestin2 Mediated Desensitization of Mammalian Odorant Receptors

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Odorant receptors comprise the biggest subfamily of G-protein-coupled receptors. While the endocytic mechanisms of other G-protein-coupled receptors have been characterized extensively, almost nothing is known about the intracellular trafficking of odorant receptors. We investigated the endocytic pathway of mammalian odorant receptors and found that these receptors bind β-arrestin2 with high affinity and are internalized via a clathrin-dependent mechanism. After prolonged odorant exposure receptors are not targeted to lysosomal degradation but accumulate in recycling endosomes. Moreover, β-arrestin2 is redistributed into the dendritic knobs of mouse olfactory receptor neurons after treatment with a complex odorant mixture. Prolonged odorant exposure resulted in accumulation of β-arrestin2 in intracellular vesicles. Adaptation of olfactory receptor neurons to odorants can be abolished by the inhibition of clathrin mediated endocytosis, showing the physiological relevance of the here described mechanism of odorant receptor desensitization. To get further insight in the mechanisms of adaptation and sensitization in the olfactory epithelium we investigate the odorant receptor trafficking and the interactions of odorant receptors with β-arrestin2 and other trafficking proteins in living cells.

Poster Session V

Identifying Regions Involved in Substrate Specificity in Olfactory Receptors by Using a Comparative Approach Across *Drosophila* Species

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The fruit fly, *Drosophila melanogaster* utilizes 62 olfactory receptors (ORs) to recognize and navigate through thousands of odorous molecules within its environment. This ability is possible because each OR can recognize a different, yet overlapping, spectrum of odorants. To our knowledge, no study to date has identified the sites within insect ORs required for odour recognition. Studies on mammalian ORs suggest the odorant binding pocket for certain odours is located within the transmembrane α-helices just below the extracellular surface. However, mammalian ORs are G protein-coupled receptors and there is increasing evidence that insect ORs are not GPCRs, but instead represent a novel class of chemosensory receptors. We are using a comparative approach among *Drosophila* species to identify sites involved in ligand binding in insect ORs. We have found that the orthologue of the *D. melanogaster* receptor DmelOR22a from *D. mauritiana*, shows a difference in affinity for heptanone with EC_{50} values differing by two orders of magnitude (EC_{50} DmelOr22a = $1.87x10^{-7}$; EC_{50} DmauOr22a = $5.41x10^{-10}$). To identify which of the 48 amino acid differences between the two receptors encode this substrate selectivity, we have constructed a range of chimeric site-specific mutant receptors. We have found that substrate selectivity for heptanone resides within the predicted 5-7 transmembrane domains. These results provide the first important clues to the location of sites involved in odorant binding in this novel class of chemoreceptor.

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Poster Session V

Odor-Driven Local Field Potential Oscillations are Temporally Dynamic, Spatially Localized and $GABA_A$ - Dependent in the Antennal Lobe of the Moth *Manduca Sexta*

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Odor-driven local field potential oscillations (LFPOs) are a common response feature in primary olfactory networks and are posited to mediate encoding by controlling spike timing. We have observed oscillatory responses in the antennal lobe (AL) of the moth *Manduca sexta* that are distinct from prior observations of this and other insects. To more carefully characterize these, we placed silicon electrode arrays into the moth AL and recorded LFPOs simultaneously from four different positions in response to a panel of alcohols and ketones. Stimulus durations ranged from 50 to 1000 ms and 20 repeats were collected for each odor and duration. Bicuculline (200 μM) was then bath applied, and the odor panel was repeated. To quantify the time-varying frequency response of the LFPO, short-time Fourier transform was used. Results were calculated for individual stimulations then averaged over all 20 repeats. Results indicate that odor drives consistent frequency modulated LFPOs that sweep from 80-100 Hz to 20-30 Hz. We observed two distinct epochs, one between ~50-110 ms and a second at ~120 ms, which lasted up to several hundred milliseconds in a stimulus-dependent manner. Time-frequency structure was odor-dependent, with longer chain odors systematically producing lower peak frequencies and slower frequency modulations. Importantly oscillatory responses were typically localized to one or two electrodes suggesting a within glomerulus process. LFPOs were disrupted by bicuculline suggesting a fast $GABA_A$ synaptic component. Given that these two oscillatory epochs relate to different phases of neural spiking responses, they likely reflect distinct synaptic interactions. Finally, focalized and modulating oscillatory responses are inconsistent with existing oscillatory-based encoding-decoding models.

Poster Session V

Postembryonic Development of the Glomerular Map in the Zebrafish

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Larval zebrafish begin to use their olfactory system soon after hatching for feeding, predator evasion and intra-species communication. Despite several advances in our understanding of the form and function of both the embryonic and mature zebrafish olfactory system, little is currently known about the postembryonic maturation of this system into a chemotopic sensory map. We have therefore employed a variety of histological techniques to track developing glomeruli from their first appearance in embryonic zebrafish throughout the first month of life. Our evidence suggests that some early protoglomeruli develop shortly before hatching and then proliferate and segregate to form approximately 100 bilaterally symmetric glomeruli within the first month of development. In addition, several glomeruli that arise during embryonic development along with these protoglomeruli appear to persist throughout postembryonic maturation of the olfactory system. We have also examined behavioral responsiveness of larval zebrafish to arrays of natural and synthetic odorants. Our results suggest that fish begin to respond to some odorants within several days after hatching, supporting the functionality of early glomeruli. The subsequent addition and segregation of new glomeruli also correlates well with the development of behavioral responsiveness to an increasing range of odorants. This work extends current knowledge of the structure and function of the olfactory system in zebrafish toward the goal of a definitive odotopic glomerular map.

Poster Session V

Genetic Dissection of Zebrafish Olfactory Circuitry Mediating Attractive Response to Amino Acids

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In teleost fishes, there are two major types of olfactory sensory neurons (OSNs) in the olfactory epithelium: microvillous and ciliated OSNs. It has been suggested that microvillous OSNs projecting axons to lateral glomeruli in the olfactory bulb (OB) mediate feeding behavior, whereas ciliated OSNs mainly targeting medial glomeruli mediate social behavior. However, the molecular, cellular, and neural-circuit mechanisms underlying such olfactory behaviors are not fully understood yet. In the present study, we introduced a Tol2 transposon-mediated gene trap method for genetic dissection of the zebrafish olfactory system. Three transgenic zebrafish lines (*Tg1*, *Tg2*, and *Tg3*) were established in which a transcriptional activator GAL4 is expressed in distinct subsets of OSNs. By crossing individual lines with the UAS-GFP reporter line, olfactory axons were fluorescently visualized which innervate some overlapping but mostly different glomeruli in the OB,

respectively. In *Tg3*, GAL4 was expressed predominantly in microvillous OSNs innervating the lateral chain of glomeruli that has been proposed to be the feeding-related and amino acid-responsive region of the OB. To genetically elucidate the functional role of these microvillous OSNs in the feeding behavior, the targeted expression of tetanus toxin light chain (TeTxLC) for neural transmission blockade was achieved in the GAL4-expressing OSNs of *Tg3* by crossing with the UAS-TeTxLC transgenic line. Silencing the microvillous OSNs by the GAL4-driven expression of TeTxLC in *Tg3* resulted in a dramatic loss of attractive response to amino acids. These findings clearly demonstrate the functional significance of a selective neural circuitry originating from the trapped OSNs in the amino acid-mediated feeding behavior of the zebrafish.

Poster Session V

Processing of Bile Salt Odor Information by Single Forebrain Neurons in the Channel Catfish

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A chemotopic map of biologically relevant odorants that includes amino acids, bile salts and nucleotides exists in the olfactory bulb (OB; Nikonov and Caprio 2001) and forebrain (FB; Nikonov et al., 2005) of the channel catfish, *Ictalurus punctatus*. The excitatory molecular receptive range (EMRR) of single OB neurons processing bile salt odorant information was previously described (Rolen and Caprio 2007). OB neurons were categorized into three groups, taurine-conjugated, non-conjugated and those more broadly-tuned (G), based on their EMRR to bile salts. Presently, we obtained EMRRs of bile salt responsive neurons from medial regions of the FB which receive synaptic input from the medial OB which processes socially relevant stimuli in fish. All recordings were performed *in vivo* using extracellular electrophysiological techniques. Excitatory thresholds of FB neurons to bile salts typically ranged between 0.01μM and 0.1μM. The EMRRs of these bile salt responsive neurons were highly similar to those previously reported for neurons within the OB using similar criteria for categorization. The data from the present study combined with those from Rolen and Caprio (2007) suggest that the neural representation of bile salt odorants is highly similar for both the OB and FB.

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Poster Session V

Heterogeneity of the Odor-Evoked Response within a Glomerulus of the Mouse Olfactory Bulb

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Anatomical studies using electron microscopy demonstrated a compartmental organization in glomeruli of the rodent olfactory bulb. Optical imaging using two-photon microscopy revealed that odor-evoked calcium signal from presynaptic axon terminals is temporally homogenous within a glomerulus (Wachowiak et al., 2004). We asked whether the dendritic tufts of principal neurons and juxtaglomerular neurons, that are postsynaptic to the olfactory receptor neurons, are also temporally homogeneous in regard to odor-evoked responses. We used two-photon microscopy combined with a multi-cell bolus loading technique and measured odor-evoked calcium signal from both cell soma of juxtaglomerular neurons and glomerular neuropils of anesthetized mice. Juxtaglomerular neurons showed three distinct types of response, an excitatory response to the onset of stimulus, an excitatory response to the offset of stimulus, and an inhibitory response to the onset of stimulus. We also found glomeruli with all three types of response when the glomerular signal was averaged over the whole glomerulus. This divergence in the glomerular response among glomeruli is inconsistent with the response measured from the presynaptic terminals which were always an excitatory response to the onset of stimulus. This result suggests a significant contribution of postsynaptic dendrites to the glomerular neuropil signals. When we examined subregions of glomeruli, we found glomeruli whose subregions showed more than one time course of response. These functionally heterogeneous subregions might correlate to the anatomical subcompartments.

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Poster Session V

Discrimination and Generalization in Natural Floral Blends

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Floral perfumes are highly variable combinations of many volatiles. These volatiles vary slightly even among flowers from the same species. In this context, pollinators must establish if a newly encountered flower is similar to a previously rewarded one or to a non-rewarded one, turning foraging decisions into fine tuned generalization-discrimination tasks. In the present study we performed behavioral experiments and calcium imaging in the antennal lobe of honey bees to study if learning modifies the perceptual boundaries used to classify a floral perfume within a rewarded or a non-rewarded category. We designed artificial blends that mimic the components and the concentration variability of two cultivars of snapdragon flowers. All designed blends share the same components but could be differentiated based on the relative concentration of the components, which was more similar within examples of the same cultivar than between them. Using the proboscis extension response (PER) paradigm, bees were conditioned using 5 different examples from one cultivar. When novel blends were presented, bees generalized the conditioned response between both cultivars. However, when bees were differentially trained such that examples of one cultivar were rewarded while the other cultivar wasn't, bees could extrapolate the discrimination to novel examples of both cultivars. Additionally, we used calcium imaging to study the representation of the components and floral blends in the antennal lobe. The space/temporal patterns of odor evoked activity in projection neurons correlate with the slight differences in the blends composition. Ongoing experiments are now aimed at comparing the neural representation of rewarded and non-rewarded cultivars in naïve and trained bees.

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Poster Session V

C-FOS Analysis Reveals Differences in Glomerular Response Profiles for Three Musk Odorants in the Rat Olfactory Bulb

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Musk odorants are one of the most important classes of fragrance ingredients used in perfumery. Commercially available musks fall into four

structurally different classes: nitro, polycyclic, macrocyclic and linear musks. Though all of them have a distinct smell, they nevertheless have a strong resemblance in their odor character known as musky. To understand the specificity and affinity of the olfactory receptor recognition for different musk compounds we analyzed glomerular response profiles for three musk odorants in adult Wistar rats: one nitro musk - Musk ketone (n = 6) and two macrocyclic musks - Cyclopentadecanone (n = 5) and Thibetolide (n = 5). Using the immunhistochemical c-fos method we analyzed odor-induced neuronal activity in the glomerular layer of the main olfactory bulb (MOB). The number and spatial position of Fos-positive glomeruli was determinate in each unit area of the bulb (statistic data analysis with the one-way analysis of variance). We found four groups of active glomeruli responding to distinct musk compounds: one group specific for Musk ketone and Thibetolide, one group specific for Musk ketone and Cyclopentadecanone and one group specific for Thibetolide and Cyclopentadecanone. Only four glomeruli responded to all three musk odorants; some glomeruli responded to only one musk odorant. Our data give strong evidence that musk odorants evoke overlapping but also significantly distinct regions of glomerular activity in the rat MOB. We, therefore, conclude that in the olfactory epithelium of the rat there are at least four olfactory receptor types which interact with chemical compounds of musk character and which can be called as ''musk receptors''.

Poster Session V

A General Theory of Olfactory Bulb Odor Representations: Regulated Self-Surround Decorrelation, Spike Synchronization, and Natural Scenes

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A great deal is known about the neurobiology and psychophysics of olfaction, but many of the established underlying phenomena lack a common theoretical footing by which they can be integrated into a single framework of odor representation and processing. I here present a general theory of olfactory bulb function and operations. The olfactory system's high sensitivity and broad dose-response functions are consequences of established pharmacological and physiological mechanisms and do not reflect special properties of odorant receptors. Multiple negative feedback circuits normalize odor-evoked activity and facilitate the concentration-independent recognition of odors. Decorrelation (contrast enhancement) among similar odorants arises from location-independent synaptic mechanisms within the glomerular layer and can be dynamically regulated by descending neuromodulatory projections; multiple predictions of this model recently have been confirmed by new electrophysiological and behavioral-pharmacological data. In contrast to the temporally unsophisticated spike trains of olfactory sensory neurons, the secondary olfactory representations mediated by mitral cells are sparser and suggest a dynamical, spike timing-sensitive precedence code generated by sniffing and cellular resonance properties and reflecting learned relationships among odor elements as opposed to their physical similarities. These principles underlie a theory of olfactory generalization that governs the perception of similarity among related odorants, including the plasticity of this perception and the observation that experimental omission of components of complex odors can have negligible effects on the results of olfactory perceptual tasks.

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Poster Session V

Respiration-Gated Formation of Gamma and Beta Neural Assemblies in the Mammalian Olfactory Bulb

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A growing body of data suggests that information coding can be achieved not only by varying neuronal firing rate but also by varying spike timing relative to network oscillations. In the olfactory bulb (OB) of freely breathing anesthetized rat, odorant stimulation induces a prominent oscillatory activity in local field potentials (LFP) in the beta (15-30 Hz) and gamma (40-80 Hz) ranges, both regimes alternating during a respiratory cycle. At the same time, mitral/tufted (M/T) cells display respiration-modulated spiking patterns. Using simultaneous recordings of M/T unitary activities and LFP activity, we analyzed for the first time the temporal relationships between M/T cell spiking activity and both OB beta and gamma oscillations. We observed that M/T cell population displays a spontaneous rhythm process which does not seem to be related to LFP oscillations occurrence even though gamma oscillations are associated with a decrease in spike frequency. Among M/T cell population, cells exhibit a respiratory pattern which pretunes instantaneous frequencies to a gamma or beta intrinsic regime. Consequently, M/T cell spikes undertake a phase-locking either with gamma or with beta LFP oscillations according to their frequency range. Our results suggest that slow respiratory dynamics pre-tune M/T cells to a preferential fast rhythm (beta or gamma) so that a spike-LFP coupling might occur when units and oscillation frequencies are in a compatible range. This double locking process might define two complementary beta- and gamma-neuronal assemblies along the time course of a respiratory cycle. Such neuronal assemblies may take part in distinct information treatment processes and fold olfactory inputs into shape, to be read by upstream structures.

Poster Session V

Correlation Between Olfactory Bulb Volume and Olfactory Function

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Involving a large number of subjects the present study aimed to investigate a possible correlation between the OB volume and specific olfactory functions. A total of 125 randomly selected subjects (58 men, 67 women), aged 19 to 79 years (mean age 37 years), participated in this study. None of them reported olfactory dysfunction. All participants received an otolaryngological investigation including a volumetric scan of the brain (MRI), and lateralized olfactory tests. All subjects underwent the mini mental state examination (MMSE) to screen for cognitive impairment. Volumetric measurements of the OBs were performed by two independent observers by manual segmentation of the coronal slices through the OBs using AMIRA 3D. Significant correlations between left OB volumes in relation to odor thresholds (left: $r_{113} = 0.19$, $p = 0.04$) as well as OB volumes in relation to odor identification (left: $r_{113} = 0.19$; right: $r_{113} = 0.25$; $p < 0.05$) were observed. In addition, OB volume decreased with age (left: $r_{113} = -0.37$; right: $r_{113} = -0.38$; $p < 0.001$). Using ''age'' as a control variable for partial correlations, correlational analyses between right OB volumes and odor identification test results were still significant (right: $r_{110} = 0.23$, $p = 0.014$). Furthermore, although men exhibited larger OB volumes than women on average, the decrease of OB volume with age was similar for men and women. In

agreement with previous research the present study confirmed the correlation between OB volume and specific olfactory functions. Furthermore, the correlation between OB volume and olfactory function is not mediated by the subjects' age. Finally, the presently data obtained in a relatively large group of subjects forms the basis for age-related normative values of OB volumes.

Poster Session V

The Firing Rate of Neurons in Piriform Cortex is Influenced by Association of Odor with Reward and Can be Altered by Learning

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The piriform cortex (PC) is the primary target of afferent input from the olfactory bulb and is believed to function in the synthesis of odor objects. Complex odors are detected as individual molecular features that activate a pattern of glomeruli in the olfactory bulb. Mitral cells transmit odor information to the PC where these signals are recombined to form the perception of complex odors, but this process is not well understood. The anterior PC receives more direct sensory input than the posterior PC, which receives more associative input. In addition to the olfactory bulb, the PC has extensive connections with higher order areas including the prefrontal, perirhinal and entorhinal cortices, and the amygdala. To detect changes in the firing pattern of neurons in the PC during odor detection, mice were implanted with electrode arrays and spiking patterns were recorded during olfactory tasks. The mice were exposed to a variety of odors, either in the absence of reward (no-reward paradigm), or in the context of a go-no go task where they were rewarded for licking to any odor different from a non-reinforced odor (water-rewarded task). Most units did not display a strong odor response unless the odor was presented within the context of the water-rewarded task; out of 98 units, 1 unit responded in the no-reward paradigm while 32 units responded in the water-rewarded task. Mice were then subjected to a go-no go task in which they learned to discriminate a mixture of two odors from one of its components. The firing rate of cells changed depending on odor valence (whether the odor predicted reward), and this response sometimes reversed when the valence of odors was reversed. We conclude that cells in the PC are highly plastic as odor meaning modulates their odor responsiveness.

Poster Session V

What Do We ''See'' of Odor Mixtures?

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Perceived odor qualities are widely different among the individuals. One of the reasons of this might come from different points to be directing attention to olfactory input. Everyday odor is usually constructed by complex odor chemicals. How do we ''see and recognize'' such complicated bottom-up olfactory information? The purpose of this study was to investigate the sensory characteristics for complex odor mixture. Specifically, we studied the strategy of how to ''see'' odor, that is whether odor mixtures would be ''globally'' perceived its odor quality or ''locally'' perceived the focused part of it. Three base odor stimuli to make a mixture of two or three combination were prepared. Each of three base odors was compounded with ten various odor chemicals and had a different odor quality from one another. Twenty-four female participants were required to evaluate intensity, preference and familiarity for seven odors and rate similarity by 0-10 scale between two from seven odors; base X, Y, Z, binary mixture XY, XZ, YZ and trinary mixture XYZ. As results, three clusters of participant groups were produced by similarity matrix and averages of odor preferences for seven odors were different among each other groups. Among every three cluster groups, each of binary mixtures was consistently located almost between two base odors of the mixture components in a two-dimensional space by MDS analysis. On the other hand, trinary mixture was near the one of binary mixtures. It suggested one of three odor bases in the mixture seemed to be ignored to ''see'' the trinary odor mixture (partly local-perception). Additionally these ignored odors were different in each participant group and related with their preferences.

Poster Session V

Mixture Interactions Among Coffee Aroma Compounds in Detection of PERI-Threshold Odors by Humans

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A previous study examined detection of peri-threshold mixtures of acetic (C_2) and butyric acid (C_4), C_2 and hexanoic acid (C_6), and C_2 and octanoic acid (C_8). Substantial interactions, i.e., departures from additivity, occurred for the C_2-C_4 mixture, but not for the other mixtures. Thus, structural similarity may play a role in mixture-interactions. However, it is possible that the C_2 and C_4 acids interact more strongly with other compounds in general. The current study included three flavor compounds very different in structure from the carboxylic acids: furfuryl mercaptan (FM), maple lactone (ML), and 3-methyl-3-sulfanylbutyl acetate (ASC). Subjects attempted to detect (2-out-of-5, forced-choice method) each flavor compound mixed with each of the four carboxylic acids (six peri-threshold concentrations of each binary mixture). An air-dilution olfactometer delivered stimuli. Stimuli were calibrated using gas chromatography-mass spectrometry. Predictions for response addition, i.e., statistical independence, were calculated based on detection of the unmixed compounds. These predictions, together with actual mixture-detection data, were submitted to a 2-way ANOVA for each combination of flavor compound and carboxylic acid: Mixture-concentration X data-type (additivity predictions vs. actual mixture detection). For FM and ASC, ANOVA revealed significant deviations from additivity for mixtures with C_2 and C_4, but not with mixtures of C_6 and C_8. There were no clear deviations from additivity for any mixtures of ML and fatty acids. These results suggest that, while molecular structure is important for mixture-interactions, carbon chain length is not the only factor involved.

Poster Session V

Perceptual Interactions in Mixtures of Odorants

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Several psychophysical studies have suggested that the odor of a mixture is not always the simple sum of the odor of the constituting odorants. Additionally, experimental studies of olfactory receptors (ORs) response to odorants using calcium imaging revealed that some odorants can act both as agonist or antagonist depending on the OR. Indeed, Sanz et al. (2005) described the odorant repertoire of a human olfactory receptor (OR1G1), identifying both agonists and antagonists. In the present study, we set out to examine whether such interactions, taking place at the early stage of a single olfactory receptor, could still be observed at the human behavioral level. We performed a psychophysical evaluation of two binary

mixtures including vanillin (OR1G1 antagonist) and 1-nonanol or 9-decen-1-ol (both OR1G1 agonists). For a binary mixture, 6 concentration levels of each component and their 36 possible combinations were evaluated by 18 trained panelists in 3 replicates. An air-dilution olfactometer allowed precise stimulus control and stimulus concentration in vapor phase were measured using gas chromatography. Psychophysical results on odor intensity revealed perceptual interactions in both binary mixtures with mixtures including a major perceptual proportion of vanillin being more likely to evidence odor suppression. Indeed, in both binary mixtures, when vanillin concentration increased, the odor intensity of the mixture fell below the intensity of vanillin alone (*i.e.* out of mixture). These findings support the idea that olfactory perceptual interactions could find their origin at the very early step of olfactory coding, namely agonist/antagonist interactions at the olfactory receptor level.

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Poster Session V

Antihypertention Effect of Odors on Awake Rats with a Particular Combination of Chemicals

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Odor stimulation has been utilized for ''aroma therapy'' since ancient times because it's been thought to have some effects on our body and spirit. Some odors indeed affect on some physical phenomenon like blood pressure, electroencephalogram or pupillary reflex as a result of activities in nervous, endocrine and circulatory systems. Blood pressure is one of the most important thing to be kept in a certain range to maintain our health. Therefore we have checked the efficiency of nine odors (aroma oils; Melissa, Clary Sage, Marjoram, Lavender, Black pepper, YlangYlang, Rose, Lemon and Grapefruit) on blood pressure with awake animal, which are known to be aroma oils used in aroma therapy. Then we found that a couple of aroma oils (Melissa, Clary Sage and Marjoram) reduced the blood pressure by 94% of control with mineral oil, although some aroma oils had no effect on it. Interestingly Grapefruit has no effect on blood pressure in this work while it was reported to have vasopressor effect on anesthetized rat. We also found that a certain proportion of components in an aroma oil, Melissa, (40% Citral and 2% Linalol) effectively reduced blood pressure of awake rat by 92% within 30-60 min, while the major content of aroma oils itself and the combination of two major components of aroma oils have no or little effect on the blood pressure.

Poster Session V

Perceptual and Semantic Learning Modify the Perception of Odor Blending Mixtures

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We investigated the influence of perceptual and semantic learning on the perception of odor blending mixtures, i.e. mixtures eliciting a different quality as compared to its components. In a first experiment, 26 subjects described the odor quality (free description and choice between attributes) of mixtures of different chemical complexity and then their components. In a second experiment, 29 subjects replicated experiment 1 but first evaluated the mixture components and then the mixtures themselves. Firstly, we compared the effect of task (free description vs. choice between attributes) and experimental procedure (Experiment 1 vs. Experiment 2) on the odor description. The results showed that both task and experimental procedure influence odor description depending on the chemical complexity of the mixtures. These findings suggested that the perception of odor blending mixtures is under both the influence of top-down (perceptual and semantic learning) and bottom-up (olfactory inputs) processes. Secondly, we demonstrated that verbal descriptions with or without semantic cues (choice between attributes or free description) can be used in parallel of typicality rating to evidence perceptual blending in odorant mixtures.

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Poster Session V

Scent Signals of Individual Genetic Identity used in Mate Choice

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Signals of individual genetic identity play a number of important roles in mate choice. Attention has focused on the highly polymorphic major histocompatibility complex (MHC) as a likely signal of genetic individuality in vertebrates because of MHC-linked discriminable scent differences in fish, rodents and humans. However, direct evidence is surprisingly limited, coming mainly from laboratory or hybrid mice that lack normal genetic variation and social experience. In wild house mice (*Mus musculus domesticus*), the major urinary protein (MUP) complex encodes specialised communication proteins that exhibit considerable variation between individuals and are much more strongly expressed in scent than MHC. In experiments that disentangle the intrinsic correlations between MHC, MUP and genetic background, we have examined whether MUP and/or MHC scents are used to recognise different individuals of the opposite sex, to avoid inbreeding with close kin, or to assess genetic heterozygosity of potential mates. In each case we find strong responses to MUP type but not to MHC. Mice avoid inbreeding using self-referent matching of MUP type but do not avoid those of the same MHC type. Recognition of individual scent owners depends on MUP but not MHC. Females also preferentially associate with MUP heterozygous males when genome-wide heterozygosity is controlled. Thus, variation in MUP genotype between individual wild mice provides a genetic identity signal in scent that underlies genetic heterozygosity assessment as well as individual and kin recognition. The lack of individual and strain variation in MUP phenotype among laboratory mice has important implications for studies that use such strains to assess mate choice or to address questions concerning the recognition of individuals, kin or sex through scent.

Poster Session V

Effects of Androstadienone and Menstrual Cycle Phase on Flirting Behavior in Random Couples

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Although the existence of human pheromones is widely accepted among layman, scant evidences for overt behavioral effects in humans exist in the

literature. Aim: The aim was to test possible effects of androstadienone on non-verbal flirtatious behavior in a controlled social-interaction situation. Method: Sixty-five male and 65 female, heterosexual individuals, aged 19-34 (mean 23 years), were randomly assigned to the experimental group (exposed to androstadienone) or the control condition. None of the women were taking hormonal contraceptives, and menstrual cycle data were collected from all. Using a double-blind experimental design, male-female pairs were instructed by a female experimenter to perform two collaborative tasks, and each pair was subsequently left alone in a room and videotaped during the task execution. The videotapes were analyzed for signs of non-verbal flirtatious behavior by two independent raters. Measures of behavior included the calculated frequencies of specific behaviors (e.g., head tilt, object caress) and several subjective ratings (e.g., level of eye contact) made independently by the raters, using visual analog scales. Results: Analyses will focus on differences in flirtatious behavior between the experimental group and the control group, as a function of gender and of menstrual cycle phase.

Poster Session V

Are there Gender Differences and Genre Dependencies in the Human Axillary Secretions Produced in Response to Visual Stimuli?

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The overall objective of this study was to test the hypotheses that the human axillary response produced when watching videos depends on genre and that this response is gender specific. This test was accomplished by evaluating the differences in the axillary secretions produced when humans watched pornographic, romantic, action or documentary videos. For this evaluation, a golden-retriever (*Canis familiaris*) was trained to recognize a target gauze pad worn while human subjects viewed the pornographic video. After sampling three test boxes, the dog exhibited a sit/stay response in front of the box containing the target. The dog first learned to correctly identify the target from unscented pads. Then, distracters, the gauze pads worn while the same subject watched the other genres of videos, were introduced as possible choices. An error analysis was used to judge the commonality between the various distracters and the target smell. In other words, the more often the dog confused a particular distracter for the target, the more similar that distracter was assumed to be to the target. For male subjects, the smell produced while watching the action video was more often confused with the pornographic target than was the smell produced from the other distracters ($p < 0.05$). However, for females, the smell produced while watching the romantic video was more often confused with the target ($p < 0.05$). These observations are consistent with the hypotheses that the axillary response is dependent on video genre and that the response produced when viewing pornographic videos is gender specific.

Poster Session V

Disruption of Olfactory Environment Impacts Close Relationships in Young Women

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Previous research suggests that a subtle olfactory component influences the relationships of young women. To explore the mechanism of this effect, 48 pairs of female undergraduate close friends participated in two interactions. In the first session, participants engaged in their regular fragrance routine. In the second, one dyad member applied an unfamiliar fragrance. Participants rated their perceptions of the interaction and their relationship quality, and analyses examined whether exposure to the unfamiliar fragrance during the second session affected these variables. A repeated measures ANOVA revealed that perceived enjoyment decreased from session one to session two, $F(1, 88) = 8.41$, $p = .005$. However, when only fragrance users were included in the analyses, there was also a session x fragrance condition interaction, $F(1, 46) = 4.01$, $p = .05$. Only participants in the unfamiliar fragrance condition reported lower levels of enjoyment in the second session ($M = 2.30$, $SD = 0.70$) compared to the first ($M = 2.70$, $SD = 0.47$), $t(22) = 3.22$, $p = .004$. Looking at perceptions of the friendship, initially, there was only a trend for ratings of closeness to decrease during the second session, $F(1, 88) = 3.33$, $p = .071$. However, when only fragrance users were included in the analyses, a significant effect emerged, $F(1, 46) = 7.06$, $p = .011$. Regardless of fragrance condition, participants reported lower levels of closeness following the second session ($M = 2.92$, $SD = 1.04$) than the first ($M = 3.03$, $SD = 1.02$). Such findings suggest that exposure to the unfamiliar fragrance during the second session dynamically and rapidly affects close relationships especially for those who wear fragrance regularly.

Poster Session V

Are Tears a Human Chemosignal?

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Emotional tearing is a uniquely human behavior who's evolutionary origin and present-day function remain unclear. We hypothesized that emotional tears may act as a chemosignal. To address this, emotional tears were obtained from two female "donors". To generate tears, donors viewed sad films in isolation, and allowed tears to collect in a vial. In a within-subjects repeated-study design, 14 healthy male subjects between 19-33 years of age participated in an experiment where they smelled (10 sniffs) either fresh tears or saline, counter-balanced for order, and then watched two ~5 minute movies, one emotionally neutral and one sad. Participants did not know the aim of the study, or that it contained tears. During this time we measured mood, psychophysiology, and endocrine state. Mood was measured by repeatedly administering the 17-qestion Ekman questionnaire. Psychophysiological measures were skin conductance, electrocardiogram, ear pulse, finger pulse, abdominal respiration, thoracic respiration, skin temperature, and body movement. Finally, endocrine state was assessed by measuring salivary levels of testosterone and cortisol. Preliminary analysis of this pilot data indicated a trend towards increased sadness after sniffing tears as compared to saline during the emotionally neutral film ($t(13) = 2.139$, $p = 0.052$). Several additional trends were evident in the mood and psychophysiological data. Because the hormone assays should ideally be conducted at the same time for all samples, this data will only be available after the intended sample of 40 participants is completed. That said, the trends evident in 14 participants to date indicate that tears may act as a human chemosignal.

Poster Session V

Eliciting and Blocking Odor-Arousal Associations

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In a series of studies, we demonstrate that a neutral odor paired with an arousing or stressful event could later trigger changes associated with the original stressor and that these effects can effectively be blocked. In study 1, participants experienced a significant increase in Heart Rate (HR) upon re-exposure to an odor paired with the Trier Social Stress Test (TSST) but not to an odor paired with relaxation instructions. Study 2 confirmed that

this effect was due to the odor-stress association by demonstrating elevations in HR to an initially neutral odor for a group that was exposed to that odor during the TSST, but not for a group that performed the TSST exposed to clean air. In Study 3, participants performing the TSST were either given a distraction task before (blocking) or after (masking) in the presence of an odor to determine whether either was effective at diminishing the arousal response upon re-exposure to the odor. Re-exposure to the odor elicited elevations in HR for the masking group, but not the blocking group. Study 4 revisited just the blocking paradigm and confirmed the efficacy of this paradigm in blocking a stress-odor association. The findings of these studies demonstrate the potency of odor-arousal conditioning while suggesting strategies for alleviating or preventing such associations.

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Poster Session V

The Necessity of Olfactory Input for Different Phases of Socially Transmitted Food Preference

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The social transmission of food preference (STFP) is an odor learning paradigm–in order to learn, the animal has to make an odor-to-odor association between the conspecific breath with the demonstrated food. The role of this olfactory input once this association is already made is unknown. In order to investigate the dynamics of the role of the olfactory input in the STFP paradigm, we temporarily lesioned the olfactory receptor cilia in female rats. When this nasal epithelial ablation is performed during acquisition, the normally conditioned preference for a food smelled on a conspecific's breath is eliminated. Impairments of learned preference persist after a week of the nasal ablation, confirming that the olfactory input is necessary for acquisition. In addition, learning is disrupted even when epithelial ablation is performed after the acquisition of the preference. Our data suggest that olfactory input is necessary to identify food in the STFP paradigm even after acquisition.

Poster Session V

Chemosignal of Fear Modulates Fear Recognition in Ambiguous Facial Expressions

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Integrating emotional cues from different senses is critical for adaptive behavior. Much of the evidence on crossmodal perception of emotions has come from studies of vision and audition. An emotion from one sense modulates how the same emotion is perceived in another sense, especially when the input to the latter sense is ambiguous. Here we address whether olfaction too causes similar sensory modulation of emotional perception in an emotion-specific way. We do so by examining the impact of a unique type of chemosignal, emotional sweat produced while subjects experienced fear, on fear recognition in facial expressions. We vary the effectiveness of the visual input by morphing between prototypical happy and fearful faces of each actor. We show that the chemosignal of fearful sweat biases women toward interpreting ambiguous expressions as more fearful, but has no effect when the facial emotions are more discernable. Our findings provide direct behavioral evidence that social chemosignals communicate emotions and demonstrate that social chemosignals modulate vision in an emotion-specific way – an effect of olfaction in humans that has been hitherto unsuspected.

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