

ABSTRACTS

List of Abstracts from the Twenty-ninth Annual Meeting of the Association for Chemoreception Sciences

Givaudan Lecture: Dr Gene Robinson

Overachievers: What Honey Bees Teach Us about Genes, Brains, and Behavior

Gene Robinson

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Honey bees have a brain the size of a grass seed, yet live together in societies that rival our own in complexity and internal cohesion. How do they stick together and work together so well? By drawing on studies from behavioral biology, neuroscience, molecular biology and genomics, this lecture will explore the secrets of their success. Topics include insect colonies as examples of self-organizing complex systems; deep conservation of neural mechanisms that couple experience with changes in brain structure; understanding the evolution of social behavior using the “evo-devo” paradigm, and a new perspective on the nature–nurture problem.

IFF Lecture

Molecular Mechanisms Underlying Taste Perception and Gastrointestinal Chemosensation

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During the past 15 years the members of my laboratory and our collaborators have identified and characterized several taste signaling elements. α -Gustducin, discovered in 1992 by Susan McLaughlin, was the first taste signaling molecule cloned from taste cells. From knockout mice, generated in 1996 by Gwen Wong, we determined that α -gustducin is a key component of signaling pathways underlying taste responses to bitter, sweet and umami compounds. Liquan Huang, Gopi Shanker, Cristian Perez, Manjiri Bakre and Raju Benard identified and characterized the roles in taste signaling for the G protein subunit G γ 13, the ion channel Trpm5, and the effector enzyme PDE1A. Peihua Jiang, Emeline Maillet and Yi Xia, in collaboration with Marianna Max and Rami Osman, have identified and characterized the multiple ligand interaction sites of the T1R2 + T1R3 sweet taste receptor. From this work we have come to a detailed molecular understanding of the nature and function of the signaling molecules and pathways underlying taste perception. Most recently, Zaza Kokrashvili and Bedrich Mosinger, in collaboration with Josephine Egan and Soraya Shirazi-Beechey, and the members of their labs have found that many of these same taste signaling elements are expressed also in glucagon-like peptide-1 (GLP-1)-expressing enteroendocrine L cells of the gut. Based on

in vivo and in vitro studies we have determined that enteroendocrine L cells of the gut “taste” glucose through the same mechanisms used by taste cells of the tongue.

Supported by NIH grants DC03055, DC03155 and DC08301.

Taste: Periphery and CNS

Characterizing the pH-Dependent Taste-Modifying Mechanism of Neoculin

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Neoculin (NCL) is a sweet protein which also has taste-modifying activity to enhance its sweetness at acidic pH. To clarify the mechanism involved, we established an assay system for the taste-modifying activity by calcium imaging analysis in vitro. The human sweet taste receptor, hT1R2-hT1R3, was functionally expressed in HEK293T cells together with optimized chimeric G α . Evaluating the cell responses to NCL under different pH conditions, we found that hT1R2-hT1R3 was activated in a pH-dependent manner as the condition was changed from pH 8 to pH 5. We then tried the His \rightarrow Ala mutation of NCL and produced the mutant using *Aspergillus oryzae*-aided expression system we devised. The cell-based assay revealed that the mutant elicited sweetness in a pH-independent manner, suggesting that the His residues in NCL play an important role in its taste-modifying activity. Also, the mutant-induced receptor activation was inhibited by NCL at neutrality. The result indicates that NCL is received by hT1R2-hT1R3 even at neutral pH and acts as antagonist. NCL can make a functional change from antagonist to agonist in the weakly acidic–neutral pH range.

This study was supported by Grant-in-Aid 16108004 from the Ministry of Education, Culture, Sports, Science and Technology in Japan.

Taste: Periphery and CNS

Claudin Expression in Taste Tissue

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Taste receptor cells are elongated and polarized cells grouped in taste buds in taste papillae. At the apical side of the taste receptor cells tight junctions maintain intercellular permeability barriers of the pore and separate apical from basolateral sides. Tight junctions are semipermeable barriers allowing specific paracellular transport through the epithelium. Claudins are a protein family conferring

specific permeability properties to tight junctions. Some Claudins reinforce the barrier and others create selective pores. There is strong evidence that paracellular transport in taste buds plays a role in taste perception, especially for salty taste. In this study, we have identified Claudins expressed in mouse taste papillae and in human fungiform papillae. By PCR we showed that 12 Claudins are expressed in mouse taste papillae-enriched tissues and 5 of those are also expressed in human fungiform papillae. By immunohistochemistry we localized Claudins 4, 6, 7 and 8 specifically in mouse taste cells. Claudin 8 is located at the borders of the taste pore, Claudin 6 inside the pore, Claudin 7 throughout the taste cells and Claudin 4 mainly at the borders of the pore but also at the basolateral membrane of the taste cells and in non-taste epithelium. According to known functions of Claudins in other tissue, we speculate that Claudin 4 and 8 might contribute to maintain a favorable NaCl gradient across the epithelium, whereas Claudin 7 would give access to Na⁺ through tight junctions to putative basolateral Na⁺ receptors. Claudin 6 may be involved in cell-cell adhesion and in pore organization.

Taste: Periphery and CNS

Type III Taste Bud Cells Express GABA-Synthetic Enzyme, GAD67

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Objectives: *g*-Aminobutyrate (GABA) is an inhibitory neurotransmitter in CNS. GABA is synthesized from L-glutamate, umami component, by the enzyme, glutamate decarboxylase (GAD, EC 4.1.1.15). Two genes are responsible for the production of GAD isoforms, GAD65 and GAD67. Recently, we have found that GAD67 is expressed in the circumvallate papillae by immunohistochemical study. In this study, we aim to further define the distribution and morphology of the cells expressing GAD67 in the taste bud. **Methods:** GAD67/GFP knock-in mouse was used. Immunohistochemical and RT-PCR analyses were employed. Antibodies and DNA primers were locally obtained. **Results:** GFP-positive cells in the circumvallate papillae were positive for antibodies against PGP9.5 and serotonin, known type III markers, but negative for gustducin, a known type II marker. RT-PCR analysis revealed that both GAD isoforms are expressed in the circumvallate papilla. We have also confirmed the expressions of GABAA and GABAB receptor subunits in the circumvallate papilla. **Conclusions:** Our present results suggest that GABA synthetic system exists in the type III taste bud cells where GABA may be functionally active as a part of chloride channel. Since type III taste bud cells are connected to the neuronal system, GAD and/or GABA may participate in the taste signal transduction.

Taste: Periphery and CNS

Molecular Markers in the Developing Solitary Tract and Nucleus

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The solitary tract (ST) and nucleus (NST) are established embryonically in rat, yet initial development of the ST and NST is not understood. To characterize embryonic (E) development, brainstems from E14, 16, 18, and 20 rats were immunoreacted with spectrin and calbindin to identify the ST and NST, and compared to expression patterns of established guidance and proliferation agents: nestin, glial fibrillary acidic protein, and sonic hedgehog (Shh), markers for radial glia, astrocytes, and a morphogen. At E14, ST axons enter the brainstem and course caudally among dense collections of radial glia that extend from medial to lateral. Astrocytes are not seen, and calbindin expression is sparse. By E16, ST axons travel more medially, just lateral to a group of neurons, presumptive NST. ST axons are embedded in a collection of radial glia, and astrocyte extensions parallel their trajectory. At E18, ST fibers are dense and bracketed by radial glia. Astrocyte extensions overlap with the ST. By E20, astrocyte extensions in the ST region are extensive. A large cluster of neurons, the NST, flanks the ST. Shh immunoprotein is intense in cells surrounding the ST from E14, and at E16–20 is especially intense in neurons located at the rostral, medial region of the NST. Thus, a structural and temporal association of both earliest appearing astrocytes and Shh with the trajectory of the ST is apparent. These interactions potentially influence the growth of ST fibers to their NST targets.

Supported by NIDCD NIH grants DC00456 (C.M.M.) and T32DC00011 (O.L.M.).

Taste: Periphery and CNS

Firing Patterns during Spontaneous Activity Predict Taste-Evoked Responses of Central Gustatory Neurons

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Gustatory neurons can be described based on several criteria, including their patterns of firing, although this approach is relatively unexplored. We therefore investigated whether the spontaneous firing patterns of central gustatory neurons are differentiable and whether they relate to responses to taste solutions. Recordings were made of neural activity, including responses to prototypical stimuli, in the nucleus of the solitary tract (NST) in rats and in C57BL/6ByJ (B6) and 129P3/J (129) mice. A preliminary analysis was also conducted for rat parabrachial nucleus (PBN) cells. Patterns of firing activity were determined by plotting distributions of interspike intervals (ISIs) during spontaneous activity. Some NST cells had a clear peak in the distribution of ISIs at less than 10 ms. In rats and B6 mice, there was a significant correlation between the percentage of ISIs less than 10 ms and the response amplitude to NaCl in the NST. In 129 mice, the percentage of short ISIs was more closely related to the amplitude of sucrose than NaCl responses. In PBN cells, distributions did not show the sharp peaks observed for the NST, and the percentage of short intervals was more closely related to the amplitude of NaCl responses than sucrose, citric acid, or quinine responses. The results indicate that short ISIs during spontaneous activity predict evoked responses to NaCl in rat and B6 mouse NST cells. Furthermore, gustatory cells can be categorized based on spontaneous firing patterns without applying taste stimuli.

Supported by NIH grants R03 DC005929 and R15 DC05326.

Taste: Periphery and CNS

Differential Spatial Representation of Taste Modalities in the Rat Gustatory Cortex

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Discrimination between foods is crucial for the nutrition and survival of animals. Remarkable progress has been made through molecular and genetic manipulations in the understanding of the coding of taste at the receptor level. However, much less is known about the cortical processing of taste sensation and the organizing principles of the gustatory cortex (GC). Using genetic tracing, it has recently 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 found that 4 of the primary taste modalities (sweet, bitter, salty and sour) are represented by distinctive spatial patterns, but that no region was specific to a single modality. In addition, we found that 2 tastants of similar hedonic value (pleasant or unpleasant) activated areas with more common regions than 2 tastants with opposite hedonic value. In summary, we propose that these specific cortical patterns can be used to discriminate among various tastants.

Taste: Periphery and CNS

Taste Receptor Polymorphisms in the Old Order Amish: Associations with Obesity, Diabetes and Related Traits

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Genetic variation in taste receptors (TRs) strongly affects nutrient intake and food preference. The expression of TRs in the gastrointestinal tract suggests that they may be involved in the nutrient-dependent regulation of metabolism, as well. We hypothesized that genetic variation in TRs may impact the development of obesity-related disease. To investigate this possibility, we genotyped >60 haplotype-tagging single nucleotide polymorphisms (SNPs) in taste-related genes, including all *TAS1R* and *TAS2R* genes, in over 1300 DNA samples from the Amish Family Diabetes Study. We identified candidate SNPs from the dbSNP and HapMap databases, and prioritized those with a minor allele frequency of ≥ 0.2 in the CEU cohort (HapMap). We then evaluated the effects of genotype on 39 obesity-related traits (with adjustments for age, sex, BMI and family structure). Nutrient intake was assessed in a subset of individuals through a food frequency survey. Several SNPs were significantly associated with obesity-related traits, including diabetes, glucose and insulin levels during oral glucose tolerance tests, weight, and eating behaviors. We conclude that common variation in taste receptor genes may influence food preference, metabolism and/or risk factors associated with obesity.

Support: NIDCD, NIDDK, NHLBI, University of Maryland SOM.

Taste: Periphery and CNS

Specific Alleles of Bitter Receptor Genes Influence Human Sensitivity to the Bitterness of Aloin and Saccharin

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Senomyx, Inc.

People display a wide range of taste sensitivities to bitter compounds. Bitter taste in humans is believed to be mediated by a family of 25 G protein-coupled receptors (hT2Rs). Despite recent progress in functional expression of hT2Rs *in vitro*, up until now *hT2R38*, a receptor for phenylthiocarbamide (PTC) was the only gene directly linked to variations in human bitter taste. Here we report that polymorphism in 2 hT2R genes results in different receptor activities and different taste sensitivities to 3 bitter molecules. Using an HEK293 cell-based assay we determined that different allele variants of these receptors vary significantly in their activation by natural plant compounds aloin and aristolochic acid. Importantly, taste tests revealed that the more responsive allele of one of these genes makes people very sensitive to the bitterness of these compounds. People who do not possess this allele do not taste these compounds at low concentrations. The same gene allele makes people more sensitive to the bitterness of an artificial sweetener, saccharin. In addition, the second gene's allele also makes people more sensitive to the bitterness of saccharin. Our findings thus reveal new examples of variations in human bitter taste and provide a molecular basis for them.

Connecting Genetics and Perceptual Variations

Genetic Variation in a Human Odorant Receptor Alters Perception of Sex Steroid-Derived Odours

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Human olfactory perception differs enormously between individuals, with large reported perceptual variations in the intensity and pleasantness of a given odor. Androst-4-en-3-one, an odorous steroid derived from testosterone, is perceived by different individuals as offensive ("sweaty, urinous"), pleasant ("sweet, floral"), or odorless. Up to 30% of humans have reduced sensitivity to androst-4-en-3-one, with 6% fitting the criteria of specific anosmia. It has been hypothesized that genetic variation in odorant receptors may account for interindividual variation in odor perception. Here we show that a human odorant receptor, OR7D4, is selectively activated *in vitro* by androst-4-en-3-one and the related odorous steroid androstadienone (androst-4-en-3-one) and does not respond to a panel of 64 other odors and 2 solvents. A common variant of this receptor (*OR7D4 WM*) contains 2 non-synonymous single nucleotide polymorphisms, resulting in 2 amino acid substitutions (R88W, T133M) and severely impaired function *in vitro*.

Subjects with *RT/WM* or *WM/WM* genotypes are less sensitive to androsthenone and androstadienone and find both odors less unpleasant than subjects with the functional *RT/RT* genotype. Our results demonstrate the first link between perception and the function of an odorant receptor *in vitro*, establishing the basis for the unraveling of olfactory coding in humans.

Connecting Genetics and Perceptual Variations

Evolution of Opsins and Their Inter-individual Variability in Humans

Jay Neitz

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In comparison to most other proteins and to other opsins, the human long-wavelength (L) and middle-wavelength (M) opsins exhibit an extraordinary degree of amino acid sequence variability. For example, the sequence variability in human rhodopsin is quite low, and the variability that has been observed has, with rare exception, been associated with disease, including progressive retinal degeneration, and stationary night blindness. Likewise, variation in the human short-wavelength sensitive (S) opsin sequence is quite rare, and all variation observed has been associated with tritan color vision deficiency. Mutations in rhodopsin and the S cone opsin arise from extremely rare random mutational events. In contrast, the human M and L opsin genes are prone to unequal homologous recombination, as evidenced by the high incidence of color vision defects in modern populations and the high frequency of female carriers. Over the course of human evolution, sequential rounds of unequal homologous recombination between normal and color defective opsin gene arrays have produced the variability in the present day L and M opsins. Evidence has accumulated indicating that the hypervariability in L and M opsins is the result of a unique combination of an unstable gene arrangement prone to an extraordinarily high mutation rate paired with a recent relaxation of selection pressure and that it is producing dire consequences for human vision.

Connecting Genetics and Perceptual Variations

Dissection of Human Sweet Taste Variation

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¹National Institutes of Health and ²Givaudan Flavors

Humans display significant variation in the TAS1R genes encoding the known sweet receptors. Evolutionary genetic analysis indicates this variation has been maintained by natural selection, suggesting it results in functional effects on these receptors. We have undertaken a combined genetic and biochemical approach to understand the effects of variation on receptor function *in vitro* and on taste perception *in vivo* in human worldwide populations. Nineteen different naturally occurring human TAS1R2 gene haplotypes were cloned into expression vectors for transfection and assay of receptor function by calcium-release assay. Psychophysical tests of human subjects for sweet taste perception were performed using an array of natural and artificial sweet substances. Testing employed repeated rank-order tests and calculation of *R*-indices, providing information on both threshold and supra-threshold responses.

Test–retest reliability of this measure was high, and it revealed that sweet taste sensitivity shows a broad unimodal distribution in the human population, suggesting substantial phenotypic variation well suited for statistical analyses. Genotyping of coding sequence variation in 48 SNPs in subjects' TAS1R genes was performed using SNPlex assays. Substantial linkage disequilibrium between SNPs exists, making it possible to obtain full genotype information by interrogation of a limited number of haplotypes.

Supported by NIH/NIDCD intramural grant Z01-000046-07 and by Givaudan Flavors Corporation.

Connecting Genetics and Perceptual Variations

Possible Genetic Basis for Human Hyperosmia to Isovaleric Acid

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Humans are highly variable in their olfactory sensitivities, with considerable evidence for a genetic contribution. Olfactory receptor (OR) segregating pseudogenes, loci that display both a functional and a non-functional allele, are excellent candidates to underlie these differences in olfactory sensitivity in the human population. To explore this hypothesis, we examined the association between olfactory detection threshold phenotypes of 4 odorants and segregating pseudogene genotypes of 43 ORs scattered throughout the human genome. A strong genetic association signal was observed between the genetic variation in the segregating pseudogene OR11H7P and sensitivity to one odorant—isovaleric acid. This association was largely derived from the significant paucity of the homozygously disrupted genotype in individuals with specific hyperosmia to this odorant. Notably, we also demonstrated a “general olfactory factor,” manifested in inter-odorant threshold concordance. Such correlation is manifested in an over-representation of individuals who are concomitantly hyperosmic to several odorants. One possible molecular explanation is genetic variation in transduction genes downstream to the receptors. Our results constitute a step forward towards deciphering the genetic basis of human olfactory variability.

Poster Session Thur AM

Activity-Dependent Expression Profiling in the Mouse Vomeronasal Organ: A Microarray Approach

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In many mammals, chemosensation represents the dominant sensory modality and conspecific chemical communication strategies control complex social and sexual behaviors. Species- and gender-specific information about individuality, social and reproductive status is conveyed by an elusive class of chemical cues—pheromones—that are detected by sensory neurons of both the main olfactory epithelium (MOE) and the vomeronasal organ (VNO). Despite the fundamental significance of social chemosignaling, the principle mechanisms of pheromone detection and processing remain mysterious. Here, we approach the problem of vomeronasal signaling from a systems biology perspective. Using the Affymetrix

GeneChip® microarray platform, we seek to determine activity-dependent mRNA expression profiles in sexually naïve male C57BL/6 mice. Animals are singly housed either in an essentially odor-free environment (control) or during repeated exposure to a variety of social stimuli (experiment). Differential analysis of vomeronasal mRNA expression patterns from both groups then aims to correlate conspecific social communication to distinct vomeronasal transcription profiles and, thus, to shed light on novel candidate signaling proteins. The resulting data should provide a solid basis for future experiments on the molecular and cellular mechanisms that govern mammalian social behavior.

Supported by the Deutsche Forschungsgemeinschaft (SP724/2-1) and by funds of the state NRW (BioChip-Initiative).

Poster Session Thur AM

Striking Differences in Evolutionary Patterns in Vomeronasal Receptors Compared To Main Olfactory Receptors

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Historically, the 2 vertebrate nasal chemosensory systems, the main olfactory system (MOS) and the vomeronasal system (VNS), were thought to detect environmental odorants and intraspecific pheromones, respectively. However, behavioral and molecular genetic studies have shown that the functional distinction between the 2 systems is not quite so clear. Here, using evolutionary genomics, we investigate possible distinctions between the 2 systems. We compare the evolution of the VNS-expressed receptors (V1Rs and V2Rs) with that of the MOS-expressed receptors (ORs and TAARs) in 7 representative vertebrates (zebrafish, frog, chicken, platypus, opossum, mouse, and dog). The phylogenies of both V1Rs and V2Rs show species-specific expansions and virtually no orthologous relationships. While the phylogenies of ORs and TAARs also have examples of species-specific expansions, these gene families show a dominant pattern of orthologous genes across a wide taxonomic range. This striking difference in evolutionary patterns of the nasal chemosensory receptors has potential functional consequences. For example, the orthologous relationships across taxa found in the MOS receptors might indicate same function of the receptors. Therefore, these receptors are not likely to be involved in species-specific functions. Similarly, the species-specific expansion patterns found in the VNS receptors support their involvement in species-specific function. This hypothesis can be tested in a functional framework.

Poster Session Thur AM

OMP Expression Is Attenuated in Vomeronasal Neurons of the Naked Mole-rat *Heterocephalus glaber*

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The naked mole rat *Heterocephalus glaber* is a eusocial rodent native to the horn of Africa. A single female mates with a few males but suppresses reproduction by all other colony members. The mechanisms by which the queen suppresses sexual behavior in colony members do

not involve the vomeronasal organ (VNO), which does not develop to the same degree as that in other rodents. To elucidate other departures from the rodent pattern, we compared olfactory marker protein (OMP) expression in VNO-associated neurons and axon fascicles between several individuals. We used immunohistochemistry on paraffin embedded tissues of 3 neonates, 4 non-breeding adult males and females, 1 breeding male, and 1 breeding female. Neuron-specific beta tubulin labeling demonstrated that the sensory epithelium is neuronal in all individuals. Conversely, OMP label was absent in most sections assayed. Possible OMP label was present in a total of 3 cells in sections from 2 individuals. Robust labeling in those sections that contained main olfactory epithelium provided an internal positive control for anti-OMP specificity. Our data suggest that OMP is either not expressed by most VNO neurons or it is expressed at levels below the resolution threshold of immunohistochemistry.

Poster Session Thur AM

Two Types of Vomeronasal Receptor-Expressing Olfactory Sensory Neurons in Goats

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Most mammals have 2 distinct olfactory epithelia, the olfactory epithelium (OE) and vomeronasal epithelium (VNE). The OE and VNE contain olfactory sensory neurons (OSNs) and vomeronasal sensory neurons (VSNs), respectively. Olfactory receptors (ORs) are generally expressed on OSNs, whereas 2 families of vomeronasal receptors (V1rs and V2rs) are expressed on VSNs. ORs, V1rs and V2rs are coupled with *G α olf*, *G α i2* and *G α o*, respectively. Previously, we reported that a goat V1r (*gV1ra1*) is expressed on small subsets of OSNs as well as VSNs. To investigate the characteristic of vomeronasal receptor-expressing OSNs in mammals, we performed double-label in situ hybridization for *gV1ra1*, *G α i2*, *G α olf*, olfactory marker protein (OMP) and growth association protein 43 (GAP43). Goat V1r-expressing OSNs are categorized into 2 types that are located in different areas of the epithelium. The first type of V1r-expressing OSN coexpressed *G α i2*, but not OMP and GAP43. The second type of V1r-expressing OSN coexpressed *G α olf* and OMP, but not *G α i2* and GAP43. These findings suggest that the 2 types of V1r-expressing OSN in goat OE function using different G protein α subunits for chemoreception.

Poster Session Thur AM

A Proteomic Analysis of Olfactory Ciliary Membranes

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The Johns Hopkins University

The cilia of olfactory sensory neurons represent a unique cellular compartment, where signal transduction components, signaling modulators, and structural components coordinate to convert odorant binding into proper electrical signals. Many of these important olfactory proteins reside at or in the ciliary membrane. However, the identities of many components of olfactory signaling, ciliary development, and ciliary maintenance remain unknown. To identify novel

proteins present in olfactory cilia membranes, we employed a direct proteomic approach. Mouse olfactory cilia membranes were isolated by hypotonic separation from the axoneme followed by sucrose density gradient ultracentrifugation. The membrane-enriched olfactory cilia proteins were separated using SDS-PAGE, and were analyzed by liquid chromatography/tandem mass spectrometry (LC-MS/MS). Preliminary analysis of proteins >50 kDa identified 94 proteins by two or more peptides. The validity of this approach was supported by the identification of membrane proteins known to be present in the olfactory cilia, such as adenylyl cyclase 3 and cyclic nucleotide-gated ion channel subunits A2 and B1. We are currently confirming the olfactory sensory neuron-specific origin of the identified proteins. We anticipate that this research will reveal previously unappreciated proteins that are important for the regulation of olfactory signal transduction and for cilia development and maintenance.

Poster Session Thur AM

Expression of OR Transgenes with Chimeric Promoters

Carey Connelly and Randall Reed

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Odorant receptors (ORs) exhibit a highly restricted expression pattern within the olfactory epithelium (OE). In mice, each mature olfactory receptor neuron expresses an OR from a single allele of one receptor gene, selected from a repertoire of over 1000 OR loci. Cells expressing any particular OR are found in a distinct domain within the epithelium. OR domains are distributed from dorsal-medial to ventrolateral across the OE. Specific DNA sequences that govern domain-restricted expression remain unknown. In this study we sought to identify important promoter features responsible for restricted expression of *M71* and *M4*, 2 ORs that reside in largely non-overlapping domains. *M71* and *M4* transgenes exhibit endogenous expression patterns when driven by their native promoter. Deletion analysis has identified elements essential for expression but is not ideal for characterizing sequences that alter the distribution of expressing cells. 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. Expression of each construct was examined in 129/B6 transgenic mice and compared to tagged parental constructs. Both chimeras express in restricted regions of the OE. The *M71*:*M4* chimera mimics the endogenous *M4* pattern, while the *M4*:*M71* chimera expression expanded and shifted ventrally compared to native *M71* and *M4*. Investigation continues whether observed glomerular convergence of LacZ axons corresponds to *M4*, *M71*, or ectopic glomeruli.

Funded by the NIDCD.

Poster Session Thur AM

Odorant Receptor Expression Profiles in Human Sperm—Part II: From Function to Behavior

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Ectopically expressed testicular odorant receptors (ORs) have been attributed a potential function in germ cell development and sperm

swimming behavior. Based on recombinant expression, functional data for 3 human testicular ORs are currently available. With respect to their physiological role in vivo, however, several crucial questions remain. First, are these ORs actually functional in human sperm? Second, do they trigger common or distinct signaling pathways, evoking similar or OR-specific behavioral responses? Third, is the “one cell—one receptor” rule also applicable to sperm? Using high resolution Ca²⁺-imaging as well as various behavioral assays, we show here that different ORs can be expressed simultaneously in one sperm cell. Spatiotemporal response parameters of odor-induced Ca²⁺ transients differ in an OR-dependent fashion and show no sign of cross-adaptation. Moreover, activation of distinct OR signaling pathways triggers different sperm swimming behaviors. Thus, our data support a concept of unique functional roles of OR signaling mechanisms at various stages of sperm development.

Supported by: DFG (T.V., M.S., H.H.), Heinrich and Alma Vogelsang Foundation (A.T., K.S.), University of Arizona Center for Insect Science NIH training grant 1K12GM00708 (J.A.R.), NSF awards IBN 01-32635/02-06775, California Sea Grant (R/F-197), NIH (2-K12-GM00708-06), and UCLA Council on Research (R.K.Z.).

Poster Session Thur AM

The Role of Fz-1 and Wnt-5a in the Mouse Olfactory Pathway

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Olfactory sensory neurons (OSNs) are broadly distributed in the olfactory epithelium (OE) yet their axons target restricted areas of olfactory bulb (OB) neuropil, glomeruli, with exceptional precision. Odor receptors, a variety of trophic and repulsive molecules, and lately OSN activity have been strongly implicated in the targeting of OSN axons. Nevertheless, how OSN axons navigate from the OE up to the OB is still not known. Formerly known as morphogens, there is increasing evidence that Wingless-Int (Wnt) molecules, signaling through their Frizzled receptors (Fz) contribute in a variety of processes, such as cell proliferation, migration and the development of neuronal circuits. In this context, the aim of the present work was to study the expression and activity of Fz-1 and Wnt-5a in the OE and OB. Immunohistochemistry of OSNs showed a very tight regulation of both molecules during development. In the adult Fz-1 expression in OSNs remains at levels compatible with a role in the regeneration. Organotypic cultures of OE showed that in the presence of high levels of Wnt-5a there is an increase in the number of dividing cells, while the number of extending processes is decreased at high concentrations and increased at lower concentrations. Together these results suggest that Wnt and Fz molecules are involved in the development as well as in the regeneration of the primary olfactory pathway.

Support contributed generously by: NIH-NIDCD to C.A.G.

Poster Session Thur AM

Development of Odorant Receptor Expression Patterns in the Mouse Septal Organ

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The rodent olfactory epithelium expresses more than 1000 odorant receptors (ORs) with distinct patterns. It is not clear how specific OR expression patterns evolve during development. Here we use the septal organ, a small patch of olfactory epithelium predominantly expressing 9 identified ORs, as a model to address this issue. The presumptive septal organ first appears at E16 and it completely separates from the main olfactory epithelium at about P7. Using in situ hybridization, we quantified the density of the septal organ cells labeled by the antisense probes of the 9 OR genes from E16 to adult. The results indicate that different OR cells have asynchronous temporal onset. MOR236-1 cells are present in the septal organ at E16; however, MOR235-1 (the closest counterpart of MOR236-1) cells do not appear until P0. In addition, different OR cell types show distinct developmental course and they reach their adult densities at different stages ranging from E16 (MOR256-17) to 4 weeks old (MOR256-3 and MOR235-1). Furthermore, early onset does not correlate with high cell density in adult. MOR236-1 and MOR256-17 cells are dominant at E16 (each contributing to ~33% of the OR positive cells) and their contributions drop to ~5% in adult. In contrast, the number of MOR256-3 cells which account for nearly 50% of the septal organ cells in adult is significantly fewer than that of MOR236-1 and MOR256-17 cells at E16. This study reveals a dynamic composition of the olfactory sensory neurons expressing different ORs during development.

Supported by NIDCD/NIH and Penn IOA.

Poster Session Thur AM

Cell Coupling in the Developing Mouse Olfactory Placode

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Cell-cell communication through gap-junctions plays an important role during prenatal brain development. Prior studies of the olfactory epithelium have shown limited amount of cell coupling in adult animals and some expression of connexins in the embryonic mouse. We used a short term culture system of olfactory placode explants from mouse embryos at E9.5 to investigate the presence and extent of cell coupling in the olfactory epithelium at E11.5. Cells were recorded electrophysiologically under perforated-patch conditions and simultaneously loaded with dye. Recorded cells were identified on a confocal microscope and characterized immunocytochemically. About 15% of cells recorded (9 of 66) showed evidence for cell coupling and visualization of the labeled cells revealed that some cells form pairs while others are connected in larger clusters of more than a dozen cells. In some cases, coupled cells exhibited voltage-sensitive sodium currents and action potentials, suggesting a neuronal phenotype, while others exhibited only potassium currents or lacked voltage-sensitive currents. Our observations indicate that there is more cell coupling in the developing olfactory organ than is present in the adult mouse and future experiments will investigate the importance of these interactions for the development of the olfactory epithelium.

Supported by NIH grant R01HD029178.

Poster Session Thur AM

Development of Bile Salt Sensitivity in the Zebrafish Olfactory System

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University of Utah

Bile salts serving as social cues are detected by the fish olfactory system. At 3 dpf, zebrafish embryos detect amino acids and bile acids in the lateral and medial olfactory bulb (OB), respectively, suggesting that spatial organization of odorant information occurs early in development. The measured increases in intracellular Ca²⁺ are due to voltage-gated calcium channel activation and precede synaptic vesicle release (SVR). We have developed a zebrafish line expressing the exocytosis indicator synaptopHluorin (spH) under control of the olfactory marker protein (OMP) promoter to specifically examine the development of bile salt sensitivity in the OB mediated by these olfactory sensory neurons (OSNs). Two to 14 dpf pOMP-SpH embryos were immobilized, embedded in 2% agarose, secured in a flow-through chamber and superfused with 2 ml/min fish Ringers. Odorants were introduced into the Ringers flow at 5 min intervals to stimulate the olfactory epithelium. SVR was detected by confocal microscopy. As early as 2 dpf (prior to hatching) the adenylate cyclase activator forskolin elicited dose-dependent SVR throughout the OB. At this same developmental stage, bile salts also elicit dose-dependent SVR but only from specific glomerular regions of the OMP-spH expression field in the developing OB. We track the development of these glomerular fields through the first 2 weeks post-fertilization. This study establishes that the bile salt information is processed spatially beginning early in development by OMP expressing OSNs.

Poster Session Thur AM

Vomeroneasal Organ Ontogeny in Tamarins

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¹Slippery Rock University, ²Auburn University, ³University of Louisville and ⁴Cleveland Metroparks Zoo

Tamarins are a diverse group of arboreal New World monkeys, some of which (genus *Saguinus*) have poorly developed vomeronasal organs at birth with few receptor neurons (VRNs) and non-patent ducts. We assess postnatal development of the vomeronasal neuroepithelium (VNNE) across different ages and species of *Saguinus*. In 15 serially sectioned heads from 5 species, the VNNE was studied in morphology and expression of olfactory marker protein (OMP), a marker of mature olfactory neurons. The VNNE at birth is thin, usually comprising one nuclear row. In adults, nuclei of VRNs are arranged in a depth of 4–6 rows. In 2 species represented by a broad age range, the VNNE is nearly 2 times longer in juveniles and 2.5 times longer in adults compared to neonates. OMP reactive cells are sparse in both subadults (mean = 320 cells/mm²) and especially adults (80 cells/mm²). These

results support our earlier finding that in *Saguinus*, few VRNs are OMP(+) and reveal that this characterizes multiple species across ages. Therefore, this may reflect a lower number of terminally differentiated VRNs in this genus compared to a diverse range of primate species that were previously shown to have a ubiquitous presence of OMP(+) VRNs. The paucity of OMP(+) cells across postnatal stages may indicate that cell turnover precedes OMP expression, or the absence of an entire lineage of OMP-expressing cells.

Poster Session Thur AM

Notch–Delta Signaling Promotes Proper Developmental Patterning of the Zebrafish Olfactory Placode

A.C. Morris, M. Meredith, D.A. Fadool and J.M. Fadool

Florida State University

The Notch–Delta signaling pathway is an important regulator of neurogenesis and boundary formation during vertebrate development; however, its role in the development of the olfactory system has not been thoroughly evaluated. We have investigated the importance of this pathway during olfactory placode formation in zebrafish. Whole mount in situ hybridization with digoxigenin-labeled antisense probes demonstrated that *Notch* and *Delta* genes are expressed in the region of the developing zebrafish olfactory placode. To examine the role of Notch and Delta in the developing olfactory system, neurogenesis and placode boundary formation were compared in wild type zebrafish embryos and in *mindbomb* (*mib*) mutants, which lack Notch–Delta signaling. Whole mount immunolabeling and in situ hybridization with markers for immature neurons demonstrated that in the *mib* mutants there is an overproduction of primary neurons in the olfactory placode, consistent with a neurogenic phenotype. These neurons, however, fail to initiate expression of OMP, suggesting that the overproduction of immature neurons occurs at the expense of later-born olfactory sensory neurons. Furthermore, immunolabeling and in situ hybridization with markers for the placodal boundary revealed that boundary formation is altered in the absence of Notch–Delta signaling. These results suggest that Notch–Delta signaling during olfactory placode development is required both for the proper differentiation of olfactory sensory neurons and for the formation of the olfactory placodal boundary.

This work is supported by NIH T32DC00044-11.

Poster Session Thur AM

Opg Is Required for the Formation of Mitral Cell Apical Dendrites

Ting-Wen Cheng and Qizhi Gong

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Precise neuronal connection ensures the information flow in the nervous system. In the olfactory system, mitral cell is the first relay to receive signals from olfactory sensory neurons (OSN). Mature mitral cells eliminate most dendritic processes and retain a single apical dendrite bearing dendritic tufts to synapse with OSN axons. In the mouse brain, Olfactory pathway gene (*Opg*) is exclusively expressed in relays of the developing olfactory system, including ol-

factory epithelium, olfactory bulb, anterior olfactory cortex, and piriform cortex. In the olfactory bulb, *Opg* is transiently expressed by mitral/tufted cell during dendritic pruning period. The expression pattern of *Opg* is not affected by odorant-evoked activity. *Opg* protein is distributed in mitral cell apical dendrites. In neuroblast cell line, *Opg* is localized in the vesicles and transported to nerve terminals. The secretion of *Opg* protein from neurons is detected by Western blotting. The effects of *Opg* in regulating mitral cell dendrite maturation were investigated in vivo by lentiviral delivery of *Opg* shRNA. *Opg* loss-of-function phenotype is lacking dendritic tufts and having degenerated dendritic processes in mitral cells. These results suggest that *Opg* may be secreted from mitral cell dendritic terminals during the remodeling period to regulate the maturation of apical dendrites. Transient expression of *Opg* is also observed in the excitatory neurons of anterior olfactory cortex and piriform cortex. These data suggest *Opg* functions in the refinement of olfactory central connections.

Supported by: NIH DC006015.

Poster Session Thur AM

Recovery of the Intrabulbar Map following Unilateral Naris Closure

Diana Cummings, Carolyn Marks and Leonardo Belluscio

National Institutes of Health

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. Anatomical studies examining the development of these projections revealed that they target broad areas of the bulb on the opposite side when first established (~P0) and refine to their adult precision by 7 weeks of age. These studies further revealed that map refinement is strictly dependent upon odorant-induced activity with no apparent critical window such that any loss of odorant-induced activity produces a broadening in 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 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. These data suggest 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.

Supported by the NIH Intramural Research Program.

Poster Session Thur AM

Dopaminergic Periglomerular Cells Form Novel Multiglomerular Circuits

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We have shown that there are 2 major circuits within the glomerular layer: an interglomerular circuit comprised of the long range connections of short axon cells; and the classic intraglomerular circuit consisting of external tufted and periglomerular (PG) cells. PG cells are thought to have synaptic interactions primarily within one glomerulus. Here we examined the dendritic projections of neurochemically identified populations of PG cells using transgenic mice where glutamic acid decarboxylase 65 (GAD65-GFP) or tyrosine hydroxylase (TH-GFP) drives green fluorescent protein (GFP). Whole cell recording was obtained from TH and GAD65 GFP positive PG cells; the neurons filled with biocytin, and the filled cells reconstructed in 3-D. GAD65+ GABAergic PG cell dendrites are mainly restricted to one glomerulus as expected. Surprisingly, however, the dendrites of DA neurons extend into up to 11 glomeruli over a radius of 2–4 glomeruli. Thus, DA GAD65+ GABAergic PG cells are primarily “uniglomerular” whereas DA neurons form a novel “multiglomerular” circuit linking small groups of neighboring glomeruli. Multiglomerular PG cells comprise a new dimension of bulb circuitry that interconnects local glomeruli. Activity mapping studies have shown that structurally similar odorants preferentially activate groups of 10–50 glomeruli, termed “modules” or “domains.” The multiglomerular circuitry reported here may be involved in such odorant modules.

Supported by NIDCD DC005676.

Poster Session Thur AM

Evidence for Signaling Megaplexes in the Olfactory Bulb

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Florida State University

Adaptor proteins are a class of macromolecules that form scaffolds between ion channels, ligand receptors, and other signaling complexes. The processing and transmission of electrical signals in neurons is dependent on the proper distribution of ion channels on the neuronal membrane, hence adaptor proteins, can govern the local excitability of a membrane. Our laboratory has investigated several adaptor proteins in the olfactory bulb (OB) and has characterized the molecular targets for interaction with the *Shaker* potassium channel Kv1.3 to cause changes in biophysical function and disruption of current suppression. In this study, we identified OB proteins from 4 adaptor classes that co-immunoprecipitate (co-IP) with Kv1.3, as well as with the receptor tyrosine kinases (RTK) Neurotrophin B (TrkB) and the insulin receptor (IR). We also demonstrate that metabotropic glutamate receptors (mGluRs) 1, 5, and 7, and *Shaker* channels Kv1.4 and Kv1.5 co-IP with Kv1.3 in the OB. Co-IP/Western analysis reveal that nearly every protein examined forms a protein–protein interaction with a *Shaker* channel, an RTK, and/or an adaptor protein. These data provide evidence for a structural complex we refer to as the Megaplex; comprised of large numbers of proteins from many classes of adaptors, ion channels, and RTK. Using immunohistochemistry and confocal microscopy, we discovered that only certain regions of the OB display high co-localization of these proteins, while other areas display a stark and well-defined lamination pattern, suggesting that Megaplexes can vary from region to region of the OB.

This work was supported by NIH DC03387 (NIDCD).

Poster Session Thur AM

Activity-Dependent Asymmetric Features of P2 Glomeruli in the Mouse Main Olfactory Bulb

Anthony Oliva and Diego Restrepo

University of Colorado at Denver and Health Sciences Center

In mice each main olfactory bulb contains 2 mirror-image glomerular maps, and responses of glomeruli to odors display mirror-image symmetry. Here we utilized gene-targeted mice expressing tau-green fluorescent protein in olfactory sensory neurons (OSNs) that express the P2 odorant receptor to allow us to visualize the mirror-image projection of these axons to medial and lateral glomeruli. We find that P2 glomeruli are asymmetric across domains in terms of volume and number. Surprisingly, we find that juxtaglomerular cells surrounding the P2 glomeruli demonstrate asymmetric activation, as measured by tyrosine hydroxylase expression or urine-stimulated Fos protein expression. Specifically, the lateral P2 glomeruli are surrounded by a larger number of activated juxtaglomerular cells. Furthermore, sensory deprivation by way of naris occlusion abolishes the asymmetry across domains in the volume and number of P2 glomeruli. These results indicate that, even though the underlying map of identified glomeruli is mirror-symmetric, activity-dependent functional and structural asymmetries exist for some glomeruli. Our findings indicate that asymmetric responses in the mirror-image glomerular maps in the main olfactory bulb help convey additional information on odor makeup that might be important for detecting subtle differences in complex odors such as urine.

Poster Session Thur AM

Expression of Connexin 36 in Second Order Neurons of the Mouse Olfactory Bulb

L. Rela and C.A. Greer

Yale University

In the olfactory bulb (OB) synchronized activity among mitral cells (MCs) is believed to be partly mediated by electrical coupling via connexin 36 (Cx36) homotypic gap junctions. To better understand the possible role of Cx36 in the developing OB, we studied its spatio-temporal distribution using RT-PCR and immunohistochemistry in CD-1 mice at embryonic day 14 (E14), E17, postnatal day 0 (P0), P6 and adult (>P50). We analyzed the subcellular localization of Cx36 immunoreactivity (IR) in MCs and tufted cells (TCs) using transgenic mice (YFP-G) expressing yellow fluorescent protein in a subpopulation of MCs/TCs. Cx36 was expressed at all ages tested, however, its spatial distribution changed with age. In adults, Cx36 IR was largely seen in puncta within the glomerular neuropil and perisomatic region of MCs, a pattern indistinguishable in the CD-1 and YFP mice. Within glomeruli, Cx36 IR colocalized with YFP positive dendritic branches of MCs/TCs. At E14 and E17, Cx36 IR was located in discrete areas which we tentatively identified as protoglomeruli. Perisomatic clusters were only found in the adult and not at E14–P6. We suggest that coupling between MCs can arise early in apical arbors as the dendrites differentiate. Coupling via the somata may occur much later and might contribute to stabilization. Changes in distribution of Cx36 IR across ages may result from maturation of chemical neurotransmission, as seen in the

hypothalamus. Cx36 knockout mice show normal MC structure, however, a role of coupling in MC maturation cannot be discarded, as other Cxs or compensatory mechanisms may be involved.

Support: generously provided by NIH-NIDCD to C.A.G.

Poster Session Thur AM

Differential Expression of Connexin mRNAs in Rat Olfactory Bulb

Elke Weiler

Ruhr-University

Neurons are not single unconnected units but communicate to each other via synapses, and even build a functional syncytium by direct connections through gap junctions. The gap junction proteins, the connexins, are numerous and divergent in their structure. This enables the system to connect cells by pores having different characteristics with regard to size and electrical polarity of the molecules passing through those channels and for electrical resistance to establish a complex neuronal network. The olfactory bulb (OB) receives input from the olfactory sensory cells, filtering this information before transmitting the modified signal into higher brain regions. This filtering function is based on neuronal connections. Therefore, we were interested, if connexins might be involved and expressed in the olfactory bulb. We investigated systematically the expression of different connexin mRNAs (Cx26, Cx29, Cx30, Cx30.2, Cx30.3, Cx31, Cx31.1, Cx32, Cx33, Cx36, Cx37, Cx39, Cx40, Cx43, Cx45, Cx46, Cx47, Cx50, Cx57) of postnatal rats by using RT-PCR and the appropriate primers. We show an expression of most of the connexins in the olfactory bulb with different expression levels. The distinct expression pattern suggests that connexins have an impact on the activity and functionality of the olfactory bulb, therefore involved in information processing and filtering processes.

This work was supported by Research Grants DFG (SFB 509, TP C4), FoRUM F208/00 M122/12(2000) and FoRUM AZ F469-2005.

Poster Session Thur AM

Viral Transsynaptic Tracing from Dual Injections in the Olfactory System Reveals Convergent and Segregated Connectivity Patterns in the Olfactory Bulb

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¹Yale University and ²National Research Council

We demonstrated in previous transsynaptic tracing work that the modular organization of the olfactory bulb (OB) glomerular information processing unit extends in a well defined column structure from the glomerulus to the deep granule cell layer after injection into either OB or piriform cortex. Single label tracing, however, could not show the degree to which the lateral network information converges between glomeruli or the degree of convergence from different sites in cortex. We therefore have used the GFP expressing strain co-injected with a PRV strain bearing a variant of a red fluorescent protein (mRFP1) to reveal convergence and divergence of columnar connectivity. These patterns are seen particularly clearly in the granule cell columns. A single granule cell column can contain separately labeled cells from 2 injection sites. Analysis using a net-

work model suggests that the sparse connectivity of granule cell columns provides greater possibilities for combinatorial inhibitory interactions between glomerular units compared to all-to-all connectivity. OB staining patterns from dual injections into anterior and posterior piriform cortex and the olfactory tubercle were computationally reconstructed. Preliminary results show an unexpected degree of cortical input segregation and medial-lateral asymmetry.

This work was supported by the NIDCD grants DC003918 (W.C.) and DC00086 (G.S.).

Poster Session Thur AM

Cellular and Synaptic Organization of the Human Olfactory Bulb

A. Maresh and C.A. Greer

Yale University

The distribution of cell types and synapses is well characterized in the rodent olfactory bulb (OB), and from that plausible models of odor processing have been constructed. Individual olfactory sensory neurons (OSNs) express only 1 of 1000 odorant receptors (ORs) and send their axons to specific synaptic targets in the OB glomerular neuropil. Each glomerulus is innervated exclusively by OSN axons expressing the same OR. The distribution of these glomeruli is conserved across animals, as is the numerical relationship between number of expressed ORs and number of glomeruli in the OB. Our objective is to extend such results to the level of the human OB to determine how its organization, and more specifically how the number and distribution of its glomeruli, compare to what has been elucidated in mice. As there are approximately 2000 glomeruli for 1000 ORs in mice, we predicted 700 glomeruli in humans based on the 350 intact OR genes identified through genomic studies. Using immunohistochemistry, the organization of cells and synapses in human OBs was evaluated and quantified. While the laminar structure of the OB is broadly conserved between species, not only does the total number of glomeruli in the human OB differ significantly from predicted, the variability among specimens is very high, thus far ranging from approximately 3000 to >6000. These results indicate that the principles of OR-homotypic axon convergence developed from mouse studies may not be readily applicable to humans, and that central processing of odor signals in the human may differ from those characterized in the mouse.

Supported in part by NIH-NIDCD to C.A.G.

Poster Session Thur AM

Expression of the Long and Short Isoforms of the Ret Receptor Tyrosine Kinase in the Main Olfactory Bulb

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Members of the GDNF family of trophic factors signal via the Ret receptor tyrosine kinase. We previously reported the immunohistochemical expression of Ret in the neuroepithelium and main olfactory bulb (OB). However, *Ret* is alternatively spliced to yield 2 main isoforms, Ret9 and Ret51, each appearing to play a distinct role in development: the Ret9 null mouse being non-viable due to renal dysgenesis, whereas the Ret51 null develops apparently normally. This

study aimed to determine which isoforms were expressed in the OB. Adult Wistar rats were cardiac perfused with 4% PFA and paraffin and frozen sections prepared for examination by DAB and immunofluorescent histochemistry. Antibodies used included a rabbit pAb to Ret9 (C-19) and a goat pAb specific for Ret51 (C-20) (Santa Cruz). The results showed the 2 isoforms were expressed in a complementary pattern, with Ret9 present on many cell types, including a significant proportion of periglomerular (PG) cells, and Ret51 prominent within glomeruli and the incoming nerve fiber layer. Double-labeling for Ret9/Ret51 showed co-expression in a small population of cells in the juxtglomerular region, although single isoform +ve PG cells were more common. Ret9 labeled a significant proportion of TH +ve PG cells whereas only rare colocalization was found with Ret51/TH. These data suggest Ret signalling via its family of ligands is important in support of some PG cells and we predict these 2 distinct Ret isoforms will have specific functions in this system.

Supported by The Garnett Passe and Rodney Williams Memorial Foundation.

Poster Session Thur AM

Dual Olfactory System without Vomeronasal Organ in a Turtle, *Trachemys scripta elegans*

Kazuyuki Taniguchi¹, Hiroyasu Ito¹, Toshihiro Oikawa¹, Satoshi Soeta², Kazumi Taniguchi³ and Yoshio Yamamoto¹

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In turtles, although the presence of main and accessory olfactory systems is electrophysiologically reported, the vomeronasal organ is absent. In the present study, therefore, the olfactory pathway of a turtle, *Trachemys scripta elegans*, was morphologically examined to obtain anatomical evidences for the presence of main and accessory olfactory systems. A total of 16 turtles were used for lectin histochemistry and electron microscopy. Their nasal cavity was divided into upper (UP) and lower chamber (LC). The UP was lined with the ordinary olfactory epithelium (OE), and the LC with the sensory epithelium (SE) similar to the amphibian OE. Similarly, the olfactory nerve bundles (ONB) were divided into dorsal (DB) and ventral branches (VB), and the olfactory bulb (OB) dorsal and ventral regions. According to lectin histochemistry, the OE of UP projected via VB of ONB to the ventral region of OB, and the SE of LC via DB of ONB to the dorsal region of OB. Electron microscopy revealed that the SE of LC contained 2 kinds of sensory cells, microvillous and ciliated. The present results demonstrated the presence of anatomically distinctive dual olfactory system in the turtle.

Poster Session Thur AM

A 3-Dimensional Representation of the Glomerular Layer of the Adult Mouse Main Olfactory Bulb

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University of Colorado at Denver and Health Sciences Center

The outer laminar layer (glomerular layer) of the main olfactory bulb (MOB) is thought to contain a topographic map of olfactory sensory neuron activation (or a sensory odor map). While many labs, using

a variety of techniques, have characterized glomerular layer activation elicited after exposure to a variety of odorants, there remains to date no simple mechanism to rigorously compare these odor maps between different labs. Our lab has previously presented a mapping technique and accompanying software capable of mapping glomeruli and generating odor maps. This technique could feasibly be used to compare odor maps generated in different labs; however, it is limited by the requirement that the olfactory bulb be sectioned along a plane perpendicular to the lateral olfactory tract. We present here the development of a "standard" space (or standard bulb) into which sections from bulbs cut in different planes can be fitted and the glomerular layers compared. This standard bulb is currently specific to 2 strains of 12 week old mice, C57 Bl6 and SVJ129 mice, and represents a 3-dimensional reconstruction of the inner glomerular layer of the MOB from multiple animals. We intend to develop the standard bulb as a method to compare and integrate data collected using diverse techniques and ultimately from different laboratories. Toward this end, we have also updated our mapping software to include the mapping method specified by the Leon and Johnson lab and intend to add a method to import the odor maps available in their Glomerular Activity Response Database.

Poster Session Thur AM

High-Throughput Imaging Data Archiving and Retrieval in Neurosciences

Nian Liu, Shin Nagayama, Rixin Wang, Max Fletcher, Arjun Masurkar, Wenhui Xiong and Wei Chen

Yale University

Aims: Two-photon microscopy techniques allow monitoring in vivo the neuronal activity of large populations of single cells in the mammalian olfactory bulb and produce large amounts of time-lapse fluorescence calcium imaging data. To begin to develop informatics tools to archive and analyze these imaging data sets, we have developed a Web-based database system, FluoreDB, to organize the experimental data and analyzed results. **Methods:** FluoreDB is an open-source and platform-independent Web application. It consists of a Java-based Web interface (<http://neurolab.med.yale.edu/fluoredb>) and an Oracle database. The imaging data files are transparently stored in a secure FTP file server. **Results:** Currently archived in FluoreDB are fluorescence calcium imaging data from the rodent olfactory bulb. Authenticated users may manage projects, experiments, and data sets. The original and any subsequently processed image files can be annotated and displayed in a hierarchical manner according to their parent-child relationships, allowing efficient data retrieval and analysis. Individual files can be made available for sharing with other designated users. **Conclusions:** FluoreDB provides a useful tool for high-throughput imaging research in neurosciences. It may also be used as a generic data tracking system for collaborative experimental research.

Supported by NIH grant K22LM008422.

Poster Session Thur AM

Interneuron EPSC Bursts Are Correlated with Tufted Cell Spike Bursts in the Superficial External Plexiform Layer of the Olfactory Bulb

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The external plexiform layer (EPL) of the olfactory bulb is the second level for olfactory processing. It contains several subtypes of interneurons and tufted cells distributed among 3 EPL sublaminae. How these subtypes contribute to olfactory processing is unknown. We have shown that EPL interneurons receive spontaneous EPSCs that exhibit sporadic increases at ~0.5 Hz, similar to the bursting frequency of some EPL tufted cells, and that superficially-located interneurons tend to exhibit autocorrelated EPSC bursts. Because EPL interneurons are known to receive morphologically excitatory synapses from mitral/tufted cells, we postulate that the EPSC bursts of superficially-located interneurons are derived from superficial tufted cells. We therefore obtained voltage clamp recordings from superficially-located EPL interneurons while simultaneously recording spike bursts from nearby tufted cells using extracellular loose-patch methods. Spike-triggered averaging showed that both spontaneous and glomerulus stimulation-evoked interneuron EPSC bursts coincided with tufted cell spike bursts. Cross-correlation analysis showed that the EPSC bursts and spike bursts were correlated. These results suggest that the excitatory synaptic input to superficially-located EPL interneurons may be derived from superficial tufted cells or from a common source providing correlated synaptic inputs to both cell types.

Support: DC006356, 007123, 00347, 008702, 003195 and RR020146.

Poster Session Thur AM

Middle Tufted and Mitral Cell Synchronization in Mouse Olfactory Bulb

Jie Ma and Graeme Lowe

Monell Chemical Senses Center

Synchronization of activity in main olfactory bulb output neurons is proposed to be an integral part of odor coding and processing. Mitral cells (MCs) whose apical dendritic tufts project to the same glomerulus can have spike bursting and single action potentials synchronized by intraglomerular glutamate spillover transmission or electrical coupling. In the glomerular layer, external tufted cells (ETCs) affiliated with the same glomerulus can also exhibit synchronous bursting activity. Middle tufted cells (MTCs) in the external plexiform layer are distinguished from mitral cells by more restricted cortical projections, shorter lateral dendrites and broader tuning to homologous series of odorants. We investigated relative spike timing of MTCs and MCs by dual patch clamp recording in mouse olfactory bulb slices. We observed synchronous spontaneous bursting in MTC–MC pairs linked to the same glomerulus. Normalized spike cross-correlograms displayed a prominent peak centered at zero lag, with width corresponding to mean burst duration. However, single action potential synchrony was not seen. Synchronous bursting was absent in MTC–MC pairs linked to different glomeruli. We propose that MTCs and MCs convey separate data streams from bulb to olfactory cortex. Glomerulus-specific coordination of these streams occurs at theta frequencies of the sniff cycle, but lack of gamma frequency synchrony rules out fast temporal integration of MTC and MC data by postsynaptic coincidence detection. This is consistent with functionally divergent projections.

Supported by NIH DC042808 (G.L.).

Poster Session Thur AM

In Vivo Whole Cell Recording of Mouse Juxtglomerular Cells

Shaolin Liu, Adam Puche and Michael Shipley

University of Maryland School of Medicine

Recent slice recording studies have revealed a significant degree of cellular and network sophistication in olfactory bulb glomeruli including distinctive morphologies, intrinsic characteristics and multiple stereotyped intra- and interglomerular circuits formed by external tufted (ET), periglomerular (PG) and short axon (SA) cells. The advantage of slice preparations is that surgical cuts, pharmacological blockade and focal electrical stimulation and drug delivery can be used to dissect specific cells and circuits. The disadvantage, however, is that it is impossible to investigate how these cells and circuits respond to odors. Thus there is a gap in understanding glomerular networks at the cellular and circuit levels and in the intact animal. To begin to close this gap we conducted whole cell recordings from JG cells in anesthetized mice. We achieved a high success rate: 1 stable recording/8 electrodes (~12%); mean recording duration \approx 15 min, maximum 45+ min. Spontaneous synaptic events (sEPSC/Ps) are indistinguishable from those recorded in slices. JG cell subtypes can be physiologically distinguished based on their firing pattern and input resistance. In voltage clamp, PG cells can be further distinguished as mono- or polysynaptic ON input based on the presence or absence of bursts of multiple spontaneous EPSCs. Odor stimuli caused a pronounced increase in both amplitude and frequency of EPSCs during stimulation. In vivo whole cell recording along with genetic, behavioral and slice approaches should further advance understanding of the roles of the glomeruli in olfactory information processing.

Supported by NIH NIDCD DC005676.

Poster Session Thur AM

The Effect of GABA Blockade on Psychophysical Measures of Odor Detection and Discrimination in the Moth *Manduca sexta*

Esther Mwilaria, Chitrita Ghatak and Kevin Daly

West Virginia University

Previous studies suggest that disruption of GABA function in the antennal lobe only affects fine odor discrimination. These studies used generalization protocols which may falsely imply that discrimination of molecularly different odors is unaffected. Using the moth *Manduca sexta* we addressed this using both differential conditioning and stimulus generalization protocols. Moths were conditioned to respond to either a monomolecular odor (CS), or differentially respond to one (CS+) but not a second (CS–) odor. Twenty-four hours after conditioning, moths were pico-injected with either saline or saline with a GABA antagonist (bicuculline or picrotoxin) directly into both antennal lobes. For generalization experiments, moths were tested with the CS odor, a molecularly similar (S) and a dissimilar (D) odor. Differentially conditioned moths were tested across a log step dilution series of the CS+ and CS–. Results show that GABA disruption increased generalization to S but not D as expected. However, differential conditioning experiments show that

discrimination of dissimilar odors is also disrupted as indicated by increased discrimination thresholds. To establish whether or not this global effect on discrimination could be attributed to changing sensitivity, moths were conditioned to a monomolecular odor and injected then tested 24 h later with the CS in a 5-log step concentration series. Results show that detection thresholds increased. This suggests that underlying the loss of discrimination is an impairment of the moths ability to detect odor.

Support: NIH-NCRR RR015574 to K.D.

Poster Session Thur AM

Impact of Sub-threshold Carboxylic Acids on Human Perception of Coffee Aroma Compounds

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In studies of odor–odor interactions, approximate additivity or mutual suppression are common results. Synergy is an exception. Yet chefs, perfumers, and others report that adding seemingly insignificant amounts of ingredients can sometimes have a noticeable impact on aroma or flavor. We investigated whether adding low levels of carboxylic acids affects the perception of coffee aroma compounds (CACs). Concentration and duration of stimuli were tightly controlled via automated olfactometry, and stimuli were calibrated via a combination of solid-phase microextraction and gas chromatography/mass spectrometry. In Experiment 1, sub-threshold concentrations of acetic and butyric acid were added to concentrations of CACs that spanned the range from just above chance-level detection to just below perfect detection. Over a wide range of CAC concentrations, proportion correct detection (2-out-of-5, forced-choice task) for the resulting mixtures exceeded both dose-addition and response-addition (synergy). In Experiment 2, sub-threshold concentrations of acetic and butyric acid were added to supra-threshold concentrations (moderate intensity) of CACs. Adding acetic acid produced small, but statistically significant increases in rated odor intensity (labeled magnitude scale) of CACs. These experiments provide empirical evidence that low levels of added compounds can indeed have a measurable impact on perception of odor mixtures.

Poster Session Thur AM

Synchronization of Diurnal Variation in Plasma Leptin Levels and Human Sweet Taste Recognition Thresholds

Yuzo Ninomiya, Yuki Nakamura, Shinya Shirotsaki, Rie Ohta, Kiyoshi Koyano, Kazuaki Nonaka and Noriatsu Shigemura

Kyushu University

Leptin is a hormone that regulates food intake, energy expenditure, and body weight. We previously found that the taste organ is one of the peripheral targets for leptin. In mice leptin suppresses gustatory responses to sweet substances through its action on functional leptin receptors expressed in taste cells. However, little is known about possible links between leptin and sweet taste in humans. In this study we examined the taste recognition threshold of non-obese humans for sucrose and glucose and found that the thresh-

olds for sugars have a diurnal variation from 0800 to 2200 h that parallels that for leptin levels, with lowest in the morning and highest in the night. This diurnal variation was not observed in thresholds for other salty, sour, bitter and umami taste stimuli suggesting that the relationship is sweet-taste selective. Furthermore, when leptin levels were phase shifted following imposition of 1 or 2 meals per day, the diurnal variation of thresholds for sugars shifted in parallel. This synchronization of diurnal variation in leptin levels and sweet taste recognition thresholds suggest a mechanistic connection between these 2 variables. Leptin may act as a modulator of sweet taste sensation in humans with diurnal variation in sweet sensitivity having a role in maintaining individual energy homeostasis.

Poster Session Thur AM

Prevalence of Chemical Sensitivity and Its Risks Factors in Teenagers: A Population-Based Study

Steven Nordin¹, Linus Andersson¹, Åke Johansson², Eva Millqvist³ and Mats Bende⁴

¹Umeå University, ²Central Hospital, ³Sahlgrenska University Hospital and ⁴Central Hospital

The prevalence and risk factors of chemical hypersensitivity to common environmental odorants and irritants have been documented for the population of adults but for the population of teenagers. The aim of the present study was therefore to investigate (1) the prevalence of (a) general chemical sensitivity (question about being bothered by odors), (b) self-reported chemical sensitivity with affective and behavioral consequences (Chemical Sensitivity Scale for Sensory Hyperreactivity, CSS-SHR), (c) capsaicin sensitivity (cough provocation) and (d) airway sensory hyperreactivity (SHR; positive capsaicin sensitivity and positive CSS-SHR), and (2) risk factors for general chemical sensitivity. A population-based sample of 401 teenagers aged 13–19 years, stratified for age and gender, were randomly selected, of which 326 (81%) responded to the questions. Of these participants, 85 were randomly recruited for capsaicin provocation. The results show that 16% of the teenagers in the general population reported general chemical sensitivity, 11% reported chemical sensitivity with affective and behavioral consequences, 13% were capsaicin sensitive, and 1% met the criteria for SHR. Risk factors for general chemical sensitivity were found to be capsaicin sensitivity and female gender but not age, smoking, anxiety or depression. These findings suggest that chemical sensitivity is a relatively common health problem also among teenagers.

Poster Session Thur AM

Retronasal Olfactory and Taste Contributions to Vegetable Liking and Intake

Valerie Duffy, J.E. Hayes, G. Napoleone and M.E. Dinehart

University of Connecticut

Previously we found bitter and sweet tastes as positive and negative predictors of preference for traditionally disliked vegetables. Here we describe retronasal and taste influences on preference for sampled vegetables (asparagus, kale, brussels sprouts) and overall vegetable intake in 116 adults who were phenotyped for propylthiouracil (PROP) and quinine bitterness. Ss sampled vegetables thrice giving

intensity and hedonic ratings—nose plugged and unplugged for flavor and hedonic ratings, nose unplugged for taste and hedonic ratings. Vegetable retronasal flavor was the difference between nose plugged and unplugged. In multiple regression analyses, those experiencing greater retronasal flavor tasted greater vegetable bitterness (i.e., congruent odor–taste pair), not necessarily greater sweetness (i.e., incongruent pair). Those tasting PROP most bitter experienced greater retronasal flavor and bitter sensations. From nose pinched to unpinched, hedonic ratings increased for asparagus, were unchanged for kale, yet were highly variable for brussels sprouts. Those reporting greater hedonic increases from pinched to unpinched had greater adiposities. In structural equation modeling, adding retronasal flavor increased the prediction of both vegetables hedonics and intake. In summary, retronasal sensations contribute to vegetable flavor directly and indirectly via vegetable bitterness. PROP supertasters may have lower vegetable preference and intake due to more intense bitter and congruent retronasal sensations.

Funded by NRICGP/USDA.

Poster Session Thur AM

Oral Contact Is Necessary for the Development of Retronasal Metallic Smell

Scott McClure and Harry Lawless

Cornell University

Nasal occlusion studies demonstrate that a major component of so-called metallic taste from oral rinses with ferrous sulfate is due to retronasal smell. We propose that ferrous sulfate initiates a rapid lipid oxidation in the mouth giving rise to metallic odors. However, at high concentrations, metallic smelling compounds such as octenone are found in solutions of ferrous sulfate, presumably due to minute amounts of oxidized lipid contaminants. This leaves the possibility that the retronasal smell from ferrous sulfate is intrinsic to the solution rather than an oxidation reaction in the mouth with lipid constituents. An experiment was conducted with an oral isolation device in which retronasal smell can be assessed after placing chemical stimuli in the mouth without liquid contact with oral receptors. Four conditions included oral contact or no oral contact crossed with nasal occlusion or normal breathing. Sucrose and citral were included as controls. 0.003 M ferrous sulfate had its highest rated intensity under the conditions of oral contact and normal breathing and only a very slight taste (presumably astringency) with oral contact but nasal occlusion. Restriction from oral contact rendered intensity judgments not different from water controls, similar to the pattern seen for sucrose. Thus both retronasal smell and oral contact appear necessary for the perception of metallic smell from ferrous sulfate, consistent with the hypothesis that ferrous sulfate generates metallic odors by lipid oxidation.

Supported by NIH DC 006223 to H.L.

Poster Session Thur AM

Olive Oil Pungency: Sensory Neuron Responses to Oleocanthal and Related Analogs

Catherine Peyrot des Gachons¹, Jeffrey Sperry², Bruce Bryant¹, Paul Breslin¹, Amos Smith^{1,2} and Gary Beauchamp¹

¹Monell Chemical Senses Center and ²University of Pennsylvania

The secoiridoids, phenolic compounds derived from terpenes, confer bitterness and pungency to olive oil. Like other food-derived oral irritants, the secoiridoids are produced by the plants as a protective mechanism to deter microbe growth and ingestion by both invertebrates and vertebrates. Recently, the main compound responsible for olive oil pungency was identified as oleocanthal, a phenolic α,β -unsaturated dialdehyde, which we have characterized as a potent antioxidant and anti-inflammatory molecule. Unlike most other plant irritants, the pungency triggered by oleocanthal is primarily sensed in the throat and not in the mouth. Moreover, the irritation potency is much higher than that of other olive oil compounds with similar structure. To understand the structural basis of oleocanthal irritation, we have conducted a structure–activity relationship study (SAR) with synthetic analogs. The compound activity was assessed by measurement of the intracellular calcium level in cultured rat trigeminal neurons. In general, both aldehyde groups, as well as unsaturation, are required to maintain full activity. These structural features have been previously shown for a variety of bioactive unsaturated dialdehyde sesquiterpenes. Introduction of a second phenolic hydroxyl group, increasing the polarity of the compound, reduces the activity. Thus high lipophilicity appears to be a prerequisite, which suggests that the irritant receptor binding site is within the cell membrane or on the cytoplasmic side of the protein.

Poster Session Thur AM

Multivariate Approach in the Investigation of Interactions between Texture and Ortho- and Retronasal Olfactory Stimuli

Natacha Roudnitsky¹, Johannes H.F. Bult², Rene A. De Wijk², Jens Reden¹ and Thomas Hummel¹

¹Smell and Taste Clinic, University of Dresden Medical School and ²A&F and Centre of Food Sciences

Perceptual interactions between texture and odor were studied using a psychophysical and an electrophysiological approach. Texture stimuli were pulses of milk-like foods with various viscosities. Butter odor stimuli were presented either orthonasally or retronasally after oral processing and before swallowing. Eighteen subjects rated odor and texture intensity. CSERPs were obtained simultaneously. For psychophysical data, odor and texture ratings were correlated; presence of a texture stimulus increased odor intensity, irrespective of odor presentation route. As for electrophysiological data, latencies and N1 amplitudes were higher for the orthonasal condition without texture or with milk than for the retronasal condition with milk. N1 (at C3, C4 and Cz) and P2 amplitudes were higher for the retronasal condition without texture than for the orthonasal condition with thickened milk. Simultaneous representation of both data indicated a positive correlation between intensity ratings and P2 amplitudes, and a negative correlation with N1 amplitude (at Fz) and latencies. The second axis was explained by latencies (at Pz) and N1 amplitudes. A cross-modal interaction between odor and texture was seen in both approaches. Electrophysiological data allow even to see an interaction between texture and odor presentation route. These interactions seemed to occur very early in the processing of this differential information.

Poster Session Thur AM

Oral Anesthesia Specifically Impairs Retronasal Olfaction

Derek J. Snyder^{1,2}, Cara J. Clark², Frank A. Catalanotto², Vicki Mayo² and Linda M. Bartoshuk²¹Yale University and ²University of Florida

Odorants reach the olfactory mucosa in 2 ways: Airborne volatiles flow orthonasally through the nostrils, while the odors of ingested foods and beverages flow retronasally up the back of the throat. While retronasal olfaction (RO), taste, and trigeminal cues contribute distinct information to the composite experience of flavor, several recent reports suggest that RO perception requires oral input. To examine interactions between oral sensation and RO, subjects ($n = 32$) sampled food items (strawberry yogurt, strawberry fruit roll-up, milk chocolate, ketchup) before and after topical anesthesia of the mouth (0.5% dyclone), which suppresses taste and oral tactile sensation. For each trial, subjects sniffed the sample to rate orthonasal olfaction, placed it in the mouth with the nose plugged to rate taste intensity, and then unplugged the nose while swallowing to rate RO. Subjects also rated the bitterness of filter papers impregnated with 6-*n*-propylthiouracil (PROP). The gLMS was used for all ratings. Following oral anesthesia, RO intensity (averaged across all foods) declined significantly ($P < 0.001$), but orthonasal intensity did not. Moreover, rising PROP intensity was associated with greater RO deficits ($P < 0.03$). These findings add to mounting evidence indicating that RO is regulated independently of orthonasal olfaction, specifically by oral cues. Further experiments exploring the relative contributions of taste and trigeminal sensations to RO are in progress (DC 00283).

Poster Session Thur AM

Identification of Air-Phase Fatty Acids: Both Retronasal and Orthonasal Failure

Richard Tamburrino and Bruce Halpern

Cornell University

Retronasal and orthonasal identifications of 3 fatty acid odorants (40% linoleic and oleic in mineral oil, 100% stearic acid), presented air-phase at room temperature (22–25 °C) twice each in random order, were made by 16 subjects (median age = 21.5 years; 10 females) on a digital computer from a display of 6 identifiers (chalk, glue, flour, linseed oil, silly putty, soap), after practice and familiarization trials with no indications given of “correct” identifications. Exhalations were detected by a microphone. **Results:** There were no significant differences across the identifiers and subjects, $P > 0.90$ (chi-square and Friedman ANOVA by ranks for repeated measures tests). However, the identification “flour” was selected for linoleic acid on 15/48 orthonasal and retronasal trials; “silly putty,” for retronasal oleic and stearic acids on 12/48 trials. Chance identifications would be 7/48. This suggests that subjects might be able to learn “correct” identifications for these fatty acids. **Conclusions:** Air-phase fatty acids are not selectively identified from a closed list by untrained subjects. Trained subjects might be able to make selective identifications.

Support from USDA Hatch NYC-191403 and a Susan Linn Sage Professorship.

Poster Session Thur AM

Detection and Identification of Metallic Odorants in a Model Solution of Ferrous Sulfate and Linoleic Acid

Kristine Yu, Harry Lawless and Terry Acree

Cornell University

After rinses with ferrous sulfate, a metallic retronasal smell is sensed that may arise from lipid oxidation in the mouth. Metallic-smelling 1-octen-3-one and 1-nonen-3-one have been detected in ferrous sulfate solutions. A model system was created to produce metallic odorants in a solution of ferrous sulfate and linoleic acid. Solid phase micro-extraction (SPME) retrieved odorants from the system at 1 and 24 h. Gas chromatography/olfactometry (GC/O) was used to identify the odorants, and dilution analysis to determine relative potency. Gas chromatography/mass spectrometry/olfactometry (GC/MS/O) compared the odorants against known standards for confirmation of identities. SPME and GC/O identified 3 possible metallic odorants, 1-hepten-3-one, 1-octen-3-one, and 1-nonen-3-one, at both time intervals, and dilution analysis revealed that the most potent metallic odor from the oxidation of linoleic acid and ferrous sulfate was 1-octen-3-one at both time periods, indicating that the metallic odorant was dependent on the substrate. Psychophysical tests are being conducted to examine the similarities between 1-octen-3-one and the metallic odor perceived after oral rinses with ferrous sulfate.

Funded by NIH DC-006223 to H.L.

Poster Session Thur AM

Investigations on Multimodal Sensory Integration: Texture, Taste, and Ortho- and Retronasal Olfactory Stimuli in Concert

Rene de Wijk^{1,2}, Harold Bult^{1,2} and Thomas Hummel³¹Wageningen Center for Food Sciences, ²Center for Innovative Consumer Studies and ³Smell and Taste Clinic

Perceptual interactions between odour and oral texture were explored in a study in which a cream odour was presented ortho- or retronasally at well-defined moments during the oral processing of milk-like foods with various viscosities. Odour pulses were presented with a computer-controlled stimulator based on air-dilution olfactometry and oral texture pulses were presented with a specially developed system of computer-controlled pumps. Odour pulses of 2 s were either presented during a 3-s period in which the oral texture stimulus filled the oral cavity, during a 3-s period in which texture stimulus was orally processed or during the swallowing of the oral stimulus. Human subjects rated the intensity of overall flavour, thickness, and creaminess. Perceived flavour intensity was reduced with increasing viscosity of the texture stimulus, irrespective of whether the odour was presented ortho- or retronasally. The odour stimulus increased intensities of thickness and creaminess, but only when the odour was presented retronasally, that is, as if the odour originated from the oral stimulus. Furthermore, these effects were most pronounced when odours coincided with swallowing, less pronounced when odours coincided with oral processing and absent for presentation during mouth filling. Results suggest that cross-modal interactions are the rule rather than an exception, provided that multi-modal sensory integration has occurred.

Poster Session Thur AM

Flavor Perception: Response Time Measures of Processing of Gustatory–Olfactory Mixtures

Kelly Burger¹, Maria G. Veldhuizen^{1,2}, Miao-Fen Wang^{1,2} and Lawrence E. Marks^{1,2}

¹John B. Pierce Laboratory and ²Yale University School of Medicine

Response times (RTs) have long served to assess the efficacy of information processing in vision and hearing, but have been used relatively infrequently in the chemical senses. Nevertheless, RTs and theoretically driven analytic procedures may provide insights into chemosensory information processing, for example, gustatory–olfactory interactions in flavor perception. We modified our automated system to deliver temporally controlled gustatory and olfactory flavorants to the mouth and to measure simple RTs: Subjects pressed a key as soon as they detected any flavor, whether gustatory or olfactory, not responding when they detected no flavor. On each trial, the stimulus could be gustatory (e.g., sucrose), olfactory (e.g., citral), the mixture (e.g., sucrose + citral), or a blank (water). Distributions of RTs for each stimulus were analyzed, following methods described by Miller, for evidence of gustatory–olfactory interactions (coactivation) in detecting the mixtures. Coactivation is assessed relative to a baseline of stochastic (probability) summation of responses to the components. Results indicate positive coactivation (additivity, superadditivity) of gustation and retronasal olfaction with the harmonious, familiar flavorant combination of sucrose + citral, but little or no positive coactivation, and possibly negative coactivation (inhibition), with the inharmonious, unfamiliar combination of MSG + citral.

Supported by NIH grant DC006688.

Poster Session Thur AM

Flavor Perception: Effects of Context on Perceived Intensity of Gustatory–Olfactory Mixtures

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Results of several studies suggest that the perceived intensity of gustatory–olfactory flavor mixtures can approximate the linear combination of the components presented alone. In those studies, the gustatory components were perceptually more intense on average than the olfactory components, a condition known to induce contextual effects in judgments of intensity. Contextual effects likely occurred in those studies, but if so, context did not affect the linear additivity. To test these inferences, 8 subjects rated, on a labeled-magnitude scale (LMS), the overall intensity of 16 flavorants in each of 2 contextual conditions; mean concentrations of sucrose were high and those of citral were low in one condition, citral high and sucrose low in the other. Results showed the intensity judgments to be roughly but not wholly additive (departures from additivity may represent nonlinear responding on the LMS). Contextual changes in mean concentrations produced differential, adaptation-like changes in perceived intensity ($P < 0.05$). From these findings and those of Rankin and Marks, we infer that contextual

adaptation likely operates separately in the gustatory and olfactory systems and precedes gustatory–olfactory addition of perceived flavor intensity.

Supported by NIH grant DC006688.

Poster Session Thur AM

Relative Impact of Taste versus Smell Dysfunctions on Quality of Life

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Clinical experience suggests that chemosensory dysfunctions impact substantially on quality of life (QOL). There have, however, been only a few systematic attempts to examine the relationship between QOL and various forms of chemosensory dysfunction in large clinical samples, and all have focused exclusively on smell dysfunctions. The Monell Smell and Taste QOL Questionnaire has been administered to 327 patients with documented chemosensory dysfunction at the time of their evaluation in our clinic (297 with primary smell dysfunction; 30 with primary taste dysfunction). The QOL questions are structured so they may be answered by individuals with no chemosensory complaint as well as by patients. Thus, patient ratings of QOL can be directly compared to those of healthy controls. Both patient groups differ significantly from controls in their enjoyment of virtually all types of foods and beverages, as well as in the variety of foods they eat. In addition, patient ratings of life enjoyment, mood, and their abilities to concentrate, work and sleep are significantly lower than those of individuals without chemosensory problems. There are also, however, interesting differences between those patients with taste versus smell dysfunctions. Of particular note, patients with disordered taste are significantly more likely to report weight loss than either patients with smell disorders or controls, supporting the idea that taste is the key determinant of what we are willing and able to eat.

Supported by NIH DC006760.

Poster Session Thur AM

Connecting Diet and Disease Risk via Food Preference

Bridget Sullivan, J.E. Hayes, P.D. Faghri and V.B. Duffy

University of Connecticut

Past research has tried to connect chemosensation, diet and disease by measuring intake, a time intensive process that often provides inaccuracies due to cognitive constraints (e.g., memory, dietary restraint). We contend that assessing preference increases the ability to make these connections and tested it among 88 middle-aged women of diverse ancestry from a worksite health program. Ss reported frequency of eating fat and fiber foods and health behaviors (e.g., exercise, smoking). On the hedonic gLMS, they reported preference for individual foods (sorted into statistically-reliable fat and fiber groups), spicy foods and non-food sensations. Blood pressure (BP) was measured; Ss reported height/weight. Over half of the Ss were overweight or obese, pre- or hypertensive, and inactive.

While preference–intake pairs were significantly correlated for fat and fiber, intakes of these foods failed to associate significantly with adiposity or BP. In bivariate analyses, those who liked fat more tended to be heavier and have higher systolic BP. Yet, in multiple regression, fat preference was a significant predictor of systolic BP ($P < 0.05$), joining age ($P = 0.001$) and adiposity ($P < 0.05$), even if controlling for non-food ratings. Independent of age, greater spicy food preference was associated with lower adiposity and BP. The fiber preference group (fruits, vegetables, whole grains) had less ability to predict health outcomes. These data continue to support that preference explains variance in health outcomes and should be an important tool for chemosensory, diet and health research.

Funded by USDA, CT DPH.

Poster Session Thur AM

The Role of Attention and Sensitization to Trigeminal and Olfactory Exposure in Chemical Intolerance

Linus Andersson¹, Mats Bende², Eva Millqvist³ and Steven Nordin¹

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Chemical intolerance (CI) is a disorder that results in severe symptoms from common environmental chemicals. The mechanisms underlying the intolerance are still largely unknown. This study investigated effects of attention and habituation/sensitization to chemical exposure in persons with self-reported CI. Trigeminal (CO₂) and olfactory (amyl acetate) stimuli were presented using a dynamic olfactometer. Event-related potentials (ERPs) were recorded from 16 CI persons and 15 controls under 2 conditions. In an attend condition, participants rated the intensities of the stimuli. In the ignore condition they were given a counting task to disregard the stimuli. The ignore condition resulted in longer trigeminal ERP latencies (P2 component) in the control group when compared with the attend condition. This effect was not found in the CI group. CI persons rated CO₂ and to some extent amyl acetate as more intense than the control group, and these differences were greatest by the end of the test session. These results suggest less or no effect of attention modulation in CI persons, which might imply that they have an attention bias to trigeminal stimuli, and have difficulties ignoring such exposures. The smaller effect of habituation in CI person is likely to be referred to a counteracting effect of sensitization. The results from this study suggest that attention and sensitization processes are important factors in CI.

The study was supported by the Swedish Asthma and Allergy Association.

Poster Session Thur AM

Gustatory Stimulation Inhibits Trigeminal Caudalis (Vc) Neuronal Responses to Noxious Electrical Stimulation of the Tongue in the Rat

R. Felizardo¹, C. Simons^{1,2}, J. Azerad¹, E. Carstens² and Y. Boucher¹

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Objective: To test the hypothesis that chorda tympani (CT) activation suppresses Vc nociceptive processing. **Methods:** Fourteen

WDR nociceptive units with lingual receptive fields were isolated in the superficial layers of Vc in pentobarbital-anesthetized rats. Neuronal responses to 3 noxious electrical stimuli delivered to the tongue (trains of 0.2 ms pulses at 1 Hz, 45 mA for 10 s) were recorded before, during and after application of a taste mixture (sucrose 0.3 M, citric acid 0.03 M, NaCl 0.1 M) at a 15 min inter-stimulus interval. An automated system continuously perfused the tongue surface with either the taste mixture or water. Vc units were also characterized by their response to chemical (pentanoic acid 200 mM), thermal (55 °C), and mechanical (nonnoxious and noxious) stimuli. Neuronal responses were quantified as spikes/30 s stimulus condition and compared using the Wilcoxon test. **Results:** Taste stimulation resulted in a 27% decrease in the mean electrically-evoked Vc response. The mean response during perfusion with water (173 spikes/30 s ± 31 SEM) was significantly reduced ($P = 0.002$) during perfusion with the taste mixture (127 ± 26 SEM) with recovery upon reapplication of water (176 ± 39). All neurons also responded to mechanical, thermal and pentanoic acid stimuli. **Conclusion:** The acute depression of Vc responses by tastant stimulation suggests that the gustatory system exerts an inhibitory effect on trigeminal nociceptive processing.

Funding: IFRO.

Poster Session Thur AM

Relationships between BMI, Perceived Pleasantness and Ad Lib Consumption of Food in Smokers and Nonsmokers

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¹John B. Pierce Laboratory and ²Yale University School of Medicine

The goals of this study were to: 1) assess whether ratings of perceived pleasantness (PP) of eating palatable foods generalize to PP of smelling the aroma of that food; 2) assess whether there is a relationship between body mass index (BMI) and PP and/or ad-lib consumption of food; and 3) to determine if these relationships vary as a function of smoking status. Forty-nine nonsmokers (NS) and 10 smokers (S) (BMI 18–37 and 19–31, respectively) used a cross-modal visual analogue scale to rate PP of eating cookies, drinking cookies and cream milkshake, and smelling (orthonasally) a cookie aroma. Due to the unequal N in these preliminary analyses, NS and S were analyzed separately. For the NS we found a significant negative correlation between BMI and the rating of PP of eating cookies and drinking cookies and cream milkshake. In contrast, no difference was observed between BMI and the rating of PP of sniffing the cookie odor or between BMI and amount of cookies consumed in the lab setting. For the S we found a significant positive relationship between the ratings of PP of eating the cookies, a negative relationship between BMI and grams eaten, and no significant relationship with PP of smelling the cookie odor. These preliminary results suggest that: 1) PP of eating foods does not generalize to PP of smelling their odors; 2) that the relationship between BMI and PP of eating food depends upon smoking status; and 3) that being a smoker decreases the likelihood of eating cookies in the lab despite higher PP.

Supported by P50 AA15632.

Poster Session Thur AM

“Bitter Taste” in the Gut? Flavor Avoidance Conditioned by Intra-gastric Denatonium in RodentsJ.I. Glendinning¹, Y.-M. Yiin², K. Ackroff², G.J. Schwartz³, R.M. Margolskee⁴ and A. Sclafani²¹Barnard College, ²Brooklyn College, ³Albert Einstein College of Medicine and ⁴Mount Sinai School of Medicine

Recent studies demonstrate that T2R bitter taste receptors and alpha-gustducin are expressed in the rodent gastrointestinal tract. This suggests that bitter tastants may act in the gut as well as the mouth to promote avoidance of potential toxins. We examined this possibility using an intra-gastric (IG) conditioned aversion procedure. First, rats (Sprague–Dawley) and mice (C57BL/6; B6) were trained with a novel flavor (CS+) paired with IG denatonium infusion and another flavor (CS–) paired with IG water infusion. We found that both species avoided the CS+ flavor in a 2-bottle test. Second, we determined if gustducin signaling in the gut mediates this conditioned avoidance. We found that gustducin knockout mice and B6 mice conditioned equally strong aversions to the denatonium-paired CS+. Third, we examined if sensory vagal fibers in the gut mediate denatonium conditioning in rats. We found that selective afferent vagotomy did not block the CS+ avoidance conditioned by IG denatonium. We conclude that IG denatonium conditions a significant flavor aversion in rats and mice, and that alpha-gustducin and vagal input are not necessary for this conditioning process.

Supported by NIH DK31135 (A.S.), DC03055 and DC03155 (R.M.M.), DK47208 (G.J.S.).

Poster Session Thur AM

Species-Specific Avoidance of Foods Containing Hydrolyzed ProteinKristin L. Field¹, Julia A. Figueroa², Alexander A. Bachmanov¹, Julie A. Mennella¹, Gary K. Beauchamp¹ and Bruce A. Kimball³¹Monell Chemical Senses Center, ²Colorado State University and ³USDA-APHIS National Wildlife Research Center

Both nutritive and chemosensory properties of a potential food contribute to an animal's decision to consume or reject it. What happens when these 2 factors appear to send conflicting signals? Prior research has found that foods containing hydrolyzed protein, and thus valuable amino acids, are avoided by humans, deer, and rats. To determine whether hydrolyzed proteins are generally avoided by mammals spanning different niches, we examined the responses of 8 rodents (*Mus musculus*, *Peromyscus maniculatus*, *P. leucopus*, *Cavia porcellus*, *Rattus norvegicus*, *Microtus townsendii*, *Thomomys mazama*, *Aplodontia rufa*), a lagomorph (*Oryctolagus cuniculus*), and a canid (*Canis latrans*) in 2-choice tests with foods containing hydrolyzed casein (HC) and hydrolyzed collagen (gelatin, GE). Each protein was paired against a cellulose-containing alternative and tested over 4 days in 16–18 subjects per species. Responses to HC- and GE-containing foods depended on species and not niche. In general, most species avoided the hydrolyzed protein-containing food, which temporal data suggest may involve learning for some species. This study contributes to better understanding

standing food selection and the potential application of hydrolyzed proteins toward reducing wildlife damage to vegetation.

Poster Session Thur AM

Olfactory Discrimination Ability of CD-1 Mice for Aliphatic Aldehydes as a Function of Stimulus ConcentrationMatthias Laska¹, Dipa Joshi² and Gordon M. Shepherd²¹Linköping University and ²Yale University School of Medicine

Functional studies suggest that the neural representations of odorants vary not only as a function of molecular structural features but also as a function of stimulus intensity. However, several species have been shown to be capable of recognizing a given odorant over a wide range of concentrations. Using an operant conditioning paradigm we therefore tested the ability of CD-1 mice to discriminate between members of a homologous series of aliphatic aldehydes (C4–C8) at 4 different concentrations. We found 1) that all mice significantly discriminated between all stimulus pairs presented at concentrations of 1, 0.01, and 0.001 ppm, respectively, with no significant differences in performance between these concentrations, 2) a significant negative correlation between discrimination performance and structural similarity of odorants in terms of difference in carbon chain length, and 3) that at a concentration of 0.0001 ppm (corresponding to the highest individual detection threshold) the majority of animals failed to discriminate between the stimuli above chance level although they were clearly able to detect them. These findings suggest that 1) CD-1 mice have an excellent discrimination ability for aliphatic aldehydes, 2) carbon chain length affected odor quality perception in a systematic manner, and 3) discrimination performance with aliphatic aldehydes already reaches a plateau when stimuli are presented at a factor of 10 above detection threshold.

G.M.S. is supported by NIH grant (5 R01 DC00086-38) and the Human Brain Project.

Poster Session Thur AM

Odortypes: Interaction of Diet and MHCKoichi Matsumura¹, Jae Kwak¹, Maryanne Curran¹, George Preti¹, Alan Willse², Jon Wahl², Kunio Yamazaki¹ and Gary Beauchamp¹¹Monell Chemical Senses Center and ²Pacific Northwest National Laboratory

Individual mice have unique odors which we have termed their odortype. Odortypes, like other phenotypes, can be influenced by genetic and environmental variation and their interaction. Variation in genes of the major histocompatibility complex (MHC) plays a central role in determining odortypes. Previous work has demonstrated that mouse odortypes can also be influenced by dietary variation. We have conducted behavioral and chemical studies designed to investigate how variation in diet interacts with MHC-regulated odortypes. Mice trained to discriminate urines from mice that differed both in diet and MHC type found the diet odor more salient. Nevertheless, when trained to discriminate mice with only MHC differences, mice easily recognized this difference in spite of dietary variation. Thus MHC odorants must survive dietary change. Chemical studies are consistent with this inference. Analysis of urinary

volatiles extracted by solid phase microextraction and analyzed by gas chromatography/mass spectrometry demonstrated that dozens of volatile compounds were influenced by the dietary change. Nevertheless, we could accurately classify mice according to MHC type regardless of diet. Thus, although there are clear diet effects on urinary volatile profiles, they have a negligible impact on either behavioral or chemical classification of MHC odortype.

Sponsored by DARPA under ARO Contract No. DAAD19-03-1-0109. Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the US Government.

Poster Session Thur AM

Component Concentration Influences Perceptual Quality of Binary Odor Mixtures

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Research into binary odor mixture perception thus far has examined how the degree of component similarity, receptor overlap, relative concentration of components, and even olfactory enrichment affect the perception of binary mixtures. These studies have aimed to categorize each binary mixture into a rigid category, configurational or elemental, but often results conflict as to which category a particular mixture belongs. A configurational mixture's components interact to create a novel percept; an elemental mixture, however, retains perceptual information about each component. In the present paper, we used a habituation/dishabituation paradigm to determine if rats could discriminate one component of a binary mixture of either perceptually-similar or -dissimilar components when the concentration of both components is varied together. Twenty Sprague-Dawley rats were presented with one of 12 different mixtures each day for 12 days for 4 consecutive habituation trials. Following habituation, a single mixture component was presented to determine if the rats discriminated the component from the mixture. We found that perception of a binary mixture changes with changing component concentration: at low (0.001 Pa) and medium (0.1 Pa) concentrations, rats perceived the mixture as configurational; however, at the highest concentration (10 Pa), rats perceived the mixture as elemental. Thus, one binary mixture can be categorized differently depending on component intensity. All animal work was in accordance with NIH guidelines.

Supported by an AAUW American Fellowship (A.M.M.).

Poster Session Thur AM

Perception of Odor Mixtures in a Newborn Mammal

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Mammalian newborns trace complex biological mixtures emitted by the mother (e.g., milk) to guide their selective approach, searching and suckling. Numerous questions remain open on how immature mammals process such highly complex mixtures to extract salient cues. Here, we take advantage of the fast odor learning induced by the mammary pheromone (MP) in newborn rabbits to assess the pups' ability to discriminate a blend from its constituents. In Exp. 1, pups (age: 2 days, $n = 25$, 5 litters) were enforced

to learn a binary mixture, considered as a "blending mixture" in humans, and then tested for their oral response to the mixture (A + B) or to constituents A or B separately. In Exp. 2, 2 other groups of pups (age: 2 days, $n = 25$ /group) were MP-conditioned to A or B, and tested for their response to the constituents and their mixture. As a result, pups strongly (>80%) responded to the odor stimulus (mixture or constituents) to which they were conditioned. But, when they first learned the mixture, they later reacted to it and also to its constituents (Exp. 1). On the other hand, after they learned one constituent, they responded to this single odorant but not to it in the mixture (Exp. 2). To sum up, rabbit newborns can extract constituents of the present blending mixture after learning the whole mixture, but they cannot generalize from a constituent of this mixture to the whole. Thus, neonatal perception of an odor mixture may involve more than the perception of its constituents.

Support by ANR grant 2005-CPER-Lorraine-125.

Poster Session Thur AM

Goldfish Can Be Conditioned to Respond to a Sex Pheromone

Peter Sorensen

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Pheromones are often defined as chemical messages that travel between members of the same species which elicit specific, adaptive responses. As such, it is generally assumed that responses to pheromones are stereotypical and associated with specialized components of the olfactory system. Indeed, many studies show that pheromone-sensitive olfactory receptor neurons are few in number and project to glomeruli within restricted portions of the bulb. However, few studies have examined central processing and it remains unclear how flexible responsiveness might actually be. We tested this question in the goldfish whose reproductive biology is regulated by 5 pheromones. Specifically, we asked whether male and female goldfish can be conditioned to respond to 17,20b-dihydroxyprogesterone (1720P), a priming pheromone released by females. Mature male and female goldfish were isolated and either 1720P or L-arginine (a food odor) injected into their tanks at random intervals, 45-s after which food pellets were added to funnels. The frequency with which fish touched or entered funnels after odor addition was measured as an indicator of learning. Males increased funnel-directed behaviors within 5 trials for 1720P demonstrating they can learn to respond to a pheromone. Rates of learning were similar for food and pheromonal odors but responses to food were stronger. Females showed the same trends with weaker responses to the pheromone. Experiments are now examining sex-specific differences to other odors to elucidate more completely flexibility in olfactory processing to natural odors.

Human Axillae: Why?

Primate Chemical Communication—An Evolutionary Perspective

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This contribution reviews recent advances in the study of primate chemical communication. It takes an explicit evolutionary

approach. Specifically, sexual selection theory is considered as a theoretical framework that can provide new insights into proximate mechanisms and ultimate causes of primate chemical communication. If chemical signals are understood as costly and as honest advertisement of signaler quality, selection pressures acting both on the senders and on the receivers of olfactory signals can be better understood. As an example for such an approach, comparative analyses of patterns of scent marking in tamarins and marmosets—small New World primates—are presented. Extending this approach to the analyses of chemical communication in other primates should ultimately also lead to a better understanding of the evolution and relevance of this mode of communication in humans.

Human Axillae: Why?

What Is the “Mal” in Malodor?

Pamela Dalton

Monell Chemical Senses Center

There is ample evidence that certain types of odors, including many volatiles emanating from the human body, evoke negative responses and avoidance. Even if such responses are ultimately determined to be the products of learning and not innate, there remains remarkable unanimity in evaluating and responding to malodors both within and across cultures. This talk will explore the historical evidence for associating certain (mal) odors with adverse outcomes, beginning with the “miasma” theory of disease, and seeks to provide a rational basis for understanding contemporary responses to body odors.

Supported by NIH-NIDCD R01 DC 0003704.

Human Axillae: Why?

The Chemistry and Biochemistry of Human Axilla Odors

Andreas Natsch

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Three classes of odorants were described in the human axilla: short chain branched acids, sulfanylalcohols and steroids. The acids and sulfanylalcohols are human-specific compounds. The acids have low volatility, but also low odor thresholds, indicating that evolution has selected for high affinity receptors to smell the conspecific. Inter-individual odor differences are determined by different ratios of these components and the same chemicals were found in European and Japanese studies. For some compounds a high frequency (30–50%) of selective anosmia was reported. This could be due to a selective loss of functional receptors and it indicates that body odors have lost importance in recent human evolution. This is paralleled by the negative reaction of the society towards body odors and odor prevention is a key target for the industry. To this aim the biochemistry of odor formation was studied. The odorants are released by skin bacteria from odorless sweat. The acids were found to be secreted as Gln-conjugates and the sulfanylalcohols are mainly linked to the dipeptide Cys–Gly, but also Cys-conjugates were found. The cleavage of the Gln-conjugates is catalyzed by an aminocyclase and the Cys-conjugates are cleaved by a b-lyase both present in skin bacteria. On a practical side, masking perfumes

could be developed based on the chemical knowledge of body odors and the biochemical findings have triggered development of compounds blocking odor release. On the scientific side, the human-specific odorants, their specific detection and the bacteria-mediated release point to an important function of chemosensory social signals in early evolutionary history of man.

Human Axillae: Why?

Fragrance Strategies Used To Mitigate Axillary Odor: A Perfumer and Sensory Perspective

Krystyna Rankin

IFF

The objective of this talk will be to show the fragrance industry's approaches to malodor control which is the largest source of fragrance business for the industry. Malodor counteractants are needed for product formulae and packaging such as adhesives used in automobiles and surfactants in household cleaning products as well as for consumer needs such as reducing body odor, pet odors, food and cooking odors and stale cigarette smoke. Various approaches are used by the fragrance industry to reduce malodor, including physical, chemical as well as sensory means. This presentation will focus on using fragrance ingredients to control the perception of all types of malodor. The presentation will outline how perfumers approach this challenging area and how sensory experts evaluate the effectiveness of fragrance ingredients in reducing the perception of malodor.

Human Axillae: Why?

Biological Significance of Axillary Odors in Humans

Charles Wysocki

Monell Chemical Senses Center

Secretions from the human underarm appear to be a source of physiologically active chemicals with pheromonal activity. The chemistry of odor production suggests a similarity between human axillary secretions and non-human mammalian odor sources, viz., lipocalins carry chemical signals used in pheromonal communication. Pheromones exist in 4 varieties, viz., primers, releasers, signalers and modulators. Primers typically affect endocrine or neuroendocrine responses. Releasers typically elicit behavioral responses. Signalers simply provide information to the smeller. Modulators convey information that modifies affect in or context of other people. Male and female axillary secretions may alter the length and timing of the menstrual cycles, which appears to be via alterations in pulses of luteinizing hormone. Male extracts also can influence female mood. The chemicals that cause these effects are unknown because, to date, there are no bioassay-guided studies that have led chemists through the complex mixture of skin-derived constituents to one or more compounds with pheromonal activity. Some signalers are influenced by biological sex and sexual orientation. Some compounds have been declared as pheromones and have been used by researchers at levels far in excess of physiological concentrations. Our studies have demonstrated that both the chemistry of axillary secretions and their effects upon other people appear to be analogous to non-human, mammalian primer, modulator and signaler pheromone. We are

currently using a bioassay-guided isolation of components from the complex axillary secretions to discover the active constituents.

Olfaction beyond the Olfactory Bulb: From Perception to Memory

Visualizing Olfactory Memories in *Drosophila* by Optical Imaging

Ron Davis, Dinghui Yu and Akalal David

Baylor College of Medicine

Recent advances in optical imaging have made possible the visualization of neural activity in the *Drosophila* brain in response to sensory stimuli. We have used transgenically supplied reporters for synaptic transmission and calcium influx to visualize these processes and the formation of cellular memory traces in the olfactory nervous system of *Drosophila* after olfactory classical conditioning. The first memory trace detected occurs in the projection neurons of the antennal lobe. This trace forms within a few minutes after training, lasts only a few minutes, and occurs as the recruitment of new sets of projection neurons into the representation of the odor used for training. A second memory trace forms in the dorsal paired medial neurons beginning at 30 min after training. This memory trace is detectable as both increased synaptic transmission and calcium influx and persists for about 2 h after training. A third memory trace was recently discovered in certain types of mushroom body neurons and forms in response to spaced training. Spaced training employs multiple training trials with a rest between each trial, and is effective at producing long-term behavioral memory. The long-term memory trace forms after the memory trace in the dorsal paired medial neurons. These data suggest that multiple memory traces are formed in different parts of the olfactory nervous system in response to olfactory classical conditioning and that each trace may guide behavior over different windows of time after conditioning.

Olfaction beyond the Olfactory Bulb: From Perception to Memory

Odor-Induced Oscillatory Dynamics in the Rat Piriform Cortex

Philippe Litaudon, Nathalie Buonviso, Tristan Cenier, Claire Martin, Julie Chapuis and Nadine Ravel

CNRS UMR 5020, Université Lyon 1, Institut Fédératif des Neurosciences

Oscillatory activities observed in the olfactory bulb (OB) have been proposed to play a key role in encoding olfactory representation. These activities, in the beta (15–35 Hz) and gamma (35–80 Hz) bands, have also been reported in the piriform cortex (PC) but their role in this structure remains largely unknown. In behaving rats, both OB and PC display an odor-induced decrease in gamma power followed by the emergence of a beta oscillatory activity (around 25 Hz). This phenomenon is strongly amplified by learning and precisely correlated with the animal performance in a discrimination task. Disruption of centrifugal connections between PC and OB prevented this modulation of beta activity and was associated with an increase in gamma activity. Similar results were observed in anesthetized rats. This preparation was thus used to study the temporal relationship between these oscillatory activities and unitary activi-

ties. Such an analysis revealed that anterior PC pyramidal cells were more tightly phase-locked with gamma oscillations than OB mitral cells. Such a result suggests that gamma oscillations could provide an appropriate time-window for coincident spike detection by pyramidal cells and could serve a gating function for bulbo-cortical transfer. Taken together, these results suggest that beta oscillatory activity could be the emergent feature of a large-scale olfactory functional network between OB and PC set up through more precisely synchronized inputs from OB to PC on gamma oscillations.

Olfaction beyond the Olfactory Bulb: From Perception to Memory

Biological Mechanism Underlying Olfactory-Discrimination Learning

Edi Barkai and Drorit Saar

University of Haifa

Learning-related cellular modifications occur not only at synapses but also in the intrinsic properties of the neurons. Learning induced enhancement in neuronal excitability has been shown in hippocampal and piriform cortex pyramidal neurons following a complex olfactory-discrimination operant conditioning task. Such enhanced excitability is manifested in reduced spike frequency adaptation that results from reduction in the slow after hyperpolarization (AHP), which develops after a burst of action potentials. The AHP reduction is maintained for up to 4 days after training completion. The post-burst AHP reduction is mediated by decreased conductance of an acetylcholine-sensitive, calcium-dependent potassium current, the slow I_{AHP} . The long-lasting reduction is mediated by a second messenger system that involves PKC and ERK activation. AHP reduction is apparent throughout the pyramidal cells neuronal population. The AHP amplitude tends to return back to its initial value within days when training is suspended. This recovery is accompanied by reduced learning capability, but not by loss of memories for learned odors. These findings suggested that AHP reduction is the mechanism that enables neuronal ensembles to enter into a state which may be best termed “learning mode.” This state lasts for up to several days and its behavioral manifestation is enhanced learning capability in tasks that depend on these particular neuronal ensembles. Specifically, enhanced neuronal excitability sets a time window in which most neurons in the relevant neuronal network are more excitable, and thus activity-dependent synaptic modifications are more likely to occur.

Olfaction beyond the Olfactory Bulb: From Perception to Memory

Learning Modifies Neural Representations of Smell in Human Olfactory Cortex

Jay Gottfried

Northwestern University

It is widely presumed that odor quality is a direct outcome of odorant molecular structure, but increasing evidence suggests that learning, experience, and context play important roles in human olfactory perception. For example, the same odorant smells entirely different depending on whether it is labeled as “mildew” or “fresh cucumber”.

A cherry odor becomes smokier in quality after being experienced together with a smoky odor. Such observations suggest that a given set of olfactory receptors activated by an odorant does not map directly onto a given odor percept. Rather, odor perception may rely on more synthetic, or integrative, mechanisms subserved by higher-order brain regions. Data presented here will explore the specific role of human piriform cortex and orbitofrontal cortex in the formation and modulation of odor quality coding. Combining olfactory psychophysical techniques and functional imaging approaches, we have found that sensory-specific information about an odorant is not static or fixed within human olfactory cortex, but is highly malleable and can be rapidly updated by sensory exposure, perceptual experience, and associative learning. This experience-dependent neural plasticity is paralleled by behavioral improvements in odor perception. Our findings provide direct evidence for the role of learning in shaping neural representations of odor quality in the human brain, a mechanism that may underlie the emergence of olfactory perceptual expertise.

Grant support: NIDCD.

Poster Session Thur PM

Chorda Tympani Responses to Sucrose–Citrate Mixtures

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University of Connecticut Health Center

Citrate is a trivalent anion and an important metabolite. To investigate the role of the citrate anion on taste responses we recorded chorda tympani (CT) responses from 8 golden hamsters (*Mesocricetus auratus*) to the following stimuli: NaCitrate (NaCit), NaCit mixed with 30 μ M amiloride, KCitrate (KCit) (all 1–10 mM, in half-log steps), 100 mM sucrose and all binary combinations of sucrose with NaCit and KCit. Responses to 500 mM NH_4Cl were used to normalize response measurements. Responses to sucrose mixtures with 1 and 3 mM KCit were smaller ($P < 0.005$) than responses to sucrose alone; at 10 mM KCit mixture responses equaled responses to sucrose alone. Thus, KCit suppressed responses to sucrose. NaCit with amiloride added produced similar suppression. Without amiloride, responses to sucrose mixtures with 1 mM NaCit were equivalent to sucrose alone and were larger than sucrose ($P < 0.005$) at 3 and 10 mM NaCit. However, mixture responses at 3 and 10 mM NaCit were still smaller ($P < 0.001$) than responses predicted by an additive response model implying some degree of response suppression. KCit alone generated little CT response and measurably suppressed baseline activity at 1 mM. Rinsing citrate containing stimuli from the tongue resulted in a concentration dependent “rebound” rinse response. We conclude that citrate suppresses taste cell function as reflected in CT nerve activity.

Supported by NIH grant DC004099.

Poster Session Thur PM

Proton Flux through NADPH Oxidase-Linked H^+ Channel (gp91^{phox}) Is Involved in Eliciting Chorda Tympani (CT) Taste Nerve Responses to Strong Acids

John A. DeSimone, Tam-Hao T. Phan, Gerard L. Heck, Shobha Mummalaneni, Gregory R. Sturz and Vijay Lyall

Virginia Commonwealth University

Sour taste transduction for strong acids involves apical H^+ flux in a subset of taste receptor cells (TRCs) via H^+ channels and a decrease in intracellular pH. However, the identity of TRC apical H^+ channels is not known. We tested the hypothesis that CT responses to HCl are elicited by apical H^+ flux via a gp91^{phox} related H^+ channel. CT responses to 20 mM HCl were monitored in the presence of gp91^{phox} modulators in rats and in wildtype (WT) and gp91^{phox} knockout (KO) mice. The results showed that rat CT responses to HCl, but not to acetic acid or CO_2 , were blocked by lingual application of gp91^{phox} blockers, Zn^{2+} , Cd^{2+} and diethylpyro-carbonate, in a dose-dependent manner. HCl CT responses, but not responses to acetic acid and CO_2 , were enhanced by gp91^{phox} activators, H_2O_2 , phorbol 12-myristate 13-acetate ester and nitrazepam. In gp91^{phox} KO mice, the HCl CT response was diminished by about 60% relative to WT mice. The residual response was inhibited by Zn^{2+} but was not affected by stimulating the tongue with 20 mM HCl + H_2O_2 . In contrast, WT and KO mice demonstrated similar responses to acetic acid and CO_2 . This suggests that in gp91^{phox} KO mice the H_2O_2 -sensitive component of the membrane H^+ conductance is absent. We conclude that about 60% of the CT response to HCl is contributed by H^+ flux via a gp91^{phox} related H^+ channel.

Supported by NIDCD grants DC-00122 (J.A.D.) and DC-005981 (V.L.).

Poster Session Thur PM

Nigericin Shifts the pH Threshold for the Chorda Tympani (CT) Taste Nerve Response from 4.5 to 6.5

Vijay Lyall, Gregory R. Sturz, Tam-Hao T. Phan, Gerard L. Heck, Shobha Mummalaneni and John A. DeSimone

Virginia Commonwealth University

To determine the relationship between the CT response and the taste receptor cell (TRC) intracellular pH (pH_i), we monitored rat CT responses in vivo and changes in TRC pH_i in vitro in the presence of acidic stimuli, before and after treating the apical membrane of TRCs with nigericin. Nigericin, a K^+ – H^+ exchanger, equilibrates pH_i and external pH (pH_o) in the presence of high extracellular K^+ . The stimulus solutions (pH s between 8.0 and 4.5) were constituted by mixing 0.15 M K_2HPO_4 and 0.15 M KH_2PO_4 or the corresponding Na^+ salts. In the absence of nigericin no CT response was observed between pH_o 7.5 and 4.5 relative to pH 8.0. After topical lingual application of nigericin (250 μ M) for 30 min, superfusing the tongue with K^+ -phosphate solution at pH 6.5 elicited a CT response. Decreasing pH_o from 6.5 to 4.9 increased the magnitude of the CT response in a dose-dependent manner with a maximum response at pH 4.9. In contrast, no CT response was obtained with Na^+ -phosphate solutions between pH 7.5 and 4.5. After nigericin treatment, decreasing apical pH_o from 8.0 to 6.5 produced the same decrease in TRC pH_i as by decreasing pH_o from 8.0 to 4.5 without nigericin. We conclude that: 1) the CT response is proportional to the decrease in TRC pH_i ; 2) nigericin shifts the pH_o threshold of the CT response from 4.5 to 6.5; and 3) a decrease in TRC pH_i is the proximate signal for sour taste transduction.

Supported by NIDCD grants DC-00122 (J.A.D.) and DC-005981 (V.L.).

Poster Session Thur PM

Lingual Co-application of Sodium and Linoleic Acid Affects Chorda Tympani Nerve Electrophysiological Responses

Jennifer Stratford¹, Kathleen Curtis² and Robert Contreras¹

¹Florida State University and ²Oklahoma State University

We previously reported that bilateral transection of the gustatory chorda tympani nerve (CTX) significantly impaired the ability of rats to detect linoleic acid (LA; a free fatty acid and main component of some dietary fats). Surprisingly, the chorda tympani nerve (CT) was unresponsive to lingual application of LA alone. LA may require a background of saliva (dilute saline) 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 salivary sodium is rinsed off). Moreover, electrophysiological studies of isolated taste receptors have shown that LA inhibits delayed rectifying potassium channels, presumably broadening action potentials, and augmenting responses to other taste stimuli. Therefore, we previously examined CT responses to co-application of monosodium glutamate (MSG) and LA. We found that LA enhances CT responses to MSG. However, MSG contains both sodium and glutamate. Therefore, the present study examined CT whole nerve responses to lingual application of dilute NaCl concentrations (55, 65, 75 mM) and to combined application of 88 μM LA and NaCl in male rats. Preliminary data indicate that CT responses to co-application of LA and NaCl were greater than those to NaCl alone. Thus, free fatty acids (such as LA) modulate gustatory responses to taste stimuli (including MSG and sodium), beginning with an initial interaction with salivary sodium.

Supported by NIH grants DC04785 and DC00044.

Poster Session Thur PM

Naturally Occurring Peptides in Mature Korean Soy Sauce Modulate TRPV1 Variant Salt Taste Receptor

M.R. Rhyu¹, A.Y. Song¹, H.Y. Kim¹, S.S. Kim¹, C. Tokunaga², T.-H.T. Phan³, G.L. Heck³, J.A. DeSimone³ and V. Lyall³

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Naturally occurring peptides fractionated by ultrafiltration (F-II, 0.5–10 kDa) from mature Korean soy sauce modulate the benzamil (Bz)-insensitive NaCl chorda tympani (CT) taste nerve responses and the human salt taste by interacting with TRPV1t in the fungiform taste receptor cells. To identify the peptide fractions that interact with TRPV1t, F-II was further separated into different molecular weight fractions: F-IIa (5–10 kDa), -b (3–5 kDa), -c (1–3 kDa), and -d (0.5–1 kDa) and their effects were tested on the Bz-insensitive NaCl rat CT responses. F-II possesses persistency, mouthfulness and kokumi; these taste effects transited to F-IIb and -c, on the other hand F-IIc showed umami. In solutions containing 100 mM NaCl + 5 μM Bz + varying concentrations of F-II, F-IIa, -b, -c, and -d, only F-II and F-IIc produced biphasic effects on the rat CT responses. The un-fractionated F-II gave

a maximum response at 0.5% and completely inhibited the response at 1.5%. In contrast, F-IIc produced a maximum enhancement at 2.5% and the peak effect was greater than that of F-II. The dose-response curves for F-IIa, -b, -c were further shifted to the right of F-IIc. We conclude that individually F-IIa, -b, -c, and -d have higher thresholds for TRPV1t but when combined excite the CT responses at significantly lower concentrations.

Supported by KFRI grant E069002 (M.R.R.) and NIDCD grant DC-005981 (V.L.).

Poster Session Thur PM

Evaluation of Maillard Reacted Peptides (MPs) as Novel Salt Taste Enhancers and Their Effect on TRPV1 Variant Salt Taste Receptor (TRPV1t)

Tadayoshi Katsumata¹, Chikara Tokunaga², Noboru Fujii², Makoto Egi², Tam-Hao T. Phan¹, Gerard L. Heck¹, John A. DeSimon¹ and Vijay Lyall¹

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In the cooking process and natural aging of foods the formation of MPs enhance food flavor and taste. MPs isolated from naturally aged products have been reported to function as salt taste modifiers. Therefore, we investigated the effect of MPs on salt taste using synthetic MPs in human sensory evaluation and in rat chorda tympani (CT) taste nerve responses. Commercially available soy protein was subjected to enzymatic hydrolysis and MP fractions were purified using ultra-filtration (1000–5000 Da) and then reacted with xylose and again purified using ultra-filtration. Between 0.001% and 0.1% MPs produced biphasic effects on salt taste in human sensory evaluation using 100 mM NaCl as the test solution. Between 0.005% and 0.01% concentration, MPs increased, and above 0.01% suppressed the salt taste. At 0.1% the relative intensity was decreased to control solution by 95.8%. Stimulating the rat tongue with 100 mM NaCl containing varying concentrations of MPs (0.1–1%) also produced biphasic effects on the benzamil (Bz)-insensitive CT response. Between 0.1% and 0.5% concentration MPs increased and above 0.5% inhibited the CT response relative to control. No effect of MPs was observed on the Bz-sensitive NaCl CT response. These results suggested that MPs modulate human salt taste by interacting with the human equivalent of TRPV1t.

Supported by Kyowa Hakko Food Specialties and DC-005981 (V.L.).

Poster Session Thur PM

Dietary Sodium Restriction Augments the Lingual Neutrophil Response to Chorda Tympani Nerve Section

Pamela Wall and Lynnette McCluskey

Medical College of Georgia

Dietary sodium restriction triggers rapid functional changes in the intact chorda tympani nerve (CT) after contralateral nerve section. At the same time, the dietary treatment suppresses the macrophage response to injury. Neutrophils also play a prominent role in inflammation, but their response to gustatory nerve injury has not been

examined. SPF Sprague–Dawley rats received unilateral CT section and/or dietary sodium restriction on day 0. At days 1, 2, or 3 post-section, tongues were collected and cryosectioned. Diaminobenzidine immunohistochemistry was performed with an anti-myeloperoxidase antibody to identify neutrophils. We counted the number of neutrophils on the ipsilateral (cut) and contralateral (intact) sides of the tongue. At 24 h after CT section, the number of neutrophils was significantly increased on both sides of the tongue in sodium-restricted but not control-fed rats. At day 2 post-section, neutrophils remained elevated bilaterally in sodium-restricted rats, and increased on the sectioned side of the tongue in control-fed rats. Neutrophil responses did not differ among different treatment groups at day 3 post-section. Therefore, the neutrophil response to injury was augmented temporally and spatially by dietary sodium restriction. Previous work has shown that sodium-restricted rats lack the robust macrophage response to nerve section. It appears that the macrophage/neutrophil balance is important for maintaining normal gustatory function in the presence of injury.

Supported by NIH DC005811.

Poster Session Thur PM

A Functional Role for IL-1 β in the Injured Peripheral Taste System

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Medical College of Georgia

The adult taste system is functionally plastic after nerve injury. When unilateral CT section is combined with dietary sodium deficiency, neural responses to sodium are reduced in the uninjured, contralateral nerve. Several studies indicate that the immune response to nerve section benefits taste function after injury, and that sodium restriction interferes with this relationship. We examined the expression of the proinflammatory cytokine, IL-1 β , and its contribution to taste function in the intact CT. SPF female, adult Sprague–Dawley rats received unilateral CT or sham sectioning, and a control or low-sodium diet. At day 2 post-section, IL-1 β levels were elevated in control-fed but not sodium-restricted rats. IL-1 β was expressed by activated macrophages and a subset of taste receptor cells, epithelial cells, neurons, and endothelial cells. A subset of taste cells also expressed the IL-1 β receptor. At days 3–4 after unilateral sectioning, recordings were made from the intact CT. Rats that received daily injections of an IL-1 β receptor antagonist exhibited decreased sodium responses compared to controls. Thus, IL-1 β is widely present in the peripheral taste system and is upregulated by nerve section. Moreover, it appears that this cytokine has the ability to maintain normal neural function after injury.

Supported by NIH DC005811.

Poster Session Thur PM

Effects of Early Chorda Tympani Transection on the Adult Rat Geniculate Ganglion

Kaeli Samson and Suzanne Sollars

University of Nebraska Omaha

Previous research has demonstrated that chorda tympani transection (CTX) results in a marked permanent disruption in the mor-

phology of fungiform papillae and taste buds, but only when the surgery is performed early in development. The current study sought to identify effects of early CTX on cells within the geniculate ganglia in aged, adult rats that received unilateral CTX (UniCTX) at 10 days of age. Ganglia were collected from adult UniCTX rats, whose surgeries were verified by tongue analysis. Of note, degeneration of fungiform papillae following neonatal CTX was shown to be life-long, lasting at least 2 years. The ganglia were sectioned (10 μ m) on a cryostat and stained using cresyl violet. The nucleoli of cell bodies were counted for both the left and right ganglia of each rat (control and experimental sides) using light microscopy and recorded using NeuroLucida (MicroBrightField) software. Results established that the geniculate ganglia on the experimental sides of these animals had lower cell counts than those of the control sides, with the experimental cell counts averaging 78.7% (approximately 400 fewer cells per rat) of the control. These findings suggest that neural loss in the peripheral gustatory system is consequent to neonatal CTX. It is possible that the permanent loss in fungiform papillae and taste buds may be the result of the loss of chorda tympani neurons that occurs following neonatal CTX. However, it is also possible that a failure of chorda tympani neurons to reinnervate taste buds may lead to a subsequent loss of neurons.

Grant support: NIDCD DC04846.

Poster Session Thur PM

Neurophysiology of Gustatory Neurons in the Rat Geniculate Ganglion

Joseph Breza and Rob Contreras

Florida State University

In anesthetized rats, we recorded the single-cell responses from 102 geniculate ganglion gustatory neurons to the basic taste stimuli (0.5 M sucrose, 0.1 M NaCl, 0.01 M citric acid, and 0.02 M quinine HCl). Two data sets were obtained: 50 neurons were tested with basic tastes at 10 °C, 25 °C, and 40 °C; and to gradual cooling and warming (1 °C/s) from those adapted temperatures; 52 neurons were tested with a range of concentrations of MSG and linoleic acid (LA), a free fatty acid. Based on the responses to the basic tastes, hierarchical cluster analysis divided neurons ($N = 102$) into 2 major categories of narrowly tuned (Sucrose-specialists, NaCl-specialists) and broadly tuned (NaCl-generalists_I, NaCl-generalists_{II}, Acid-generalists, and QHCl-generalists) groups. MSG was an effective stimulus for the majority of neuron groups; however none of the neurons responded best to MSG and none were responsive to LA. Further examination of the neuron groups revealed that NaCl-generalists_{II} and QHCl-generalists may be members of the same neuron group as they were the only types relatively unresponsive to citric acid or MSG. In general, the taste responses of broadly tuned neurons decreased systematically to the basic stimuli with decreasing adapted temperatures. Only NaCl-specialists and Acid-generalists were affected by gradual changes in temperature. At present, the data suggest that the rat chorda tympani consists of 5 physiological groups uniquely responsive to the basic taste stimuli and differentially influenced by background temperature, and temperature itself is a stimulus for 2 of the 5 neuron groups.

NIH R01 DC04875.

Poster Session Thur PM**Quantitative Trait Loci (QTL) Underlying Taste Bud Number in Recombinant Inbred Strains of Mice**

David J. Reiner, Taha A. Jan, Cheng Xiang Li, John D. Boughter, Lu Lu, Robert W. Williams and Robert S. Waters

University of Tennessee Health Science

Quantitative trait loci (QTL) analysis is an important tool for studying complex traits. We previously used QTL methods to describe genetic factors underlying variability of tongue size in BXD recombinant inbred mice. Suggestive loci were identified on chromosome 7 influencing tongue length and on chromosomes 9 and 16 affecting tongue weight. We extended these findings in additional strains of BXD mice, as well as conducted a novel investigation into genetic factors underlying variability in taste papillae size and number. In a robust set of 43 BXD RI strains we confirmed significant QTLs (*P* Supported by NIH grant to R.S.W.).

Poster Session Thur PM**PROP Taster Status and the Rejection of Foods with Added Tastants**John Prescott¹, Yun Mi Lee² and Kwang-Ok Kim²¹James Cook University and ²Ewha Women's University

It has been established that PROP tasting predicts both sensitivity to food qualities as well as overall food rejections. Using the Consumer Rejection Threshold (CRT) method, the present study aimed to assess whether incremental changes in the taste intensity of samples of familiar food products would lead to earlier rejection of these products, relative to a control samples, by PROP tasters than by PROP non-tasters. Three hundred and five Korean university students rated the intensity of 0.0032 M solutions of PROP using the gLMS. Using forced choice procedures, detection thresholds and CRTs were then evaluated for 7 concentration increments of citric acid in orange juice, caffeine in coffee, and sodium chloride in beef soup. The CRT requires the subject to indicate which of 2 samples they prefer, but is otherwise identical to a 2-AFC detection task. For each food, tasters were more sensitive to variations in tastants than non-tasters. Tasters also rejected more bitter coffee and more sour orange juice at lower concentration of caffeine and citric acid, respectively, than non-tasters. Tasters rejected less salty beef soup more readily than did non-tasters. PROP tasters also gave higher intensity ratings of bitterness in coffee and saltiness in beef soup, but not for the sourness of orange juice. These results further indicate close relationships between PROP taster status and preferences in food systems, particularly with respect to optimal taste intensities.

Poster Session Thur PM**Strain-Specific Asymmetrical Taste Generalization between Quinine and Denatonium in Mice**David Blizard¹, Margaret Colby¹, Thomas Hettinger² and Marion Frank²¹Penn State and ²University of Connecticut Health Center

Taste qualities of denatonium and quinine were investigated in 2 inbred strains of mice differing in aversion for the 2 compounds. C57BL/6J and BALB/cByJ male mice were made averse to either denatonium or quinine by pairing ingestion of these compounds with LiCl. Like golden hamsters, BALB/cByJ mice (denatonium aversion threshold, 0.3 mM; quinine aversion threshold, 0.1 mM) exhibited reciprocal generalization between the 2 compounds. C57BL/6J mice (denatonium aversion threshold, 3 mM; quinine aversion threshold, 0.03 mM) generalized an aversion from quinine to denatonium but not in the reciprocal direction. The lack of generalization from denatonium to quinine extended to concentrations as high as 1 mM quinine. Previous research using operant procedures has not been able to train rats to discriminate between these 2 solutions. Thus, BALB/cByJ mice, hamsters and rats react similarly to denatonium and quinine. The fact that there was reciprocal generalization between denatonium and quinine in BALB/cByJ and unidirectional generalization from quinine to denatonium in C57BL/6J shows that these 2 compounds have stimulus properties in common in both strains. Possible reasons for the strain difference in reciprocity of generalization will be discussed.

Supported by NIH grant, DC004099.

Poster Session Thur PM**Haplotypes of the Bitter Receptor TAS2R38 and Their Relationship to Bitter Perception of PROP in Children, Adolescents and Adults**

Julie A. Mennella, Fulkio Duke, M. Yanina Pepino, Emily Perlman, Catherine Forestell and Danielle R. Reed

Monell Chemical Senses Center

Common genetic variants of the bitter taste receptor gene TAS2R38 are related to PROP sensitivity. In this study, we recruited a genetically diverse, large urban sample (*N* = 760; 54% Black, 30% White; 26% Other) whose ages ranged from 5 to 55 years. PROP thresholds and genotype for the 3 TAS2R38 single nucleotide polymorphisms (A49P, V262A and I296V) were determined for each subject. Haplotypes were inferred by expectation-maximization methods using an algorithm implemented by the computer program fastPHASE. Three common and 6 rare haplotype groups were identified, all of which were related to PROP threshold (*P* < 0.001). Although previous cell-based assessment of TAS2R38 receptor haplotypes suggest that the third SNP position (I296V) has either a subtle or no effect on sensitivity to a fixed PROP concentration, subjects with haplotype variants that differed in this position demonstrated marked differences in taste perception. Age was related to bitter sensitivity but only in those with the PAV/AVI haplotype (*N* = 231). Children with this haplotype could taste PROP at lower thresholds than adolescents (11–15 years) or adults with the same haplotype (*P* = 0.05). We conclude that the TAS2R38 haplotype is more predictive than each individual SNP for PROP perception, and age interacts with TAS2R38 heterozygosity, with the switch from child- to adult-like bitter perception in heterozygous individuals occurring around the time of puberty.

Supported by NIH grants HD37119 and AA09523.

Poster Session Thur PM

Complex Genetics of Taste Responses to Saccharin

Natalia Bosak, Cailu Lin, Xia Li, Maria Theodorides, Zakiyyah Smith, Dani Reed, Gary Beauchamp and Alexander Bachmanov

Monell Chemical Senses Center

Inbred mouse strains differ in their responses to sweet taste stimuli in part due to allelic variation of the *Sac* (*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, and to eliminate the effect of the *Tas1r3* locus, we crossed B6 inbred mice with 129.B6-*Tas1r3* congenic mice; thus, all mice in this cross had only the B6 *Tas1r3* allele. Despite the genetic identity at the *Tas1r3* locus, mice from the F₂ generation varied widely in their 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 large phenotypical divergence between these 2 lines. This result demonstrates that genes other than *Tas1r3* affect saccharin consumption. Mice from the 4th generation of selective breeding were only genotyped with markers in chromosomal regions linked to saccharin intake in their progenitors, B6x129.B6-*Tas1r3* F₂. For all chromosomes (Chr) tested, there was a significant divergence of frequencies of alleles in these regions. Mice from the High line accumulated B6 alleles in Chr1, 3 and 13, and 129 alleles in Chr2 and 7 (mice from the Low line accumulated alleles from the opposite strain at these locations). This observation suggests a complex genetic architecture for behavioral taste responses to saccharin with each parental inbred strain carrying several loci increasing or decreasing the trait. Supported by NIH grant DC00882.

Poster Session Thur PM

NaCl Taste Thresholds in 13 Inbred Mouse Strains

Yutaka Ishiwatari^{1,2} and Alexander Bachmanov¹

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Molecular mechanisms of salty taste in humans and other animals are poorly understood. We use genetic approaches to study these mechanisms. Previously, we developed a simple behavioral procedure to estimate NaCl detection threshold. This procedure involves conditioning mice to avoid LiCl and then examining avoidance of NaCl solutions presented in the ascending order of concentrations in 2 bottle preference tests. Using this procedure, we estimated NaCl taste thresholds of mice from 13 genealogically divergent inbred strains. These strains included mice that have been previously reported to have large differences in 1) amiloride sensitivity of chorda tympani (CT) nerve responses to NaCl and 2) NaCl preferences. We found substantial variation in NaCl detection thresholds among the tested inbred strains. There were no significant correlations of NaCl detection thresholds with amiloride sensitivity of CT responses to NaCl or with NaCl preferences. To exclude a possibility that strain differences in learning or memory affected differences in NaCl detection thresholds, we used a similar procedure to estimate taste thresholds of citric acid in 4 inbred strains with similar acid sensitivity in preference tests, but with large differences in NaCl taste thresholds. Citric acid taste thresholds were similar in these

4 strains. This suggests that our technique measures taste quality-specific detection thresholds that are likely to represent peripheral taste responsiveness. The strain differences in NaCl taste sensitivity found in this study provide a basis for genetic analysis of this phenotype.

Poster Session Thur PM

Polymorphisms of ENaC α Subunit Are Associated with Strain Differences in Amiloride Sensitive NaCl Responses in Mice

Noriatsu Shigemura¹, Tadahiro Ohkuri¹, Chiharu Sadamitsu¹, Keiko Yasumatsu¹, Ryusuke Yoshida¹, Gary K Beauchamp², Alexander A. Bachmanov² and Yuzo Ninomiya¹

¹Kyushu University and ²Monell Chemical Senses Center

Amiloride, an epithelial Na⁺ channel blocker, is known to inhibit responses to NaCl of taste cells and the chorda tympani (CT) nerve innervating the anterior tongue in various mammalian species. In mice, amiloride sensitivity varies among strains; C57BL/6 (B6) exhibited inhibition of NaCl responses by amiloride to ~50% of control, whereas only weak inhibition (~20%) was observed in 129P3/J (129). The amiloride-sensitive epithelial Na⁺ channel (ENaC) expressed in taste cells is a potent candidate to play a role in the salt taste transduction. In this study, using B6, 129 strains and their F₂ hybrids, we investigated possible relationships of the amiloride sensitivity with single nucleotide polymorphisms (SNPs) and mRNA levels of 3 ENaC subunits (α , β , γ) in the anterior tongue. Sequencing detected an SNP resulted in an amino acid substitution, R616W in α subunit. No SNP was found in β and γ subunits. F₂ hybrid mice were divided into 3 groups according to their R616W genotypes (129/129, B6/129 and B6/B6). Responses of the CT nerve to NaCl decreased after amiloride treatment in B6 and F₂ (B6/129 and B6/B6), whereas only weak inhibition was evident in 129 and F₂ (129/129). No significant difference in the mRNA levels of ENaC subunits between B6 and 129 was observed. These results suggest that R616W of α ENaC may be one of the factors responsible for mouse strain differences in amiloride sensitive NaCl responses.

Poster Session Thur PM

Fatty Acid Taste in Obesity-Prone and -Resistant Rats: Strain and Sex Differences

D. Pittman¹, K. Smith¹, M. Crawley¹, C. Corbin¹, D. Hansen², K. Frasier² and T. Gilbertson²

¹Wofford College and ²Utah State University

Previously we have shown that Sprague-Dawley rats can detect free fatty acids (FFAs) using gustatory cues and FFAs produce more inhibition of outward K⁺ currents in TRCs from obesity-resistant rats (S5B/P1) than obesity-prone rats (O-M). This study assessed in vivo differences in gustatory sensitivity to FFAs between males and females of the S5B/P1 and O-M strains of rat. Taste aversions to 100 μ M linoleic acid were conditioned for 3 days. Conditioned and generalized avoidances were assessed using 15 s trials of 2.5–100 μ M linoleic acid, 50–100 μ M oleic acid, and 100 μ M lauric acid in the MS-160 Davis Rig on 3 testing days. All rats showed robust

avoidance of ≥ 2.5 μM linoleic acid on test day 1 with extinction on subsequent test days. On test day 2 differences in sensitivity to the FFAs were evident between the S5B/P1 and O-M strains as well as males and females. In contrast to our hypothesis based upon electrophysiological recordings, O-M rats had stronger aversions to linoleic acid than the S5B/P1 rats. Within each strain, female rats had stronger aversions to linoleic acid than the male rats. Both strains and sexes showed a generalized avoidance of 100 μM oleic but only the female O-M and S5B/P1 rats showed a generalized avoidance of 100 μM lauric acid. Our data suggest that obese-prone and female rats have a greater gustatory sensitivity to FFAs than obese-resistant and male rats.

Funded by Fullerton Foundation Community of Scholars (D.P.) and NIH DK59611 (T.G.).

Poster Session Thur PM

Relationship between Odor Identification Ability Using the Brief Smell Identification Test (BSIT) and Epidemiological Variables in Persons in a High Arsenic Area

J. Prah, E. Hudgens and R. Calderon

US Environmental Protection Agency

We obtained BSIT scores from 906 (538 female), aged 45–92, persons in a study of arsenic (As) exposure in Fallon NV. All subjects signed a consent form. The inorganic arsenic concentrations in drinking water ranged from <10 to >300 ppb. We observed significant correlations between BSIT score gender ($P < 0.0001$), ever smoke ($P < 0.0002$), age ($P < 0.0001$), pack years ($P < 0.0002$), and urinary As ($P = 0.021$). The correlation of BSIT and asthma ($P = 0.0554$) neared significance. We have previously reported a significant relationship between urinary As and BSIT in Inner Mongolia. Logistic regression of other data revealed relationships between BSIT score and diesel exposure ($P = 0.0443$), age at menopause ($P = 0.0002$), radiation treatment ($P = 0.0134$), and use of statins ($P = 0.0204$). “Ever having had” cancer neared significance ($P = 0.0576$). We have confirmed the deleterious effects of smoking on the sense of smell and the greater male vulnerability to odor impairment. The effect of As on olfaction has been hypothesized to be mediated by As trioxide, an anticancer drug, to downregulate telomerase activity upon which olfactory receptor cell regeneration depends. This may explain the trend for “ever having had” and radiation therapy for cancer to score less well. The effect of statins on odor score and age of menopause require further exploration. The effect of statins has been reported, though a mechanism for this effect has not been defined. Menopausal effects may be due to mucosal changes.

The study sponsor was US EPA. This is an abstract of a proposed presentation and does not reflect EPA policy.

Poster Session Thur PM

Role of Cytochrome P450 in the Nasal Inflammatory Process

Karen Yee¹, Beverly Cowart¹, Edmund Pribitkin², Hakan Ozdener¹ and Nancy Rawson¹

¹Monell Chemical Senses Center and ²Thomas Jefferson University

The olfactory mucosa (OM) provides a defense against airborne pollutants, carcinogens, bacteria, and inhaled toxicants. Nasal cytochrome P450 enzymes play a key role in this process by metabolizing a host of xenobiotics (e.g., drugs, toxicants, and carcinogens) as well as natural chemicals (e.g., steroids, retinoids). As a part of our ongoing clinical research on chronic rhinosinusitis (CRS), we examined the effects of inflammation on cytochrome-expressing cells in OM of CRS patients. In control biopsies, we observed co-localization of cyp2A5-immunoreactivity and ck18-immunoreactivity (cyp2A5 + ck18-ir) in supporting cells (SC) and mucosal glands (MGs). Immunofluorescence examination of CRS biopsies revealed varying degrees of loss of cyp2A5 + ck18-ir SCs and MGs, with severe inflammation resulting in keratinization of the superficial epithelium and disappearance of cyp2A5 + ck18-ir SCs and MGs. The loss of cyp2A5-ir SCs and MGs could result in accumulation of inhaled toxicants and chronic exacerbation of epithelial damage and remodeling, as well as altered odorant metabolism contributing to olfactory dysfunction. In addition, the loss of SCs is likely to affect the functional ability of any remaining olfactory sensory neurons.

This study was funded in part by NIH DC006760 and DC000014.

Poster Session Thur PM

Chemosensory Function in Students Exposed to Formaldehyde in the Veterinary School Laboratory

Laurence Jacquot, Tamika Wilson, Laura Sitvarin and Pamela Dalton

Monell Chemical Senses Center

Impairment of chemosensory function in humans has been associated with occupational exposure to inhaled pollutants or toxic substances. Despite that, little is known about the mechanisms underlying chemical-induced olfactory dysfunction. The aim of this study is to assess the chemosensory impact of repetitive exposure to formaldehyde, a pungent-smelling gas, among veterinary students enrolled in gross anatomy laboratory. Personal exposure values for each participant were collected using passive monitors. Overall, subjects were exposed to formaldehyde for 2 h/day, 4 days/week for 12 weeks. Changes in chemosensory function were evaluated in 4 sessions: 1) before exposure (Visit 1) and 2) after 4, 8 and 12 weeks of exposure (Visit 2 to Visit 4). Olfactory function was assessed using a standardized battery that included tests of olfactory thresholds for phenylethyl alcohol (PEA), limonene and formaldehyde and tests of odor identification ability. Lateralization thresholds for formaldehyde were also obtained to evaluate the changes in trigeminal sensation. Overall, results showed modifications of olfactory function throughout the exposure period, especially between Visit 1 and Visit 4.

Supported by NIH-NIDCD DC P50-DC006760.

Poster Session Thur PM

The Influence of Pentoxifylline on Olfactory Function

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Dresden Medical School

Background: Signal transduction in the olfactory epithelium is carried out by adenosine 3',5'-cyclic monophosphate (cAMP) as the second messenger. An elevated intracellular cAMP level causes calcium influx ensuing depolarisation of the neuron. cAMP is degraded by phosphodiesterases. **Aim:** This study aimed to investigate a possible impact of pentoxifylline—an unspecific phosphodiesterase inhibitor—on olfactory function. **Method:** We studied olfactory function in 19 patients with sensorineural hearing disorder treated with pentoxifylline and in 19 healthy age and sex matched controls. Olfactory function was assessed by the TDI-score by means of the “Sniffin’ Sticks” test battery, nasal airflow was measured by anterior rhinomanometry before and 1–2 h after administration of pentoxifylline in patients and on 2 occasions in healthy controls. **Results:** The interval between the 2 tests did not differ between patients and controls. Nasal airflow did not change in relation to drug administration in the patients’ group. TDI-score significantly increased in patients treated with pentoxifylline. Olfactory test results revealed a significant decrease in odor threshold in patients, while odor discrimination and odor identification did not change significantly. Furthermore, the odor threshold shift towards lower concentrations was age-dependent, being significantly more pronounced in younger patients. Further studies are needed to address the question whether pentoxifylline can improve olfactory function in patients with smell disorder.

Poster Session Thur PM

Immune Cell Profile in the Olfactory Epithelium of Patients with Chronic Nasal Inflammation

P. Feng¹, K.K. Yee¹, B.J. Cowart¹, E.A. Pribitkin² and N.E. Rawson¹

¹Monell Chemical Senses Center and ²Thomas Jefferson University

Chronic rhinosinusitis (CRS) is one of the most common human diseases, and may be related to abnormal immune response. CRS is a major cause of olfactory loss and current therapies are often ineffective in treating this sensory impairment, even when some relief of other clinical symptoms is achieved. To understand the role of nasal immunity in the pathogenesis of CRS and associated olfactory dysfunction, we are using immunocytochemistry to determine the profile and distribution of various immune cells in biopsies of the olfactory mucosa of CRS patients taken prior to and after treatment. Patient tissue exhibited a distinct profile of immune cells: CD3+ T lymphocytes were predominant, compared to CD19+ B cells, both in the epithelium and the lamina propria. In the epithelium, CD8+ T cells were more prevalent compared to CD4+ T cells, resulting in an abnormally low ratio of CD4+ T cells (helper)/CD8+ T cells. This shift suggests that an atypical cellular immune response may contribute to CRS-related pathogenesis. The density of macrophages (CD64+) and dendritic cells (CD11c+) varied among patients, and dendritic cell density tended to decline with treatment. Given the critical role of dendritic cells in immune regulation, a decrease in this cell population suggests that the treatment was having the expected effect of reducing the inflammatory response. In spite of this, olfactory performance and the pathology of the sensory epithelium did not consistently improve, and in some cases deteriorated further.

Funded in part by NIH DC006760.

Poster Session Thur PM

Inflammatory Changes following Repetitive Exposure to Formaldehyde Vapor

Ryan McDermott, Tamika Wilson, Kai Zhao and Pamela Dalton

Monell Chemical Senses Center

Formaldehyde is a ubiquitous air contaminant that is both naturally occurring and man-made. Many potentially adverse human health effects have been associated with exposure to formaldehyde, ranging from eye and upper airway irritation and occupational asthma to nasal cancers. Both animal and human studies have shown that even low-level exposures to formaldehyde vapor can produce significant impairments in olfactory function. Although mucosal inflammation is one likely mechanism underlying functional changes, the cross-sectional nature of existing studies precludes charting the co-development of symptoms and inflammatory markers. In this study, markers of nasal mucosal inflammation (nasal nitric oxide, cytokine profiles) and mucociliary clearance (saccharine transit time) were obtained from medical students having exposure to formaldehyde (2+ h/day, 4 days/week) in gross anatomy lab. Assessments were made at the start of the semester and at 4 week intervals, while passive dosimeters were used to obtain personal exposure levels continuously throughout the semester. Elevations in biomarkers were positively associated with increases in exposure concentrations and duration, underscoring the need for personal monitoring in studies linking chemical exposures to functional changes.

Supported by NIH-NIDCD P50 DC 006760.

Poster Session Thur PM

Lateralized versus Bilateral Olfactory Testing in Clinical Settings

Antje Welge-Luessen, Birgit Merz and Markus Wolfensberger

University Hospital Basel

Objectives: Olfactory testing is usually performed birhinally. Examining a large number of patients using a lateralized testing paradigm, we aimed to get additional information concerning the lateralization of olfactory disorders and possibly testing recommendations. **Methods:** A total of 357 patients (posttraumatic disorder: $n = 159$; postviral $n = 71$; sinunasal disease $n = 38$; idiopathic $n = 89$) were bilaterally examined using the extended “Sniffin’ Sticks” test battery (threshold [T], discrimination [D], identification [I]; sum: TDI-score). Statistical analysis was performed using SPSS 12 for Windows. **Results:** In the extended testing 27% of all patients showed side differences of 6 or more points in the TDI score. Moreover, a side difference in one of the tests correlated with side differences in the other tests. Only threshold testing showed significant differences between the different groups of patients in lateralized testing. **Conclusions:** In approximately one quarter of patients with olfactory disorders lateralized testing reveals significant side differences. Using the extended test battery and knowing the positive correlation between the tests, the following approach should be discussed: Depending on the results from initially lateralized

threshold testing further olfactory testing should be performed either in a lateralized or birhinal fashion.

Poster Session Thur PM

Olfactory Function and Occurrence of Olfactory Event Related Potentials in Rhinologic Clinic

Philippe Rombaux¹, André Mouraux² and Bernard Bertrand²

¹Cliniques Saint Luc University Louvain and ²Cliniques Saint Luc

To evaluate the probability to detect olfactory event related potentials (OERPs) in patients with an olfactory dysfunction. **Materials and methods:** Prospective study of 65 patients with different origin of their olfactory loss. Orthonasal olfactory function was assessed with the Sniffin' Stick test (orthonasal score; maximal score 48) and retronasal olfactory function (retronasal score; maximal score 20) with odorized powders presented intraorally. Olfactory event related potentials were obtained after presentation of selective olfactory stimulus; 2-phenyl ethyl alcohol. **Results:** Based on orthonasal testing, 32 and 33 patients were diagnosed with functional anosmia and hyposmia respectively. Twenty-two patients from the hyposmic group demonstrated reliable OERPs. There were no OERPs recorded in the anosmic group. Prevalence of OERPs in a cohort of patients with olfactory dysfunction is 33.8% (22/65). Median score (expressed as the percentage of the maximal score that could be theoretically obtained) where OERP is recorded is 50% (24/48) with orthonasal testing and 80% (16/20) with retronasal testing. **Conclusions:** Patients with olfactory dysfunction usually demonstrate OERPs in a third of the cases. When olfactory dysfunction is in the range that separates normosmic subjects from anosmic patients, patients may have identifiable OERPs.

Poster Session Thur PM

The Usefulness of Olfactory Biopsies in Patients with Olfactory Loss

Martin Witt, Katja Bormann, Volker Gudziol and Heinz Reichmann

University of Technology

Olfactory loss may result from head trauma, nasal surgery or in patients with chronic rhinosinusitis or neurodegenerative disorders such as Parkinson's disease. In fact, impaired olfactory function may constitute one of the earliest symptoms of PD. However, it is still unclear to what degree changes of the olfactory epithelium may contribute to dysosmia in these patients and if these changes are different from those of other hyposmic patients. Biopsies of 7 individuals diagnosed with PD (mean age: 76 years), and 18 hyposmic/anosmic (mean age: 46 years) and normosmic (mean age: 31 years) controls were taken from the insertion of the middle turbinate and opposite septal areas. More cranial areas were omitted. For immunohistochemistry, antibodies against OMP, neurotubulin, PGP 9.5 were applied to paraffin embedded tissue sections. Immunohistochemical findings showed irregular patchy areas of olfactory-like epithelium positive for PGP 9.5 and neurotubulin, but mostly negative for OMP. The probability to obtain OMP-positive samples was not different in normosmic or hyposmic/anosmic subjects. This

suggests that biopsies are not a straightforward diagnostic tool in patients with olfactory loss. While being aware of the small sample size, we carefully conclude from these data that PD-related olfactory impairment is not associated with major changes in the olfactory epithelium.

Poster Session Thur PM

Treatment of Post-infectious Olfactory Disorders with Minocycline: A Double-Blind, Placebo-Controlled Study

Jens Reden¹, Birgit Herting¹, Robert C. Kern² and Katja Lill¹

¹University of Dresden Medical School and ²Northwestern University

A common cause for a decreased olfactory function is an infection of the upper respiratory tract. In this case, a loss of olfactory receptor cells could be found, following an increased apoptosis. Considering this pathomechanism, a therapeutical effect of an anti-apoptotic treatment, for example, minocycline, could be assumed. In the present double-blinded study we investigated the effect of minocycline on the olfactory function by means of the change of the results in a standardized olfactory test ("Sniffin' Sticks"). Fifty-five patients with olfactory dysfunction participated, 26 of them received the verum (16 female, 10 male; 2 × 50 mg/day), and 29 the placebo (19 female, 10 male), each for a period of 21 days. At the end of treatment 53% of the patients reported subjective improvement of olfactory function. Objectively, in 18% of the patients an improvement could be found by means of an increase of more than 6 points in the test score. This result was not affected by the treatment with minocycline/placebo; changes in olfactory function were not significantly different between groups ($P = 0.681$). In conclusion, treatment with minocycline at a dosage of 2 × 50 mg/day over a period of 21 days cannot be recommended for post-infectious olfactory loss.

Poster Session Thur PM

Reduced Olfactory Sensitivity in Subjects with Depressive Symptoms

Olga Pollatos, Albrecht Jessica, Rainer Kopietz, Jennifer Linn, Veronika Schoepf, Anna Maria Kleemann, Tatjana Schreder, Rainer Schandry and Martin Wiesmann

Ludwig-Maximilians-University of Munich

Clinical studies suggest that olfactory sensitivity is reduced in major depression. Nevertheless, only little is known about the relationship between depressive symptoms and olfactory functions in healthy subjects. We investigated the association between depressive symptoms and olfactory performance in 48 healthy subjects (14 males). First depressive symptoms were assessed using the BDI, following by olfactory testing. Olfactory threshold and discrimination performance was assessed as well as emotional arousal and pleasantness during the testing procedure. We observed a significant negative correlation between olfactory sensitivity and depressive symptoms while olfactory discrimination was not related to depressive symptoms. We conclude that the observed relation between reduced olfactory sensitivity and depressive symptoms could be mediated by

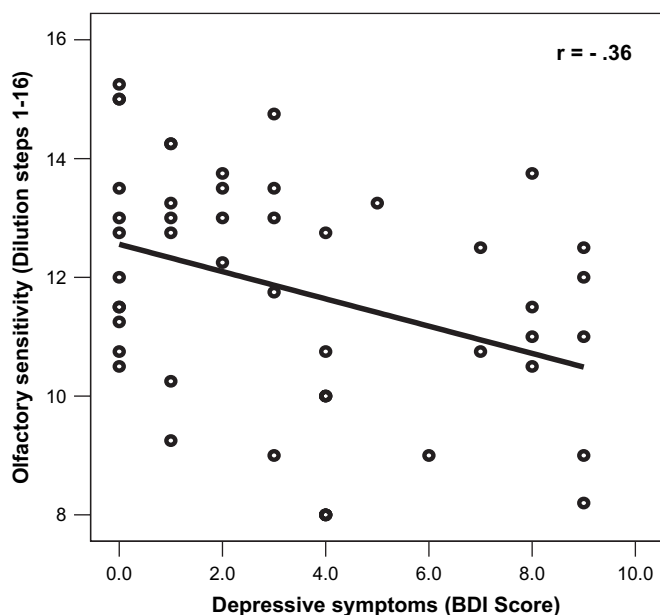


Figure 1 Scatter plot between depressive symptoms and olfactory sensitivity.

functional deviations within brain structures subserving primary olfactory processing such as amygdala and piriform cortex which is in line with results showing abnormal activity pattern in the amygdala and other brain regions in depression.

Poster Session Thur PM

Olfactory Perception of the Odorant Bourgeonal by Infertile and Fertile Men

Eva Kemper¹, Petra Spornraft-Ragaller¹, Hanns Hatt² and Thomas Hummel¹

¹University of Dresden and ²Ruhr-University Bochum

Aim: Aim of the study was to investigate whether patients with idiopathic infertility would exhibit a decreased olfactory sensitivity towards bourgeonal, an agonist for the human olfactory receptor hOR17-4. hOR17-4 has been suggested to play a role in sperm chemotaxis and thus in fertility. **Methods:** Participants were 17 patients with idiopathic infertility participated (age 23–43 years) and 22 controls (all fathers; age 23–41 years). Following a standardized interview, a 12-item odor identification test (“Sniffin’ Sticks”) ascertained normosmia. Odor thresholds for bourgeonal, helional, and phenylethylalcohol were determined in a 3-alternative forced choice paradigm using logistic regression. Intensity ratings were additionally assessed for the odors. **Results:** ANOVAs indicated that patients exhibited a specifically decreased sensitivity towards bourgeonal/decreased intensity ratings of bourgeonal. **Conclusions:** The results suggest that idiopathic infertile men tend to be less sensitive towards the odor of bourgeonal but not to that of other floral odors. It may be speculated that the decreased olfactory sensitivity relates to a decreased functionality of hOR17-4, which in turn may relate to idiopathic infertility.

Poster Session Thur PM

Discrimination of Odor Mixtures: Effects of Stimulation Time, Composition and Training Protocol

Patricia Fernandez, Nicole Rennell, Gregory Deleo, Locatelli Fernando and Brian Smith

ASU

Recognition and discrimination of floral scents by honeybees is a good model for how animals learn about odors. After learning about the relationship between a complex, multicomponent floral odor and carbohydrate or protein rewards offered by flowers, odor recognition is affected by factors such as carbon chain length, functional group, chain shape and concentrations of the components. Recently, there has been considerable debate as to whether stimulation times affect detectability and discriminability of odors. In order to test this hypothesis, we used proboscis extension response conditioning to examine the degree of generalization and discrimination of different ratios of binary odor mixtures (1-hexanol and 2-octanone). We first conditioned bees to either 9:1 or 1:9 ratios. In this protocol, honeybees generalize broadly to all other tested ratios (9:1, 7:3, 5:5, 3:7, 1:9). Discrimination conditioning (9:1+ vs 1:9–) produced steeper decreases in response from the reinforced (+) to the non-reinforced (–) mixture. Reinforcement of 5:5 (+) versus 9:1 or 1:9 (–) produced a peak shift in the response away from the unreinforced mixture, indicating an interaction of positive and negative generalization gradients. We are currently evaluating how shortening stimulus duration affects discrimination via the interaction of these gradients. These data are also being correlated to neural activity patterns in the antennal lobes using calcium imaging.

Supported by DC007997 from NIH-NIDCD.

Poster Session Thur PM

Pyrimidines and Micosporin-Like Amino Acids Function as Alarm Cues in the Defensive Secretions of the Sea Hare *Aplysia californica*

Cynthia Kicklighter¹, Michiya Kamio², Markus Germann² and Charles Derby²

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The sea hare *Aplysia californica* releases ink and opaline, which chemically defends it from attacking predators. In addition to modifying predator behavior, ink and opaline also affect conspecifics by functioning as alarm cues. When *A. californica* juveniles are presented with ink or opaline from other individuals, they exhibit alarm behaviors such as moving away and galloping. Thus, the release of secretions by an attacked sea hare signals to nearby conspecifics that a predator is nearby and evasive behaviors should be produced. We examined this alarm response, including the stimulus specificity and the molecules mediating it. Either ink or opaline alone evokes the full alarm response, but other sea hare secretions or odors from other species do not. Thus, ink and opaline specifically act as alarm cues to nearby conspecifics. We isolated and identified the alarm cues in ink as the base uracil and the nucleosides uridine and cytidine. The 3 alarm cues in opaline are mycosporin-like amino acids, one of which is asterina 330. Ink or opaline without its respective alarm cue molecules does not elicit

a significant frequency of alarm behaviors. Thus, these molecules are necessary and sufficient to produce alarm responses. *Aplysia californica* anti-predator behaviors are also elicited by ink from congeners and from a squid and octopus, due to the presence of uracil and uridine in them. Thus, these alarm cues may be common among ink-releasing mollusks.

Supported by NSF IBN-0324435 and -0614685.

Poster Session Thur PM

Vertebrate Pheromones Affect Female Receptivity in Salamanders

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Vertebrate pheromones that affect female receptivity have been documented in plethodontid salamanders. The source of the plethodontid courtship pheromone is the male's submandibular (mental) gland, which produces a multi-protein secretion. In work with our main study species, *Plethodon shermani* (the red-legged salamander), courtship encounters were staged in which a female received either a pheromone treatment or a saline (control) treatment. Average courtship duration was reduced for pairs in which the female received the pheromone treatment. Thus, the extract of protein secretions obtained from male mental glands acted to increase sexual receptivity in females. In a second experiment, one particular protein in the gland secretion, plethodontid receptivity factor (PRF), was found to act alone to increase female receptivity. In a third experiment, an additional protein, termed "plethodontid modulating factor" (PMF), acted in the opposite manner to "reduce" female receptivity. The natural composition of mental gland secretions includes both PRF and PMF. Although these 2 protein pheromones separately produce opposing messages, the combined effect of both proteins is to increase female receptivity.

This work was supported by the National Science Foundation research grants 0110666, 0416724 and 0416834.

Poster Session Thur PM

Sex Differences in Chemo-investigative Behavior in a Plethodontid Salamander (*Plethodon shermani*)

Stephanie Schubert and Sarah Woodley

Duquesne University

In the terrestrial red-legged salamander (*P. shermani*), the volume of the vomeronasal organ (VNO) in males is 1.7× larger than the volume of the VNO in females, even though males are slightly smaller in body length than females. We hypothesized that the sex difference in VNO volume reflects sex differences in behavioral and vomeronasal responses to chemosensory cues. Nose tapping (a chemo-investigative behavior that brings substrate-borne chemosensory stimuli into the lumen of the VNO) in response to a substrate moistened with water or female-derived body rinses was greater in males than in females. There was no increase in nose tapping in response to a substrate moistened with male-derived body rinses. Responsiveness of the VNO was measured using agmatine uptake as a marker of sensory neuron activation. In both sexes, chemosensory

cues derived from females (cutaneous granular gland secretions) and chemosensory cues derived from males (hedonic mental gland extract) activated more VNO cells than the saline control solution. However, levels of VNO cell activation did not differ between the sexes in response to these 2 chemosensory cues. These results indicate that males chemo-investigate their environment more than females, particularly when female-derived chemosensory cues are present. Future studies will measure VNO responsiveness to additional female-derived chemosensory cues in males and females.

Poster Session Thur PM

Newborn Mice Prefer Odors Indicating Closer Genetic Relatedness

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Previous evidence indicates that adult rodents recognize familiar individuals irrespective of relatedness (but including close kin) but a separate mechanism enables discriminations based on genetic relatedness without prior familiarity. Adult mice can assess the extent of their genetic relatedness to unfamiliar individuals using perceptual similarities between their individual odors. In 2-choice preference tests between odors of paternal aunts versus unrelated conspecifics and conspecifics versus heterospecifics, newborn mice selected the odor of the more genetically similar lactating female even without prior direct exposure to adults with the tested genotypes. These preferences demonstrate that the genetic relatedness assessment process is already present in newborns and that experience with parental odors is not necessary for genetic relatedness distinctions. It remains to be determined whether exposure to odors of other fetuses in the womb or littermates shortly after birth affects this genetic relatedness assessment mechanism.

Poster Session Thur PM

The Identification of Attractive Volatiles in the Aged Male Mouse Urine

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In many species, older males are often preferred mates because they carry "good" genes that account for their viability. How females discern a male's age is a matter of question. However, for animals that rely heavily on chemical communication there is some indication that an animal's age can be determined by its scent. To determine whether inbred female mice prefer the scent of the aged male more than that of adult males, we conducted a preference test and a chemical analysis of the urine of adult (4–8 months) and aged (15–20 months) mice. Our results demonstrated that female mice prefer the odor of aged males rather than adult males. To determine the chemical basis for these preferences, studies were performed using FID-GC and GC-MS in conjunction with HS-SPME. These analyses detected differences in the amount of urinary volatiles between 2 different age groups. The most prominent differences involved significantly greater amount of 2-sec-butyl-4,5-dihydrothiazole (BT), isopropyl-4,5-dihydrothiazole (IT), and 2,3-dehydro-exo-brevicomin (DB), and a significantly

lower level of 6-hydroxy-6-methyl-3-heptanone in the aged mice urine. A behavioral study of the ultrafiltrated mice urine suggested that female mice were attracted to the odor of aged male mice due to, either one of BT, IT, and DB or in combination.

Poster Session Thur PM

Scent Over-marking: Selective Targeting of Rivals by Males and Use in Mate-Choice Decisions by Females

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Scent over-marking, in which one individual deposits a scent mark that at least partially overlaps the scent mark of another individual, is a distinct category of behavior and may have unique functions. In some species, over-marking is a type of male-male competition by which males demonstrate their relative vigor. If so, 2 hypotheses follow: 1) adult males should selectively target the scent marks of other adult male rivals and 2) over-marks should have some function, such as influencing female mate choice decisions. We tested these hypotheses in golden hamsters, *Mesocricetus auratus*. Using a new method that distinguishes between targeted over-marking and over-marking by chance, we found that adult males selectively targeted the scent marks of other adult males but did not target the marks of females, juvenile males, castrated males, or littermate brothers. Females did not selectively target flank marks of other individuals. Regarding functions of over-marking, we found in 2 experiments with different designs that, after exposure to male over-marks, pre-estrous and estrous females preferred the top-scent male over the bottom scent male as measured by close approach and vaginal scent marking, a form of sexual advertisement. Females in other states showed no preferences. These results indicate that males selectively target the flank marks of adult male rivals and that sexually receptive females use the results of flank over-marking competitions in mate-choice decisions.

Supported by NSF grant to R.J.

Poster Session Thur PM

NKCC1 Deletion Does Not Affect Olfactory Sensitivity in Behaviorally-Trained Mice

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When olfactory receptor neurons respond to odors, a depolarizing Cl⁻ efflux is a substantial part of the response. This requires that the resting neuron accumulate Cl⁻ against an electrochemical gradient. In isolated olfactory receptor neurons, the Na⁺-K⁺-2Cl⁻ cotransporter NKCC1 is essential for Cl⁻ accumulation. In intact epithelium, however, a robust electrical olfactory response persists in mice lacking NKCC1. To determine whether NKCC1 is required for normal olfactory perception, olfactory sensitivity was compared in knockout mice carrying a null mutation for NKCC1 with normal, wild-type littermates. Using operant behavioral techniques, olfactory sensitivity was assessed using commercial, liquid-dilution olfactometry (Knosys). Discrimination thresholds for simple odor-

ants lylal, lilial, cineole, 1-heptanol, ethyl acetate and propanol were compared in KO and WT animals, as were thresholds to binary mixtures of lylal and lilial. Regardless of the stimulus conditions employed, no systematic differences in behavioral thresholds were evident between KO and WT animals. We conclude that NKCC1 does not play a crucial role in olfactory perception.

This work was supported by NIDCD grant R01 DC00926.

Poster Session Thur PM

The Loss of the cAMP Mediated Odor Response: Is "Clean Air" Responsible?

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University of Vermont

A well-established principle of neuroscience is "use it or lose it." Recently animal housing conditions have been changed to be cleaner by increasing the air filtration and flow, thus reducing the amount of odors animals are exposed to. Here we present data that in these "odor-deprived mice" the OE has undergone a reduction of the odor induced cAMP mediated responses. Cyclic AMP is the odor transduction pathway that mediates many of the odor responses in mouse olfactory sensory neurons (OSNs). Using gramicidin perforated patch clamp we stimulated OSNs with IBMX/ forskolin to activate the cAMP pathway. Our findings show decreased responses of OSNs from mice in clean air conditions (13%), compared to OSNs from mice in an odor-enriched environment (50%). Additionally, using the same techniques on mudpuppies (aquatic salamanders) produced robust responses, ruling out chemical or experimental problem. Furthermore, pilot behavioral data showed differences between mice in odor enriched environment (unfiltered cages, lower air turnover) and those in "clean" air conditions. Mice were placed in a new, unused cage, and the first 3 min of their behavior were analyzed. Mice from enriched conditions spent more time rearing, and engaging in "search" behavior, whereas those in 'clean' air conditions spent more time digging in their bedding. These combined data suggest that the housing condition of mice is affecting their primary olfactory sensory system, down-regulating odor responses similar to the surgical closing of naris.

Supported by RO1-DC006939 and NIH-P20RR16435.

Poster Session Thur PM

Noradrenergic Modulation in the Olfactory Bulb Influences Spontaneous Olfactory Discrimination in Adult Rats

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The mammalian main olfactory bulb (OB) receives a significant noradrenergic input from the locus coeruleus. Norepinephrine (NE) is involved in acquisition of conditioned odor preferences and has a modulatory effect on attention. However, the action of NE on olfactory discrimination remains unknown. We tested the role of NE in the olfactory bulb of cannulated rats by bilateral injections of vehicle (6 μ l saline), the alpha NE receptor antagonist phentolamine (3.15 or 10 mM), the beta NE receptor antagonist

alprenolol (12 or 120 mM), the alpha1 NE receptor antagonist prozasin (1 or 1.2 mM) and the alpha2 NE receptor antagonist yohimbine (1.10⁻³ or 1.10⁻⁵ mM) 20 min before the behavioral tasks. Antagonist effects on spontaneous odor discrimination were tested in the absence of reward conditioning using a habituation task. We found that local blockade of NE receptors in the OB did not affect the habituation to an odorant across sequential presentations separated by 5 min intertrial intervals. Spontaneous discrimination between chemically related odorants was impaired when noradrenergic receptors, and in particular alpha1 receptors, were blocked by local antagonist infusion into the OB. Interestingly, spontaneous discrimination was improved in our experiments when beta receptors were blocked. These findings indicate that NE release in the OB, via activation of alpha receptors, improves discrimination among similar odorants.

Supported by a Marie Curie Fellowship to N.M. and CRCNS R01DC008702 (NIDCD) to C.L. and M.E.

Poster Session Thur PM

Odor Sample Time: Simple versus Difficult Discrimination Tasks

Burton Slotnick

University of South Florida

Odor sample time on S+ and S- trials was measured for mice trained on a variety of detection and discrimination problems in an olfactometer using a go, no-go discrimination procedure. Odor sample time was defined as the interval between operation of odor control valves and head removal from the odor sampling port (reinforcement tube located outside of odor port). Delay from valve operation to delivery of odor to subject is unknown but estimated at less than 200 ms. In simple odor detection tasks (e.g., vapor from 0.5% ethyl acetate solution vs air) mean odor sample time was 388 ms. Mice sampled longer on S- than S+ trials initially but with continued training sample time on both types of trials were equivalent. Simple 2-odor discrimination tasks produced a small (~50 ms) increase in sample times but more difficult odor mixture discrimination tasks or odor intensity difference tasks resulted in a greater, abrupt, and sustained increase in S+ and smaller increase in S- sample times. The magnitude of this increase in sample time was related to task difficulty as indexed by response accuracy.

Supported in part by NIH grant DC04671.

Poster Session Thur PM

BACE1 Is Necessary for Normal Olfactory Behavior in Mice

Jurgen Ziesmann, Sharon R. Gracey, Emily M. Maarschalk, Loren A. Martin, Dipti D. Patel, Jennifer Somers, Robyn J. Sumpter and Anthony S. Walls

Azusa Pacific University

BACE1 is a key enzyme in the pathology of Alzheimer's disease. One of the first signs of Alzheimer's is a change in the sense of smell. We investigated the potential role of BACE1 in olfaction, comparing olfactory behavior of heterozygous (+/-) and knockout (-/-)

BACE1 mice with the behavior of wild type C57BL/6 (+/+) mice. BACE1 -/- adults are viable, but show some cognitive deficits. Therefore, tests of olfaction which involve conditioning or habituation, and hence require functioning memory capabilities, could not be used. In our tests we measured spontaneous behavior of mice in response to odorants. No learning or training of the mice is necessary. The first test uses exploratory behavior of novel objects. Male mice were allowed to explore 2 amber vials in an open field, with or without odorant (male or female urine or water control). BACE1+/+ males explore the vial with female urine significantly longer than -/- mice. ($F_{(2,61)} = 4.34, P = 0.022$). They also explore urine from a female longer than that from a male, while -/- mice show no such preference. The second test uses typical foraging behavior of 12 h food deprived mice. +/+ Mice find a food pellet buried in bedding quicker than +/- or -/- mice ($F_{(2,61)} = 5.30, P = 0.008$). The same result is observed when the food is presented within a background of vanillin, a novel odorant to the mice ($F_{(2,61)} = 7.34, P = 0.001$). However, all knockout mice found the food pellets in all tests indicating that the olfactory system is at least partially functional in these mice.

Poster Session Thur PM

Chemosensory Basis for an Ecological Paradigm in the Rocky Intertidal

Graham Ferrier, Steven Kim, Cheryl Ann Zimmer and Richard Zimmer

University of California

The rocky intertidal has provided stellar examples of predators stabilizing prey populations, but still elusive is how predators locate preferred prey in this dynamic habitat. In a seminal work, Murdoch predicted that predatory whelks (*Acanthinucella spirata*) switch prey species depending on relative abundances. At sites along the southern California coast, we revisited this hypothesis and showed 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 is explained by their ability to exploit an insoluble proteinaceous cue. A protein complex of ~200 kDa was extracted from *B. glandula*, purified, and bound to nitrocellulose membranes. Portions of membranes treated with the extracted protein caused arrestment in *A. spirata*, but there was no significant effect of equivalent preparations from alternative prey (mussels, *Mytilus* spp.; turban snails, *Tegula funebris*). Proteins from each species also were imbedded in gel (at natural prey concentrations) and placed in acid-washed heat-treated barnacle tests. *Acanthinucella spirata* fed in 100% of all feeding-preference trials, but only on *B. glandula* infused gels. Selective predation by *A. spirata* is therefore determined by the detection of insoluble proteins bound to surfaces of live barnacles.

Poster Session Thur PM

The Role of Vomeronasal Organ in Mediating Responses to Predator Odor

Vera Voznessenskaya, Maria Klyuchnikova and Anna Voznesenskaya

Institute of Ecology and Evolution

In our earlier studies we examined the influence of predator chemical cues derived from feral cat urine on reproductive output of rodents: rats and mice. Animals responded to cat odor with reduced litter size and skewed sex ratio. The reduction in litter size in rodents exposed to cat urine was attributable to suppressed progesterone levels affecting the implantation of embryos. In the current study we investigated the role of vomeronasal organ (VNO) in mediating the response to predator chemical cues in rats (*Rattus norvegicus*) and mice (*Mus musculus*). We used 2 basic approaches: vomeronasal surgery (VNX) followed by histochemical verification and immunohistochemistry (*c-fos*). VNX completely suppressed the response to cat urine in rats ($n = 12$) and mice ($n = 12$). At the same time we observed reductions in litter size in sham operated rats ($n = 12$, $P < 0.001$) and mice ($n = 12$, $P < 0.001$). Fos-positive cells were counted in accessory olfactory bulb (AOB) of mice after 40 min of intermittent stimulation with cat urine ($n = 8$). Consistent pattern of activation was observed in rostral part of AOB. Also we observed fos-positive cells in apical zone (VIR) of sensory epithelium of mice VNO ($n = 10$) after 90 min of stimulation with cat urine. Behavioral and immunohistochemical data indicate the involvement of VNO in mediating responses to predator chemical cues in rodents.

Supported by RFBR 04-04-48723 and RAS, Program "Biological Resources" #3.1.5.

Poster Session Thur PM

Transgenic Mice Expressing an Inducible Cyclic AMP Reporter

Joung Woul Kim, Craig Roberts, Stephanie Berg, Stephen Roper and Nirupa Chaudhari

University of Miami Miller School of Medicine

Cyclic AMP (cAMP) is a ubiquitous second messenger in chemosensory tissues. Sweet, bitter and umami tastants alter cAMP levels in taste cell. The significance of cAMP signals in taste buds remains uncertain pending the ability to monitor cAMP dynamics in individual cells and in real-time. To achieve this, we introduced a cAMP reporter in the genome of mice by pronuclear DNA injection. The 2 subunits of the reporter were first cloned into a single tetracycline-inducible vector, pBI. Three such pBI-cAMP transgenic mouse lines were then evaluated for expression of the cAMP reporter by crossing against 2 different lines of transactivator mice, hCMV-rtTA and Ins2-rtTA. Reporter expression was induced with doxycycline. In pBI-cAMP \times hCMV-rtTA mice, some abdominal skeletal muscles, ovary, and pancreatic acini showed fluorescence indicative of the cAMP reporter. In pBI-cAMP \times Ins2-rtTA mice, reporter expression was limited to β -cells of pancreatic islets. We observe that elevating cellular cAMP levels with forskolin or IBMX reproducibly produces a graded change of the fluorescence resonance energy transfer (FRET) signal, demonstrating that the transgenically expressed cAMP reporter functions as expected. This highly flexible system for detecting cAMP dynamics will be a powerful tool for dissecting the role of cAMP in chemosensory cells such as those in the taste bud.

Supported by NIH/NIDCD (F31DC007591 to C.R.; R01DC006308 to N.C.; R01DC000374 to S.R.).

Poster Session Thur PM

Artificial Sweeteners Stimulate Sensory Neurons through Activation of TRPV1 Receptors

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Throughout the world many people use artificial sweeteners (AS) for the sole purpose of reducing their caloric intake. These molecules include saccharin, aspartame, acesulfame-K and cyclamate. Despite the caloric advantage they provide, their use is characterized by an aversive aftertaste that has been described as bitter and/or metallic. From a sensory point of view, it is now known that bitterness and burning are closely related. We have investigated whether this class of molecules could stimulate sensory neurons by activating TRPV1 receptors. These receptors, which are found throughout the oral cavity, are known to respond to a large number of structurally different chemicals ranging from vanilloids such as capsaicin, the pungent molecule in hot chilli peppers, to protons, alcohols, terpenes, aldehydes and lipids. Therefore, we tested if TRPV1 would also be responsive to artificial sweeteners by using Fura-2-based Ca^{2+} imaging on TRPV1 receptors heterologously expressed in HEK293 cells and dissociated primary sensory neurons from dorsal root ganglia. Our results show that AS activate TRPV1 receptors in a dose dependent manner with saccharin being the most potent agonist. AS stimulate capsaicin sensitive sensory neurons and their induced activation could be blocked by SB366791, a specific TRPV1 inhibitor. Our results not only present a novel modulation of TRPV1 channels by providing a molecular connection between sweeteners and nociception, but they also might explain some of the off tastes of these compounds.

Poster Session Thur PM

Recording Chemosensory Responses in Pancreatic Islets from Transgenic Mice Expressing a cAMP Reporter

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cAMP is a second messenger in a variety of chemosensory receptors, including glucose-sensitive pancreatic β cells. We have developed a mouse strain expressing a cAMP reporter in a tissue-specific and tetracycline-inducible manner. This reporter enables us to image real-time changes in single-cell cAMP levels by fluorescence resonance energy transfer (FRET). We crossed this mouse with another strain expressing tet-activator in beta cells of the pancreas. We induced expression of the cAMP reporter by injecting the progeny mice with doxycycline. When islets from such mice were stimulated with the adenylate cyclase activator, forskolin (10 μ M), we observed reliable changes in reporter fluorescence in individual cells corresponding to changes in intracellular cAMP. Such islets responded to increasing concentrations of glucose (5.5–35 mM) with an increase in cAMP levels. The half maximum of 9 mM glucose for the cAMP response corresponds well with reported glucose concentrations that elicit insulin release from whole islets. Stimulating pancreatic islets with glucose is known to drive Ca^{2+} influx into

B-cells. When we simultaneously imaged both second messengers, we found that Δ cAMP precedes and is independent of Δ Ca²⁺. The flexibility of the tet-system will enable cAMP reporter expression in numerous cell types, including those which mediate gustatory and olfactory transduction.

Supported by NIH/NIDCD (F31DC007591 to C.R.; R01DC006021 to N.C.; R01DC000374 to S.R.).

Poster Session Thur PM

Functional Characterization of Odorant Receptor Mediated Signaling Mechanisms in Prostate Cancer Cells

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Ruhr-University Bochum

Olfactory receptors (ORs) comprise the largest superfamily of G-protein coupled receptors. In addition to their expression in the olfactory epithelium, ORs are shown to be expressed in other tissues, such as testis. Here, we describe cloning and functional expression of a human OR family member that is known to be expressed in prostate epithelial cells (prostate specific G-protein coupled receptor, PSGR). We employed Ca²⁺ imaging in HEK293 cells to identify potential PSGR ligands and characterized the receptor's molecular receptive field. As PSGR was found to be overexpressed in prostate cancer, we investigated whether the identified ligands induce responses in a prostate cancer epithelial cell line (LNCaP). Imaging techniques revealed an increase in intracellular Ca²⁺ via depletion of intracellular stores. This was confirmed in patch clamp experiments. Further electrophysiological analysis will shed light on the signal transduction molecules/channels involved in this pathway. Treatment of LNCaP cells with the PSGR ligand also resulted in a time dependent activation of members of the MAPK family. Additionally, the ligand was found to be a potent inhibitor of cell proliferation and induced apoptosis. Similar results were obtained from primary prostate cancer cells from resection specimens. Thus, our results suggest that PSGR could provide a novel therapeutic target for the treatment of prostate cancer.

Poster Session Thur PM

The Reception of ESP Peptides in Rodent Vomeronasal System

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The University of Tokyo

We recently identified a sex-specific peptide, named ESP1, in tears of male mice, which are received in vomeronasal organ (VNO). The secreted ESP1 appears to be transferred to the female VNO wherein it elicits an electrical response in vomeronasal sensory neurons (VSNs), suggesting that ESP1 is a candidate sex-pheromone in mice. The ESP1 gene is a member of a multigene family (the ESP family) that consists of 38 homologous genes in mouse genome. Among this family, 15 genes are expressed in exocrine glands, 2 out of which show sexually dimorphic expression pattern. Thus, the expression of ESP1 is induced exclusively in adult male mice, while ESP36 expression is suppressed in adult males. Moreover,

ESP1 induced c-Fos expression in VSNs expressing V2Rp5, suggesting that ESP1 is received by the specific receptor. Interestingly, the expression level of V2Rp5 proteins was different between sexes. Additionally, 7 orthologous ESP genes were found in rat genome. Five of them were expressed in exocrine glands, one of which is expressed only in female rats. These results indicate that ESP peptides possess pheromonal information including sex and species in rodents. Elucidation of the function of ESP peptides and their receptive mechanism in the vomeronasal system will provide a molecular basis for the pheromonal communication in rodents.

Supported by PROBRAIN, Japan.

Poster Session Thur PM

Termination of Lingual Nerve Afferents near a Small Subset of Neurons in Rostral Nucleus of the Solitary Tract (NTS) That Express Fos-Like Immunoreactivity (Fli) following Electrical Stimulation of the Chorda Tympani Nerve

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Previous studies have revealed overlapping projections in the rostral NTS from chorda tympani (CT, VIIbis) and trigeminal (V) afferents in hamsters and rats, but details of this convergence at the cellular level are lacking. The aim of this study was to visualize NTS neurons receiving convergent trigeminal and gustatory inputs in male rats. The bidirectional tracer BDA (10 kDa) was applied to the central cut end of the lingual nerve proximal to its separation from the CT. One week later, the ipsilateral CT nerve was electrically stimulated to induce, after which rats were perfused and brainstems processed for double-labeling of BDA and FLI. BDA-labeled fibers and terminal boutons were observed mainly laterally, with numerous thin fibers and en passant terminals in the central part of NTS. Following CT stimulation, FLI was observed mainly in the central third of the rostral NTS ipsilaterally, with less FLI at the rostro-lateral pole. FLI was also observed bilaterally in the caudal-most aspect of NTS. In central and lateral aspects of rostral NTS, a small number of neurons expressing FLI were in very close proximity to BDA-labeled terminal boutons, consistent with the possibility that they receive convergent input from gustatory and trigeminal afferents. The overlapping distributions of FLI and BDA labelling are in agreement with previous tracer studies.

Poster Session Thur PM

Microvillar Non-olfactory Cells in the Main Olfactory Epithelium

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Previously we noted that in transgenic mice where the TRPM5 promoter drives expression of green fluorescent protein (GFP) there are

2 populations of cells expressing GFP. One population represents olfactory sensory neurons (OSNs). Here we describe morphology and immunocytochemical properties of the other population. These cells are short (<15 μm) with soma located in the apical layer among nuclei of supporting cells. They are scattered throughout the entire olfactory epithelium. A prominent feature is their apex bearing several microvilli. In contrast to OSNs, axonal processes are absent in most of the cells. Antibodies against the neuronal marker PGP9.5 which labels OSNs failed to react with these short cells, suggesting that they may not be neurons. Consistently, antibodies against OMP and CNGA2 did not label these cells; neither did antibodies against PLC $\beta 2$ and α -gustducin which label solitary chemosensory cells. At the electron microscopic level, 2 types of microvillar cells were reported which differ in morphology of microvilli and cell organelles. EM-immunocytochemistry reveals that the type bearing "wavy" microvilli and containing centrioles in their dendrites are GFP-positive. Our data indicate a population of non-neuronal cells in the olfactory epithelium.

(Supported by grants DC06828 (W.L.); DC00566, DC04657, DC06070 (D.R.); DC07732 (A.H.); and DC03055, DC03155 (R.M.).

Poster Session Thur PM

Nasal Solitary Chemoreceptor Cells Respond to Denatonium But Not to Other Classic Bitter or Trigeminal Stimulants

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Nasal trigeminal chemosensitivity in mice and rats is mediated in part by epithelial solitary chemoreceptor cells (SCCs). Histological evidence suggests that mature SCCs express many elements of the bitter taste transduction pathway including T2R receptors, the G protein α -gustducin, PLC $\beta 2$, and TRPM5. This has led to speculation that SCCs are the receptor cells that mediate trigeminal nerve responses to bitter receptor ligands. We used calcium imaging to ask if SCCs respond to classic bitter or trigeminal stimulants. SCCs from the anterior nasal cavity were isolated from transgenic mice in which green fluorescent protein (GFP) expression was driven by the TRPM5 promoter. Isolated cells were exposed to a variety of test stimuli to determine which substances caused an increase in intracellular Ca^{2+} . GFP positive cells respond with increased intracellular Ca^{2+} to the bitter receptor ligand denatonium, and this response is blocked by the PLC inhibitor U73122. In addition SCCs respond to the PLC activator 3M3FBS, and the neuromodulators ATP and ACh, but do not respond to other bitter or trigeminal stimulants. These results suggest that TRPM5-expressing nasal SCCs broadly express the receptor for denatonium. The mechanisms by which the trigeminal nerve responds to other bitter receptor ligands, for example, cycloheximide, remain enigmatic.

Support: NIDCD grants NRSA DC008275-01, RO1 DC 006070 and P30 DC 04657.

Poster Session Thur PM

Chemically-Mediated Gamete Interactions in a Sea Urchin: A Model Organism for Studies of Fertilization and Embryogenesis

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University of California

The sea urchin, *Arbacia punctulata*, is a keystone system in the field of developmental biology, and an important model for human fertilization. Twenty years ago, a 14 amino-acid oligopeptide isolated from *A. punctulata* eggs ("resact") was claimed to elicit sperm chemotaxis, and has since served as a paradigm for sperm-egg communication. Several studies have noted the impact of this chemical on various aspects of sperm physiology, such as respiration, calcium ion influx, and membrane potentials. No study, however, has confirmed the chemotaxis of sperm to live eggs. This investigation compared sperm locomotory performance and behavior near single *Arbacia* eggs and in the presence of synthetic resact or egg extracts. Preliminary results indicate that concentrated extracts and resact stimulated rapid changes in the direction of the helical path, inducing an accumulation of sperm within the chemical gradient. However, sperm failed to orient or navigate to single eggs. These initial results suggest that resact does not perform as a long-distance chemotactic signal for single eggs, but acts through kinesis to keep sperm in the vicinity of egg clusters that naturally form on the spines of females during spawning events. Thus, resact may serve as a cooperative signal to increase the probability of sperm-egg encounters for multiple cells rather than a guide for sperm to locate individual eggs, and may be an inappropriate model for signaling in single-egg systems such as human fertilization.

Poster Session Thur PM

The Elephant as an Ideal Olfactory Model Organism

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¹HortResearch, ²University of Auckland, ³OGI School of Science and Engineering, Oregon Health and Science University and ⁴September

The last 20 years has been a productive time for studies on olfaction, helped in no small way by technological developments in the analysis of volatile compounds. The role of behavior as a tool for guiding bioassay-directed fractionation of semiochemical-containing extracts is now a well-defined principle and a particularly successful approach for identifying the individual components and active blends involved in chemical signalling in animals including mammals. The Asian elephant *Elephas maximus* has been the subject of our study from which through careful analysis of elicited responses to compounds presented to wild and captive animals, we have been able to define the role of several compounds present in various elephant glandular secretions that initiate key behaviors in conspecifics that are important for maintaining social interactions and indeed for ensuring reproductive success of this endangered animal. Our work argues that elephants are a compelling model for olfaction assisted by its demonstrative trunk and the spatial-temporal separation of events due to its large size. Our recent finding that the ratio of enantiomeric forms of one of its defined

pheromones differentially modulates behavioral activities depending on gender and life stage is evidence of detail that rivals, if not surpasses, that of model rodent species.

NSF Award to L.E.L. Rasmussen and D.G. #0544191.

Poster Session Thur PM

Defense through Chemoreception: An L-Amino Acid Oxidase in the Ink of Sea Hares Deters Predators through Their Chemical Senses

Michiya Kamio, Cynthia Kicklighter, Ko-Chun Ko, Matt Nusbaum, Juan Aggio, Melissa Hutchins and Charles Derby

Georgia State University

Sea hares release a purple ink upon predatory attack, which can deter consumption by many predators. One ubiquitous compound in the ink is an L-amino acid oxidase. The chemistry of this enzymatic pathway is complex, producing α -keto acids and carboxylic acids derived from L-lysine and L-arginine, hydrogen peroxide, ammonium, and others. L-Amino acid oxidases have antimicrobial and antitumor effects, but anti-predatory effects are unknown to date. Here, we show that the products of "escapin," the L-amino acid oxidase of the sea hare *Aplysia californica*, acts on chemosensory systems of some crustaceans to produce anti-predatory effects. Against spiny lobsters (*Panulirus interruptus*), some escapin products suppress food searching behavior. However, none of the escapin products deter feeding by spiny lobsters. Escapin products also do not deter sea anemone (*Anthopleura sola*) or fish (senorita wrasse *Oxyjulis californica*, pinfish *Lagodon rhomboides*, mummichog *Fundulus heteroclitus*). However, they do deter feeding by a crab (*Callinectes sapidus*). Overall, our results show that the L-amino acid oxidase of sea hares produces compounds that affect the chemosensory systems and feeding behavior of some predators. This is the first demonstration of the anti-predatory effects of sea hare L-amino acid oxidases.

Supported by NSF IBN-0324435 and IBN-0614685.

Poster Session Thur PM

Membrane-Associated Mitochondria Contribute to Depolarization-Mediated Swimming Behavior in *Paramecium*

Wade Bell¹, Eri Kamura¹ and Richard Hallworth²

¹Virginia Military Institute and ²Creighton University

Paramecium swimming behavior is regulated by changes in membrane potential that control the rate and direction of ciliary beating. Multiple currents have been identified both electrophysiologically and genetically suggesting that Na⁺, K⁺ and Ca²⁺ channels and/or pumps are the primary effectors of membrane potential regulation in these ciliates. We previously identified a ryanodine-sensitive calcium flux in *Paramecium* that can be observed under depolarizing conditions such as an increase in external K⁺ concentration. Surprisingly, attempts to localize these channels led not to the endoplasmic reticulum (the normal site of ryanodine receptor/channels) but to mitochondria. Here we present both microscopic

and biochemical evidence that mitochondria are indeed the organelles that possess this ryanodine-sensitive channel in *Paramecium*. In addition, our recent work suggests that 2 subpopulations of mitochondria exist in *Paramecium*. Those mitochondria in close association with the membrane have ryanodine sensitive receptors while those located in proximity to the endoplasmic reticulum do not. These results confirm recent studies that demonstrate subpopulations of mitochondria can exist in the same cell and play different roles in cellular homeostasis.

Supported by a VMI Research Grant in Aid.

Neural Coding in the Chemical Senses

Neural Coding in the Chemical Senses: Networks and Systems

Christian Lemon

University of Tennessee Health Science Center

The neural pathways that represent gustatory and olfactory information are composed of dynamic, interactive networks of neurons arranged in series, recurrently and in parallel along the neuraxis. During stimulus processing, neurons within and between networks engage one another in a time-dependent fashion to shape and evolve neural representations of tastes or odors as related to variables such as environmental factors, physiologic status of the organism and experience. Establishing the operational principles of networks for taste and olfaction will be critical for delineating the logic of neural coding and information processing in these senses. "Network processing" ultimately constitutes the mechanism by which the nervous system gives rise to chemosensory perception and guides behavior. This symposium will focus on gustatory and olfactory neural coding as viewed from a systems/network perspective. The participants are active researchers in this area and each brings a unique approach to this topic.

Neural Coding in the Chemical Senses

Neural Modulation of Central Taste Processing

Robert Lundy

University of Louisville

Animals respond to a wide variety of sensory stimuli on a daily basis. The central nervous system processes sensory information in such a way that an identical stimulus may elicit distinct reactions under different environmental and behavioral conditions. This multifarious responsiveness implies that modulatory systems in the brain are essential components of the neural circuitry underlying adaptive behavioral output. Lower-order sensory nuclei are reciprocally connected with the forebrain, and this anatomical arrangement provides a substrate for altering the input available for "higher order processing." Through facilitative and inhibitory mechanisms, centrifugal projections can selectively shape the response characteristics of sensory neurons at each stage of the system. Such altered neural representations of sensory stimuli reflect the neural plasticity associated with processes like learning and motivation. This presentation will discuss how descending forebrain projections influence taste processing in the brainstem.

Neural Coding in the Chemical Senses

The Role of Sampling Behavior in Shaping Odor Coding in Awake Animals

Matt Wachowiak, Justus Verhagen and Daniel Wesson

Boston University

The neural code for odors is initially determined by receptor expression patterns of olfactory receptor neurons (ORNs) and their projections to olfactory bulb glomeruli. While these features are invariant over a short time-scale, odor coding by higher-order neurons can be very plastic, with response properties changing with behavioral state and over time-scales of minutes or seconds. Much of this plasticity is attributed to centrifugal modulation or other network phenomena. Another possibility is that changes in odor sampling behavior (sniffing) alter ORN responses and, subsequently, higher-order coding. We have investigated how sniffing alters odor coding by imaging input to the olfactory bulb in awake rats during different sniffing behaviors. Sniffing dramatically alters both spatial and temporal patterns of ORN input. At low sniff frequencies typical of resting animals, ORNs respond with short bursts of activity after each inhalation and encode information about all odorants present. In contrast, at higher frequencies typical of active investigation, ORNs do not burst after each sniff, rapidly adapt to continued odor presentation, and primarily encode information about changes in the olfactory environment. This frequency-dependence occurs independent of stimulus valence or novelty, and can be recreated by “playing back” natural sniff patterns in the anesthetized animal. Thus, sniffing alone can alter odor coding by ORNs and significantly transform odor representations. These changes likely also shape olfactory processing in the olfactory bulb and beyond.

Funded by NIH DC06441 and DC008197.

Neural Coding in the Chemical Senses

Cortical Networks and the Processing of Tastes

Donald Katz, Lauren Jones and Alfredo Fontanini

Brandeis University

Analyses of single-neuron responses to taste stimuli have revealed many properties of the gustatory system, and have engendered a range of theories as to the nature of taste coding. A full accounting for how tastes are processed, however, requires an analysis of taste networks themselves—that is, of the interactions between neurons that reside within those networks. Here, we present just such a network analysis, showing that ensembles of neurons in gustatory cortex respond to taste administration in a coherent, dynamic manner. These coherent dynamics—sequences of stable states—are taste-specific and reliable, unfolding in anticipation of taste-specific behavioral responses. Because the rate at which they unfold varies from trial to trial, however, they make use of information that is treated as “noise” in more traditional analyses, and thus do a much better job of identifying tastes on a single-trial basis than across-trial averages. This simple, dynamical characterization captures important, novel facets of taste codes, by treating simultaneously active neurons as part of a unified network process.

Neural Coding in the Chemical Senses

Encoding Odor Plumes with a Temporally Structured Neural Representation

Mark Stopfer

National Institutes of Health

Sensory neural systems use spatio-temporal coding mechanisms to represent stimuli. These time-varying response patterns sometimes endure longer than the stimulus. Can the temporal structure of a stimulus interfere with, or even disrupt the spatio-temporal structure of the neural representation? We investigated this potential confound in the locust olfactory system. We made intracellular and extracellular recordings from olfactory interneurons while presenting odorants as natural plumes within a small wind tunnel. The plume stimuli elicited odor-specific spiking patterns from first-order interneurons (projection neurons, PNs) that could outlast the brief odor filament interactions with the antenna. When controlled odor pulses odors were presented in plume-like trains of nearly overlapping pulses, responses of PNs changed reliably and often greatly with pulse position, as responses to one pulse interfered with subsequent responses. However, using the responses of an ensemble of PNs together, we could accurately classify the odorants, while characterizing the temporal properties of the stimulus. Further, we found that second-order, follower neurons (Kenyon cells) showed firing patterns consistent with the information in the PN ensemble. Thus, ensemble based spatio-temporal coding could disambiguate complex and potentially confounding temporally structured sensory stimuli, providing an invariant response to a stimulus presented in various ways.

Gastrointestinal Chemosensation

Gene Expression Analysis Shows That Intestinal Taste Receptor-Like Cells Regulate Multiple Physiological Processes

Sami Damak, Johannes le Coutre, Carole Bezençon, Andreas Fürholz, Frederic Raymond and Robert Mansourian

Nestlé Research Center

The chemical nature of food in the gastrointestinal (GI) tract plays an important role in how GI physiological functions are affected. However, the cells and receptors that allow the GI tract to chemically analyse its contents are largely unknown. It has been suggested that solitary cells expressing taste receptors and signalling proteins act as GI chemosensors. We carried out a microarray study of the transcriptome of Trpm5 expressing intestinal cells that have been labelled with eGFP in transgenic mice, sorted by FACS, and compared it to the transcriptome of intestinal cells that do not express eGFP. The findings of the study are: 1) Most eGFP+ cells look like and express markers of tufted cells. 2) Most proteins involved in taste signal transduction are expressed in eGFP+ cells, although sometimes the isoforms are different. 3) A subset of eGFP+ cells have synapses with afferent and efferent nerves. 4) POMC and GRP, 2 hormones that play a role in food intake control, are expressed specifically in eGFP+ cells. 5) Several genes that play a role in inflammation, including cox-1 and cox-2, are expressed

specifically in eGFP+ cells. Furthermore, these cells express the entire biosynthesis pathway of leucotriene C4, an eicosanoid also involved in modulation of intestinal smooth muscle contraction. 6) Finally, angiotensinogen, renin and the succinate receptor are expressed in the eGFP+ cells, suggesting a role in the regulation of water and sodium transport, vasomotricity and blood pressure.

Gastrointestinal Chemosensation

Glutamate Receptors in the Gastrointestinal Tract

Ana San Gabriel¹, Takami Maekawa¹, Hisayuki Uneyama¹, Sumio Yoshie² and Kunio Torii¹

¹Ajinomoto Co., Inc. and ²Nippon Dental University

Like in the oral cavity, the gastrointestinal (GI) tract also responds to chemical signals at the lumen from the diet. Whereas taste perception confers cognitive discrimination, sensorial information activates the vagal afferent fibers that innervate the gastric mucosa in the stomach. We have already reported that this response is specific to L-glutamate. Other amino acids do not evoke this stimulation of the vagus nerve. Therefore, in this study, we examine the existence of potential glutamate receptors in the GI tract by RT-PCR, Western blot and immunohistochemical analysis within the gastric lumen. In addition, we will discuss the effects that L-glutamate has in stomach physiology.

Gastrointestinal Chemosensation

Taste Receptor Signaling in Enteroendocrine Cells of the Mammalian Gut

Enrique Rozengurt

UCLA School of Medicine

We found that taste receptors of the T1R (sweet) and T2R (bitter) families as well as the alpha subunit of the taste-specific G protein gustducin (Galphagust) are expressed prominently in the mouse and human GI tract. Double-immunofluorescence studies demonstrated the existence of solitary Galphagust-positive cells containing chomogranin A immunoreactivity (a marker of endocrine cells) in the epithelium of the GI mucosa. In the mouse and human colon, Galphagust-positive cells can be identified as enteroendocrine L cells, as shown by the co-localization of Galphagust with peptide YY (PYY) and GLP-1 immunoreactivity. We also demonstrated the expression of taste transducers, including receptors (e.g., multiple T2Rs and T1Rs), G proteins (Galphagust and Galphat-2) and effectors (PLCbeta2 and TRPM5) in enteroendocrine STC-1 cells. Addition of bitter stimuli, including denatonium benzoate (DB) or phenylthiocarbamide (PTC) or amino acids to STC-1 cells initiates a rapid increase in the intracellular concentration of Ca²⁺ by stimulating Ca²⁺ influx via L-type voltage-sensitive Ca²⁺ channels. The expression and function of taste receptors in specific cells of the lining of the GI mucosa and the unraveling of the signal transduction pathways that mediate their biological effects in these cells opens new avenues for understanding molecular sensing in the GI tract and for developing novel therapeutic compounds that modify the function of these receptors in the gut.

Gastrointestinal Chemosensation

Glucose Sensing and Regulation of Intestinal Glucose Absorption

Soraya Shirazi-Beechey

University of Liverpool

Sodium/glucose co-transporter isoform 1, or SGLT1, is expressed on the luminal membrane of intestinal absorptive cells (enterocytes). It transports dietary sugars from the lumen of the intestine into enterocytes. Regulation of this protein is important for the provision of glucose to the body, and therefore has nutritional and clinical significance. It has been shown that the expression of SGLT1 is regulated by luminal monosaccharides, this regulation is independent of glucose metabolism, and is initiated through glucose sensing. To date, the components of sensing system have remained unidentified. We show that taste receptors T1Rs and α subunit of G protein gustducin ($G\alpha_{gust}$) are expressed in enteroendocrine cells. Using knockout mice we demonstrate that the sweet taste receptor subunit T1R3, and $G\alpha_{gust}$ are required for the intestinal sensing of luminal sugars and subsequent upregulation of SGLT1 expression. We also report that mice fed artificial sweeteners, commonly employed in food and beverages, display a significant increase in SGLT1 expression resulting in an increased capacity to absorb dietary sugars. Furthermore, we show that the artificial sweetener, sucralose, acts via sweet taste receptor to stimulate secretion of glucagons like peptides implicated in upregulation of intestinal SGLT1. The involvement of gut-expressed sweet taste receptor and gustducin in sensing dietary sugars and sweeteners makes them potential targets for therapeutic modulation of the capacity of the gut to absorb sugars.

Gastrointestinal Chemosensation

Taste Receptors and Gustducin in Gut Regulate GLP-1 Secretion

Z. Kokrashvili¹, H.J. Jang², M.J. Theodorakis², O.D. Carlson², B.J. Kim², J. Zhou², H.H. Kim², X. Xu², S.L. Chan², M. Juhaszova², M. Bernier², B. Mosinger¹, J.M. Egan² and R.F. Margolskee¹

¹Mount Sinai School of Medicine and ²National Institutes of Health

Glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (GIP) are hormones secreted from enteroendocrine cells of the gut that enhance insulin secretion after oral intake of glucose. How luminal glucose elicits the release of GLP-1 and GIP from enteroendocrine cells was previously unknown. That intravenous glucose does not induce GLP-1 secretion led to the conclusion that glucose acts on the luminal surface of the gut to stimulate L-type enteroendocrine cells to secrete GLP-1. We sought to identify the glucose-sensing mechanism underlying this response. We have found that L cells express T1R taste receptors, gustducin and several other taste transduction elements. Ingestion of glucose by gustducin null mice revealed deficiencies in these mice in secretion of GLP-1, and in regulation of plasma insulin and glucose. Glucose-dependent GLP-1 secretion from isolated intestine or intestinal villi from gustducin null mice was severely decreased, indicating that expression of gustducin in intestinal cells is required for the normal

release of GLP-1 from L cells. Additional experiments with T1R3 null mice and enteroendocrine cell lines implicate T1R3-containing sweet receptors in regulating GLP-1 release. We conclude that L cells of the gut use T1R receptors and gustducin to “taste” glucose.

Supported by NIH grants DC03055 (R.F.M.), DK73248 (B.M.) and the Intramural Research Program of the NIA/NIH (J.M.E.).

Gastrointestinal Chemosensation

Flavor Preferences Modified by the Post-oral Actions of Tastants

A. Sclafani

Brooklyn College, City University of New York

Food preferences are determined not only by the orosensory (taste, odor, texture) properties of food but also by the post-oral actions of nutrients. Flavor preferences are conditioned in rodents by intra-gastric (IG) nutrient infusions but little is known about the post-oral sensors involved. The discovery of taste signaling proteins in the GI tract raises the possibility that food preferences are influenced by sensory input from “taste” cells in the gut. This was examined by comparing the oral and post-oral responses of mice to different sweeteners. C57BL/6 (B6) and 129 mice express different T1R3 sweet receptors, and differ in their oral intake of sucrose. Yet, when trained to associate a novel flavor (CS+) with IG sucrose infusions, the 2 strains displayed similar CS+ preferences over a flavor paired with IG water infusions. Gustducin knockout mice also consumed less sucrose than B6 wildtype mice in oral tests, but did not differ from B6 mice in their flavor conditioning response to IG sucrose. The role of gut T1R3 receptors in flavor conditioning was further studied by comparing the response of B6 mice to IG infusions of sucrose and noncaloric sucralose; both are ligands of T1R3. Whereas IG sucrose conditioned a CS+ preference, IG sucralose conditioned a CS+ avoidance. This latter result was unexpected because naive B6 mice preferred sucralose and sucrose equally in 2-bottle tests. Together, these results suggest that T1R3 and gustducin signaling in the gut do not mediate post-oral preference conditioning by sugars. Rather, gut T1R3 and/or gustducin signaling may mediate the satiety or niemiety response to sugars in the lower gut.

Supported by DK31135.

Poster Session Fri AM

Ligand Binding to the N-Terminal Domain of Mouse T1R1

Stephan Vigues¹, Graeme L. Conn² and Steven D. Munger¹

¹University of Maryland School of Medicine and ²University of Manchester

T1R1 and T1R3 function as a heteromeric receptor to detect umami stimuli such as monosodium glutamate (MSG) and other L-amino acids. The T1R1:T1R3 receptor is highly selective for L-amino acids over D-amino acids. To better understand the structural basis of amino acid recognition by the gustatory system, we measured the binding of amino acids to T1R taste receptors. Because T1R3 is part of both the umami and sweet taste receptors, we hypothesized that T1R1 contains a binding site for L-amino acids. As

with T1R2 and T1R3, we took advantage of the fact that T1R1 is a class C GPCR, whose members possess a long N-terminal domain (NTD) that is the primary site of ligand binding. We expressed the C57BL/6J mouse T1R1 NTD as a fusion protein in bacteria, and purified the T1R1 NTD protein by affinity chromatography. Synchrotron radiation circular dichroism spectroscopy showed the purified protein to be stable and folded in solution. We next measured the binding of amino acids to T1R1 NTD using steady-state fluorescence spectroscopy to measure changes in intrinsic tryptophan fluorescence, which is indicative of ligand binding. MSG bound the T1R1 NTD with a K_d of 22.2 ± 7.4 μ M. This interaction was specific: the NTD of T1R2, a subunit of the sweet taste receptor, did not bind this L-amino acid, and T1R1 NTD did not bind the sweet stimulus glucose. These findings indicate that T1R1 binds L-amino acids within the extracellular NTD.

Support: DC007317 (S.V.), DC005786 (S.D.M.).

Poster Session Fri AM

Sweet Taste Associated with mGluR4 Agonist L-AP4 in Rats

Benjamin Eschle, Meghan Eddy, Jessica Longobardo and Eugene Delay

University of Vermont

It is generally thought that members of the T1R family dimerize to form taste selective G-protein-coupled receptors. T1R1 + T1R3 detects umami stimuli, for example, monosodium glutamate (MSG) and L-amino acids but not sweet stimuli, whereas T1R2 + T1R3 detects sweet but not umami stimuli. Another putative taste receptor, *t*-mGluR4, also detects MSG. Conditioned taste aversion (CTA) studies show that MSG and the potent mGluR4 agonist L-2-amino-4-phosphonobutyrate (L-AP4) elicit similar tastes. Curiously, in rats a CTA to MSG generalizes to sucrose and to other sweet substances, suggesting that glutamate taste has a sweet component. This raises the question of whether L-AP4 also possesses sweet qualities. We used CTA methods to test for perceptual similarities between the tastes of L-AP4 and sucrose. Amiloride was in all solutions to reduce sodium taste during conditioning and testing. Rats were conditioned by exposing them to 100 mM sucrose and then injecting them with NaCl or LiCl. The rats were then tested with sucrose and L-AP4 and licks emitted in 10-s trials were counted. The CTA to sucrose generalized strongly to low concentrations of L-AP4. These results indicate that both substances elicit similar tastes. They also suggest that afferent signaling for these 2 substances may share transduction mechanisms, down-stream signal processes, or interact at some point in the neural pathways for each taste signal.

Supported by NSF IOB-0450350 (E.D.).

Poster Session Fri AM

Cyclamate Inhibits the Mouse Sweet Taste Receptor

Peihua Jiang, Marianna Max and Robert F. Margolskee

Mount Sinai

The mammalian sweet receptor is a heterodimer of T1R2 and T1R3. Each T1R subunit is a Family C G protein-coupled receptor

(GPCR) with a large extracellular domain (the “Venus Fly Trap Module [VFTM]) and a cysteine rich domain (CRD) that links the VFTM to the 7 transmembrane helix domain (TMD). Previous studies using chimeric and mutant receptors indicate that different sweet compounds interact with various sites of T1R2 and T1R3. One sweet compound, cyclamate, tastes sweet to humans but not to mice. Human sweet receptor responsiveness to cyclamate depends mainly upon Arg790 within T1R3’s TMD. However, the computationally-predicted binding site for cyclamate within T1R3’s TMD is distant from Arg790, indicating that the mouse sweet receptor’s unresponsiveness to cyclamate might not be due to a primary defect in binding cyclamate, but instead to a failure to adopt the active conformation upon binding cyclamate. Consistent with this idea, we found that cyclamate inhibited activation of mouse T1R2 + T1R3 by other sweeteners, that is, cyclamate is inferred to bind to the mouse sweet receptor, but in doing so prevents its activation by other sweeteners. Using mouse/human chimeric receptors we determined that cyclamate’s inhibitory effect depends on the TMD of mouse T1R3. Consistent with these in vitro results, we found that cyclamate inhibited behavioral responses of mice to other sweeteners. We conclude that cyclamate binds to the mouse sweet receptor but instead of activating it as it does the human receptor, it inhibits the mouse receptor.

Supported by NIH grants DC07984 (P.J.), DC03155 and DC08301 (R.F.M.), DC06696 (M.M.). P.J. is a Revson Fellow.

Poster Session Fri AM

Brazzein Variants and the Brazzein–Taste Receptor Interaction

Eric Walters¹, Tiffany Otto², Zheyuan Jin², Jon Rumbley² and Göran Hellekant²

¹Rosalind Franklin University of Medicine and Science and

²University of Minnesota Medical School

Brazzein is a small protein (54 amino acids) derived from the African plant *Pentadiplandra brazzeana*. It is 37 500 times as sweet as 2% sucrose on a molar basis. We have previously used data from 23 brazzein mutants in combination with molecular modeling to propose a model for brazzein interaction with the taste receptor T1R2–T1R3 ligand binding domain. The purpose of this study was to prepare additional brazzein mutants that further explore the way in which brazzein interacts with the receptor. We report taste data on 8 new mutants and 4 new double mutants. The results are consistent with the previously proposed model.

NIH R01 DC006016.

Poster Session Fri AM

Probing the Sweet Receptor’s Transmembrane Domain Ligand Binding Pocket with Cyclamate Analogs

Y. Xia¹, P. Jiang¹, E.F. Thompson², W.J. Spillane², R.F. Margolskee¹ and M. Max¹

¹Mount Sinai School of Medicine and ²National University of Ireland

The artificial sweetener sodium cyclamate (cyclohexylsulfamate) tastes sweet to humans, but not to mice. When expressed in vitro,

the human sweet receptor (hT1R2 + hT1R3) responds to cyclamate, but the mouse receptor does not. Using chimeric receptors, mutagenesis and molecular modeling, we have shown that cyclamate interacts with the transmembrane domain (TMD) of hT1R3. Experimental and computational data indicated substantial overlap in the binding pockets within hT1R3 for the agonist cyclamate and the inverse agonist lactisole. To further characterize this pocket, we examined responses of heterologously-expressed wildtype and mutant sweet receptors to a panel of 19 cyclamate analogs of varying degrees of sweetness: both agonist and antagonist activity were measured. Interestingly, several of the cyclamate analogs acted as antagonists of the wildtype receptor. Furthermore, the rank order of potency of the cyclamate agonists was altered in some of the hT1R3 mutants. From these studies we have identified specific residues of hT1R3 critical for the interaction with cyclamate and more finely localized the site at which cyclamates bind within hT1R3’s TMD binding pocket. Our data provide restraints with which to refine computational models of the ligand binding pocket within the TMD of hT1R3 and suggest a possible mechanism of activation.

Supported by NIH grants DC07984 (P.J.), DC03155 and DC08301 (R.F.M.), DC06696 (M.M.) and by grants from the NUI, Galway Millennium and Corrib Funds (W.J.M.). P.J. is a Revson Fellow.

Poster Session Fri AM

Histidine Residues Play a Crucial Role in Taste-Modifying Activity of Miraculin: Verification by Site-Directed Mutagenesis

Keisuke Ito, Yuji Morita, Ken-ichiro Nakajima, Tomiko Asakura, Akiko Shimizu-Ibukawa, Katsuyoshi Masuda, Masaji Ishiguro, Tohru Terada, Jun-ichi Maruyama, Katsuhiko Kitamoto, Takumi Misaka and Keiko Abe

The University of Tokyo

Miraculin, a taste-modifying protein, has a unique property of converting sourness to sweetness. Recently, we reported on X-ray crystal structure analysis and expression-assay system construction with the other taste-modifying protein, neoculin, but the mechanism still remains unclear. In the present study aimed at defining the structure–function relationship of miraculin, we constructed an expression system using *Aspergillus oryzae*, and produced recombinant miraculin by site-directed mutagenesis. For the production, alpha-amylase with a KEX2 cleavage site was fused upstream of native miraculin and the resulting fusant was expressed. After purification, 0.8 mg of recombinant miraculin was obtained from 1 l of culture medium. The purified miraculin had a strong taste-modifying activity just like native miraculin did. Using this expression system, we produced the 2 site-directed mutants, H30A and H30-60A, to identify responsible residues for the taste-modifying activity. It resulted that both the mutants were sensory-inactive. This suggests that the histidine residues play a crucial role in the taste-modifying activity of miraculin.

This study was supported by Grant-in-Aid 16108004 from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Poster Session Fri AM

Structure–Function Studies on MNEI: What Makes Monellin Sweet?Jeanette Hobbs¹, Steve Munger² and Graeme Conn¹¹Faculty of Life Sciences and ²University of Maryland School of Medicine

Monellin is one of a small number of proteins that are perceived as intensely sweet by humans and some Old World primates. Despite extensive characterisation, the basis of their sweetness, such as the molecular details of their interaction with the sweet taste receptor T1R2:T1R3, remains 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. Circular dichroism (CD) spectroscopic analysis and X-ray crystal structures of wild-type and mutant MNEI proteins will be presented, providing insight into: 1) conformational flexibility in MNEI, including a network of 15 discrete side-chain conformations involving residues critical for sweetness; 2) the role of these key residues in maintaining a functional MNEI structure or, potentially, their direct interaction with the sweet taste receptor, T1R2:T1R3; 3) electrostatic interactions of MNEI with the largely negatively charged surface of the sweet taste receptor, through the location of bound negative ions in the MNEI structure. The next major challenge will be to establish methods to quantitate the interaction between wild-type and mutant MNEI and T1R proteins. This would allow direct correlation of taste test, binding affinity and sweet protein structure, to provide a complete view of what makes a sweet protein sweet.

Support: NIDCD (DC 05786).

Poster Session Fri AM

Gymnemic Acid Interactions with Sweet Taste ReceptorsAlexey Kuposov¹, Eric Walters², Xia Li³ and Göran Hellekant¹¹University of Minnesota Medical School, ²Rosalind Franklin University of Medicine and Science and ³Monell Chemical Senses Center

Gymnemic acid is the earliest described modifier of sweet taste, and is also the most powerful blocker of sweet taste. It is a triterpene that is produced by a shrub, *Gymnema sylvestre*, and it can be extracted from its leaves. Eighteen variants have been described, but only 12 exert the sweet blocking effects. When GA (1–4; 10–18) is applied to the human tongue in an amount less than 1 mg it will block sweetness for up to 1 h. These effects occur in humans and chimpanzees, but not in rhesus monkeys. The purpose of this study was to compare modeled taste receptor T1R2–T1R3 ligand binding domains of human and rhesus monkey (in which gymnemic acid does not block sweet taste) to identify the likely binding site for gymnemic acid in humans. The results suggest a way in which gymnemic acid may block sweet taste.

NIH R01 DC006016.

Poster Session Fri AM

X-Ray Crystal Structure Analysis and Molecular Dynamics Simulation with Neoculin: Insights into Its Sweetness and Taste-Modifying Activity

Yuji Morita, Akiko Shimizu-Ibuka, Tohru Terada, Tomiko Asakura, Ken-ichiro Nakajima, Keisuke Ito, So Iwata, Takumi Misaka and Keiko Abe

The University of Tokyo

The fruit of *Curculigo latifolia* contains a heterodimeric protein, neoculin, which has both sweetness and a taste-modifying activity that converts sourness to sweetness. Here, we report the crystal structure of neoculin analyzed at 2.76 Å resolution. This is the first well-defined tertiary structure of a taste-modifying protein of this kind. The overall structure is quite similar to those of monocot mannose-binding lectins. However, crucial topological differences are observed in the C-terminal regions of both the 2 subunits. In neoculin, the C-terminal tails turn up to form loops fixed by inter-subunit disulfide bonds that are not observed in the lectins, each of the corresponding regions of the lectins showing that the C-terminal tail of the one subunit stretches straight over the surface of the other. Molecular dynamics simulation based on the crystallographic results suggests that neoculin adopts a widely “open” conformation at acidic pH, while unprotonated neoculin at neutral pH is in a “closed” conformation. Observing changes in tertiary structures of neoculin by monitoring its intrinsic tryptophan-derived fluorescence in the pH range causing clear taste modification, we found that the conformational change of neoculin is associated with its taste-modifying activity.

Poster Session Fri AM

Analysis of Sweet Taste Receptor Gene (*Tas1r2*) in Species of FeliformiaXia Li¹, Dieter Glaser², Weihua Li¹, Gary Beauchamp^{1,3} and Joseph Brand^{1,3}¹Monell Chemical Senses Center, ²Anthropological Institute and Museum, University of Zürich and ³University of Pennsylvania

Sweet taste is transduced primarily by one receptor, a dimer of 2 related proteins, T1R2 and T1R3 (gene symbols *Tas1r2* and *Tas1r3*). Mutations in either protein could have a spectrum of consequences, from little or no alterations, to complete loss of activity of the receptor. Previous behavioral studies demonstrated that animals in Felidae, including domestic cats and wild cats, are indifferent to sweet stimuli, and likely cannot perceive their taste. We argued that this indifference could be explained by the observation that in cats the sweet receptor gene, *Tas1r2*, is a pseudogene showing many stop codons, deletions and substitutions. To explore when the alteration of *Tas1r2* occurred, we examined the sequences of *Tas1r2* in other species of Feliformia, for example, civet, mongoose and hyena by PCR, and performed taste-tests on selected species to evaluate their preference for sweet stimuli. Neither stop codons nor deletions were detected in *Tas1r2* of these species, this being consistent with our behavioral tests and with their omnivorous behavior. These observations suggest that *Tas1r2* is functional in these other species of Feliformia and that the pseudogenization of *Tas1r2*

in cats occurred in the lineage of Felidae after it split from other families of Feliformia around 30–35 MYA.

This work was supported in part by NIH grant R01DC00882 (G.B.).

Poster Session Fri AM

Polymorphisms in the *Tas1r3* Gene Alter Taste Responses to Sweeteners: Evidence from 129.B6-*Tas1r3* Congenic Mice

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The *Tas1r3* gene encodes the T1R3 receptor protein involved in transduction of sweet taste. To characterize the genetic architecture of sweet taste responsiveness and the ligand specificity of the T1R3 receptor, we analyzed taste responses of 129.B6-*Tas1r3* congenic mice to a variety of sweeteners, using 3 different measures: responses of the chorda tympani nerve, initial licking responses, and sweetener consumption in 24-h preference tests. The results were generally consistent across the 3 measures of taste responsiveness. Allelic variation in *Tas1r3* influenced responses to noncaloric sweeteners, caloric sweeteners, sugar alcohols, and some amino acids. *Tas1r3* genotype did not affect responses to glucose polymers, several amino acids, NaCl, HCl, quinine, monosodium glutamate or IMP. Thus, allelic variation of the *Tas1r3* gene affects taste responses to many caloric and noncaloric sweeteners, but not all carbohydrates. In addition, we found that the genetic architecture of sweet taste responsiveness changes depending on the measure of taste response and intensity of the sweet taste stimulus. Variation in the T1R3 receptor influences peripheral taste responsiveness over the wide range of sweetener concentrations, but behavioral responses to more potent sweeteners increasingly depend on mechanisms that override input from the peripheral taste system.

Poster Session Fri AM

Sucrose Taste-Responsive Neurons Are Located in the Dorsal Rostral Part of Hamster Solitary Nucleus, but Receive Predominately Convergent Input from Two or More Tastants: An In-Vivo Intracellular Study

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The in-vivo intracellular method can be used to study taste input of individual subdivisions of solitary tract (NST) neurons, identify morphology of recorded cells, and localize cells within the nucleus. In the present study, we tested the hypothesis that NST is gustotopically organized by focusing, on cell type, location, and input specificity of sucrose-responsive cells in NST. Intracellular microelectrodes were used to record NST neurons, while the tongue was stimulated with anodal current or application of tastants (sucrose, NaCl, citric acid, and quinine). When possible, the cell was labeled with biocytin. Three hours after labeling, brains were removed, sectioned, processed for biocytin, and counterstained with cytochrome oxidase. From a set of 49 taste responsive neurons, 18 cells

were labeled, and 12 of these responded to sucrose. Of sucrose-responsive cells, 75% were localized along the dorsal border of NST, and 75% were classified as multipolar-type neurons (20% elongated-cell type, 5% ovoid-cell type). In contrast, only 16% of the sucrose responsive neurons were driven by sucrose alone, 25% responded to 2 tastants, 50% to 3 tastants, and 16% to all 4 tastants. These results favor a general gustotopic organization for NST that includes topography and morphology, but not specificity of taste input.

Supported by DC000066 to D.S. and R.W.

Poster Session Fri AM

Responses to Taste Mixtures in the Nucleus of the Solitary Tract of the Rat

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To study neural coding of complex taste stimuli, electrophysiological responses to 4 tastants: sucrose (S), NaCl (N), HCl (H), and quinine (Q), and their binary, undiluted mixtures (NH, NS, NQ, HS, HQ and SQ) were recorded from 46 single cells in the nucleus of the solitary tract (NTS) of the anesthetized rat. For 30 of these cells, all 10 stimuli were repeated at least 6 times (range 6–16; median = 9). Among all 46 cells, the most effective binary mixture almost always ($n = 45$; 98%) included the most effect single tastant, but in only 25 cells (54%) was the second most effective single tastant a component of the best mixture. Of the 30 cells with multiple replications, 14 (43%) showed significantly larger ($P < 0.05$) responses to a mixture than to any single tastant. These responses were $47 \pm 7\%$ SEM (mean = 9.5 ± 1.8 spikes/s) greater than their responses to the most effective single tastant. Different mixture interactions were present in different classes of cells (defined by hierarchical cluster analyses). In N specialists ($n = 19$), S enhanced H and Q responses. In N generalists ($n = 7$), responses to mixtures were nearly equal to responses to their most effective components. For NH generalists (responded best to NH; $n = 10$), S suppressed N, but enhanced H responses. Q suppressed S responses only in S specialists ($n = 6$), but suppressed H responses in all other cell classes. Multidimensional scaling analyses showed poor discrimination among mixtures containing N. Results impact our understanding of local NTS circuitry and coding mechanisms subserving analytical abilities in gustation.

Supported by NIDCD grants DC006914 and DC005219 to P.D.L.

Poster Session Fri AM

Effects of Microstimulation at Different NST Locations

Nicole Kinzeler and Susan Travers

Ohio State University

Bitter stimuli elicit a discrete distribution of Fos-like immunoreactivity (FLI) in the dorsomedial rNST. IXth nerve transection eliminates this Fos pattern and the rejection (gaping) reflex, but decerebration eliminates neither, suggesting that the dorsomedial solitary nucleus could be a trigger zone for reflex rejection. To test this, microelectrodes were implanted into different rNST regions, and the oral motor effects of electrical stimulation (100 Hz, 0.2 ms) were tested

with a range of intensities (0–80 μA) and train durations (0.1–24.3 s). After testing, 30 mM quinine was infused to elicit FLI. Microstimulation was effective in inducing oromotor behaviors at low currents ($\sim 20 \mu\text{A}$), with licking occurring more frequently (88% of sites) compared to gaping (33%). All sites that elicited gaping also elicited licking. Sites in the rostral half of rNST had a lower threshold for evoking oral behaviors than sites further caudal. However, when placements were divided into those $\leq 250 \mu\text{M}$ from the center of the Fos ($n = 11$), and those more distant ($n = 10$), only 2/11 sites near the Fos elicited consistent gaping, compared to 6/10 more lateral sites ($P < 0.01$). These results are contrary to our hypothesis and suggest an alternative, but unknown, function for the Fos cells. In a subset of animals ($n = 8$), irregular patterns of electrical stimulation were used to mimic rNST responses elicited by sucrose and quinine. Preliminary observations reveal no behavioral differences for the various temporal patterns.

Supported by DC00417 and T32-DE014320.

Poster Session Fri AM

Convergent Excitatory and Inhibitory Inputs to the Reticular Formation from the Rostral Nucleus of the Solitary Tract

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Multiple lines of evidence suggest that the reticular formation (RF) subjacent to the anterior nucleus of the solitary tract (rNST) forms an interface between gustatory input and oromotor output. We have begun to explore this interface using calcium imaging and patch clamp recording in a neonatal rat brainstem slice preparation. Calcium imaging studies indicate that many neurons receiving excitatory input from the rNST are under some degree of tonic inhibition as the addition of bicuculline and strychnine to the bath greatly enhanced the number of RF neurons activated by rNST electrical stimulation. Patch clamp studies from identified prehypoglossal RF neurons revealed that some inhibition originated from within the rNST. Several RF neurons that had purely inhibitory currents in response to stimulation of the rNST showed excitatory currents when GABA_A antagonists were added to the bath (3/4). Less direct evidence came from other RF neurons with purely excitatory currents following rNST stimulation that showed enhanced excitatory responses following GABA_A antagonist drug application (5/8). Evidence for mixed inputs to the RF neurons also included neurons with purely excitatory responses to rNST stimulation that switched to inhibitory responses following application of non-NMDA antagonists (4/10). Mixed inputs from the rNST could provide a substrate for the release of inhibition that has been postulated to mediate the oral rejection gape response.

Supported by NIH DC000417 and DK 56373.

Poster Session Fri AM

Neurotransmitters in Brainstem Gustatory Reflex Circuitry

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The primary vagal gustatory center of the goldfish is the vagal lobe, a complex laminated structure including both sensory and motor layers. These are equivalent to the vagal gustatory portion of the nuc. of solitary tract and nuc. ambiguus, respectively. Topographically organized reflex connections link the sensory to the motor layers of the lobe. We used Ca⁺⁺ imaging and histochemistry to characterize the neurotransmitters of this reflex circuit. Vagal motoneurons were retrogradely labeled by calcium-green dextran injections into the vagus nerve. In slice preparations, intracellular Ca⁺⁺ levels of motoneurons increased following electrical stimulation from the sensory zone. These Ca⁺⁺ responses were suppressed by the AMPA/kainate receptors antagonist, DNQX, indicating that AMPA/kainate receptors are crucial to this response. The presence of such receptors is also demonstrated by kainate-activated cobalt labeling of the motoneurons. In Mg⁺⁺-free aCSF, Ca⁺⁺ responses were enhanced and these enhanced responses were suppressed by the NMDA receptor antagonist AP-5. These results suggest that the vagal motoneurons receive glutamatergic inputs from the reflex interneurons via both the AMPA/kainate and NMDA receptors. Both GABA_A (bicuculin) and glycine receptor antagonists (strychnine) enhance Ca⁺⁺ responses. Vagal motoneurons also are surrounded by GAD and gephyrin (anchoring protein for GABA_A and glycine receptors) immunopositive punctate. These results suggest tonic GABA and glycinergic inhibition of the motoneurons which is relieved by activation of the glutamatergic reflex system.

Supported by NIH Grant DC 00147.

Poster Session Fri AM

The Involvement of Delta-Opioid Receptors in the Mediation of Synaptic Transmission between the Fiber Terminals of the Solitary Tract and the Neurons in the Rostral Portion of the NST That Project to the Gustatory PbN

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In the present experiments the effect of δ -opioid receptor agonists and antagonists on afferent synaptic transmission between the afferent fiber terminals of the solitary tract (ST) and the PbN projecting neurons in the rostral gustatory zone of the NST was investigated using whole-cell recording techniques in the hamster brainstem slices. ST-evoked EPSCs were significantly reduced (24%) by met-enkephalin (MetE) and/or SNC80, a selective δ -opioid receptor agonist. The effects of MetE were abolished by the presence of a non-selective opioid receptor antagonist, naltrexone hydrochloride (NTX), and naltrindole hydrochloride (NTD), a selective δ -opioid receptor antagonist. The effects of SNC80 were completely abolished by NTD, and BNTX, a selective δ_1 -opioid receptor antagonist. Neltriben mesylate (NTB), a selective δ_2 -opioid receptor antagonist had minimal effects on δ -opioid receptor responses evoked by ST stimulation. Western immunoblot demonstrated the presence of δ -opioid receptor protein in the rostral portion of NST. Single-cell RT-PCR experiments showed that δ_1 -opioid receptor mRNA is present within the PbN-projecting NST neurons whose ST-evoked EPSCs were reduced by SNC80, and were abolished by presence of NTD. These studies demonstrate that δ -opioid receptors modulate ST-evoked

EPSCs of Pbn-projecting NST neurons by means of postsynaptic mechanisms in the hamster.

Supported by NIDCD006623 to C.-S.L.

Poster Session Fri AM

Membrane Properties of Rostral NST Neurons Projecting to the Parabrachial Nucleus in Rats

Takeshi Suwabe and Robert Bradley

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The rostral nucleus of the solitary tract (rNST) processes gustatory information for relay to several brainstem and rostral locations with diverse functions. Separate populations of rNST neurons project to either parabrachial (PBN) or brainstem sites but not both. While there is information on the anatomy of rNST neurons with different projection patterns little is known about their neurobiology. We hypothesized that rNST neurons that project to the same nucleus would have similar membrane properties. To test this hypothesis, we performed whole-cell recordings from rat rNST neurons identified by retrograde transport of DiI-microinjected into the PBN. Whole cell recordings of the PBN projecting neurons were performed under current and voltage clamp and the recording electrode also contained Lucifer Yellow to fill the neuron for later morphological analysis. The rNST-PBN projection neurons exhibited various repetitive discharge patterns elicited by injection of depolarizing current following hyperpolarizing current steps under current clamp conditions. An A-current was observed in the rNST neurons under voltage clamp condition whose amplitude varied from neurons to neuron. PBN projecting rNST neurons could not be separated into distinct groups based on repetitive discharge characteristics and A current amplitude. These preliminary data indicate that PBN projecting rNST neurons have heterogeneous membrane properties.

Support contributed by: NIH grant DC000288 to R.B.

Poster Session Fri AM

Reverse Microdialysis of Iontropic Glutamate Receptor Blockers into the Parabrachial Nucleus Reduces Taste Reactivity Behaviors in Conscious Rats

Michael King and Tricia Dorne

Stetson University

Microinjection of glutamate into the waist area of the parabrachial nucleus (W) in conscious rats elicits taste reactivity (TR) behaviors. To address the hypothesis that glutamate in W plays a role in TR responses to tastants we introduced the ionotropic glutamate receptor blocker kynurenic acid (KYN) or 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) into W in conscious rats by reverse microdialysis prior to intra-oral infusion of 0.01 M NaCl (N), 0.01 M sucrose, 0.03 M HCl and 0.003 M quinine (Q). Microdialysis guide cannula (CMA Dialysis) and intra-oral cannula were surgically implanted in 11 male Wistar rats under pentobarbital anesthesia. Following a week of recovery and 3 days of habituation to the behavioral arena, 10 mM KYN ($n = 5$) or CNQX ($n = 6$) was dialyzed into W for 1–2 h at a rate of 1 μ l/min. Each tastant was infused into the oral cavity at 0.233 ml/min for 2 min

before, during and after drug dialysis. KYN significantly reduced the number of ingestive TR behaviors to each tastant by 52–77% and aversive responses to Q by 83%. CNQX also decreased ingestive TR responses to all tastants, but did not significantly alter aversive responses to Q. Overall, CNQX shifted the TR response profile to include a higher percentage of aversive behaviors. This effect was particularly prominent on the response to N, that included gapes and chin rubs during CNQX dialysis. These data suggest that AMPA/kainate receptors in W play a role in the initiation and type of TR responses to intra-oral tastants.

NIH R01 DC07854.

Poster Session Fri AM

Parabrachial Responses to Bitter Taste Stimuli

Laura Geran and Susan Travers

Ohio State University

Gustatory responses were recorded from 57 single units in the parabrachial nucleus. Stimuli included 4 bitter tastants (quinine, denatonium, PROP and cycloheximide) and chemicals representing each of the 4 remaining putative taste qualities (sour, salty, sweet and umami). When possible, neurons were also tested for receptive field (RF), variability, and concentration. Hierarchical cluster analysis revealed a bitter-best (B-best) cluster of 15 cells (26% of the total). Although RFs for B-best cells in a previous study in NST were overwhelmingly foliate in origin, 7 of the B-best cells in the current study received bitter input both from the foliates and nasoincisor ducts. Neurons in the acid-best cluster were also more likely to have posterior or mixed RFs than those in other best-stimulus groups (4 of 5 neurons, compared with 4 out of 27 for the umami/sucrose and salt/acid clusters combined). B-best PBN cells appeared to be more homogenous than those in the NST, forming a single B-best cluster. Furthermore, an across-neuron correlation revealed that 3 of the bitter stimuli correlated significantly with one another ($r > 0.76$, $P < 0.05$) but not with representatives of other taste qualities. However, similar to the NST, quinine was not significantly correlated with any other stimulus tested. Entropy values for B-best cells did not change significantly from NST to PBN (mean $H = 0.33$, $P > 0.9$), suggesting that although there was apparent RF convergence from the brainstem to the pons, chemospecificity across taste qualities was not affected for these units.

Supported by NIDCD grant DC00716 to S.T.

Poster Session Fri AM

Altered Pontine Gustatory Coding of Sucrose Concentrations in a Rat Model of Obesity

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Overconsumption induced by high palatability may contribute to obesity. Recently, we have demonstrated an increased avidity for sweet in the Otsuka Long Evans Tokushima Fatty (OLETF) rat that lacks the CCK-1 receptors, hyperphagic and develops obesity and type-2 diabetes. To investigate central taste processing in this strain, we used extracellular single unit recording in the pontine

parabrachial nucleus while stimulating the tongue with various concentrations of sucrose (0.01–1.5 M) in addition to a standard stimulus array. The analyses included 844 taste responses recorded from 179 neurons in age-matched (~26 weeks) prediabetic, obese OLETF ($n = 4$) and lean LETO control ($n = 3$) rats. We found more sodium-best (66% vs 48%) but less sucrose-best (14% vs 32%) or sucrose specific (3% vs 11%) neurons in OLETF compared to LETO. Obese rats demonstrated a right-ward shift in their concentration-response functions based on all sucrose responses compared to lean: increased threshold (0.37 ± 0.05 M vs 0.23 ± 0.2 M, $P < 0.05$), higher maximum concentration (0.96 ± 0.07 M vs 0.56 ± 0.5 M, $P < 0.0001$), and broader dynamic response range (0.59 ± 0.07 M vs 0.33 ± 0.6 M, $P < 0.01$). Further analysis revealed that the proportion of sweet responses carried by NaCl-best neurons and the sucrose-best neurons was higher in obese than in lean cohorts (2.15 vs 0.97). The altered concentration effects may result from a reduced number of narrowly tuned sucrose-sensitive units or an altered across-pattern code, and may contribute to overeating in this strain.

Supported by NIH grant DK065709, PSU Diabetes Center and PA TSF.

Poster Session Fri AM

Tactile Effects on Taste Localization

Juyun Lim and Barry Green

John B. Pierce Laboratory

Although taste is always accompanied by tactile stimulation, little is known about how touch interacts with taste. One exception is evidence that taste can be 'referred' to nearby tactile stimulation. It was recently found that spatial discrimination of taste is poorer for bitterness than for other tastes independent of perceived intensity. We hypothesized this may have been caused by greater referral of bitterness to touch. The present study tested this hypothesis by comparing localization of QSO₄ and sucrose under conditions that maximized and minimized the opportunity for referral. In both conditions stimulation was produced by 5 cotton swabs spaced 1 cm apart and arranged in an arc to enable simultaneous contact with the front edge of the tongue. Only one swab contained the taste stimulus while the rest were saturated with deionized water. In both conditions the swabs were stroked up-and-down against the tongue 5 times. Ss were asked to identify which swab contained the taste stimulus 1) 5 s after the fifth stroke (*localization condition*) and 2) immediately at the end of the fifth stroke, with the swabs still in contact with the tongue (*referral condition*). Taste intensity was rated under the same conditions. For both stimuli, localization tended to be poorer in the referral condition. However, consistent with the hypothesis, the difference between conditions was significant only for bitterness. This result implies that there is a closer perceptual association between touch and bitterness than between touch and other tastes.

Supported in part by NIH grant DC005002.

Poster Session Fri AM

The Effect of Intraoral Trigeminal Stimulation on Orthonasal Olfaction

Johannes Frasnelli, Carina Oehrns and Marilyn Jones-Gotman

MNI

Intranasal trigeminal stimulation leads to altered perception of odors. In daily life, however, trigeminal stimulation occurs mainly in the mouth; for example, upon ingestion of spicy food. In this study we stimulated the intraoral trigeminal system of healthy subjects and investigated the effect of this stimulation on a number of orthonasal olfactory tasks. Both types of stimulus were completely separated physically from each other. Forty subjects (20 women) participated; they were tested on 3 different days. Intraoral trigeminal stimulation was achieved by application of a Capsaicin solution and of CO₂ enriched (sparkling) water; still water was used as a control. Subjects were instructed to keep the liquids in their mouth during testing. Olfactory tasks included PEA threshold measurement, a perithreshold intensity discrimination test, an odor quality discrimination test, and intensity ratings. In addition, 2 visual control tasks were performed. Both Capsaicin and CO₂ led to a clear trigeminal perception. Capsaicin was mainly described as burning, whereas CO₂ evoked mainly a tingling sensation. The results in the olfactory tasks were not significantly influenced by the presence of a trigeminal stimulant in the mouth. We observed that odor intensity ratings became lower over the 3 testing days. This effect was amplified when Capsaicin was the trigeminal stimulant. To conclude, no direct effect of intraoral trigeminal stimulation on olfactory perception could be observed, possibly due to low sensitivity of the measures used or possibly to an order effect.

Poster Session Fri AM

Responsiveness of the Human Nasal Epithelium to Trigeminal Stimuli

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Smell and Taste Clinic

Aim: The study was designed to investigate the differential responsiveness of the human nasal epithelium to trigeminal stimuli using electrophysiological mucosal recordings. **Methods:** A total of 60 subjects participated (30 m, 30 f; age range 19–40 years). Chemical stimuli were menthol (60% v/v), CO₂ (60% v/v), ethanol (50% v/v), and cinnamonaldehyde (30% v/v) (total airflow 6 l/min). Stimulus duration was 500 ms, the average interstimulus interval was approximately 40 s. Electrophysiological recordings using tubular electrodes (outer diameter 0.8 mm) were made from 5 intranasal sites: the anterior septum, the posterior septum, the middle turbinate, the lower turbinate, and the lateral wall of the olfactory cleft. Electrode positioning was made under endoscopic control. **Results:** Significant differences between recording sites were only found for menthol. Post hoc testing revealed that response amplitudes were largest at the anterior septum and the lower turbinate compared to the posterior septum. Correspondingly to these changes, response amplitudes to menthol were significantly smaller than those to the 3 other stimulants at the posterior septum and at the olfactory cleft, but not at the other recording sites. **Conclusions:** The present data indicate a differential responsiveness of the nasal epithelium to chemical stimuli. In turn, this suggests a differential topographical distribution of trigeminal chemoreceptors.

This research was supported by Philip Morris USA Inc.

Poster Session Fri AM

Retronasal and Oral-Cavity Identification of Trigeminal Odorants

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Single concentrations of 6 odorants (eugenol, heptyl alcohol, nonanal, 1-octanol, *dl*-menthol, valeric acid) selected to be trigeminal stimuli, presented in random order 3 times each in vapor-phase either retronasally (RETRO) or oral-cavity-only (OCO), were identified (ID) on a digital computer by 20 subjects (ages 18–35; 9 female) from a list of 9 identifiers, with 1 or 2 identifiers correct for each odorant. OCO was produced by a nose clip and exhalation from the mouth. Exhalations were detected by a microphone.

Results: Median % RETRO correct ID [correct ID in brackets] were: eugenol, 100% [cloves or spice]; heptyl alcohol, 67% [cleaner]; nonanal, 58% [citrus or floral]; 1-octanol, 71% [citrus or cleaner]; *dl*-menthol, 100% [ointment or peppermint]; valeric acid, 67% [rancid or sweat]. Median % OCO correct ID were all 0%, except *dl*-menthol OCO median correct ID was 67%. **Conclusions:** Many vapor-phase “trigeminal” odorants can be identified only when access to the nasal cavity (RETRO) occurs, but substantial correct ID of vapor-phase *dl*-menthol also occurs when restricted to the oral cavity (OCO). Odorants similar to *dl*-menthol may contribute to flavor from both the oral and nasal cavities.

Support from USDA Hatch NYC-191403 and a Susan Linn Sage Professorship.

Poster Session Fri AM

The Neural Correlates of Capsaicin versus Pure Taste in Humans

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We used functional magnetic resonance imaging to investigate the neural correlates of oral capsaicin stimulation relative to the neural representation of taste in humans. Fourteen healthy right-handed subjects were scanned on a 3T magnet while receiving 5 different solutions: capsaicin, sweet, salty, bitter and tasteless. We used our standard long-event related design with the modification that all capsaicin trials were followed by 5 tasteless trials. Subjects were pre-screened to ensure that perceived burn consistently returned to baseline by the 5th presentation of the tasteless solution. We entered contrasts of each stimulus minus its baseline into a second-level ANOVA using SPM5. As predicted, capsaicin and the gustatory stimuli activated similar regions of insula and overlying operculum. However, capsaicin uniquely activated dorsal anterior cingulate cortex, VPM thalamus, the paracentral lobule, and a region of ventrolateral PFC. We also performed conjunction analyses of the capsaicin-baseline contrast with each taste-baseline contrast. In support of behavioral data indicating that bitter and capsaicin uniquely interact, we found areas of rolandic and frontal operculum, as well as caudolateral OFC, that were unique to the capsaicin + bitter conjunction. In general, we found that capsaicin and bitter preferentially ac-

tivated frontal operculum, whereas sweet and salty preferentially activated parietal operculum.

This work was supported by NIDCD R01 DC005002-06.

Poster Session Fri AM

Odor and Ocular Detection of *t*-Butyl Acetate and *n*-Butyl Acetate: Implications for Environmental Regulation and Chemosensory Science

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In study 1, Ss screened for the health of their airways sought to detect the odors of *t*-butyl acetate (TBAC) and *n*-butyl acetate (NBAC), compounds in existing odor compilations. A vapor delivery device presented analytically confirmed concentrations. An S made hundreds of judgments per compound per day, enough to create a psychometric function. Fifty percent detection above chance (threshold) occurred at 2 and 8 ppb for NBAC and TBAC, respectively, with a range among Ss of only 24 to 1. This study yielded the highest measured sensitivity to the compounds. In study 2, Ss sought to detect vapor with the eye via localization (right vs left eye). Fifty percent detection occurred at 113 and 177 ppm for NBAC and TBAC, respectively, more than 10 000× that for odor detection. The functions for chemesthetic detection (feel) increased much more steeply than those for odor. The range of differences among Ss equaled sixfold. The present results on odor detection point out the poor state of archival data for decisions regarding odor detectability and highlight how existing databases can mislead environmental and safety professionals. Despite sincere attempts to “clean” the databases, they rarely exhibit accuracy better than ±1000% and differ systematically from one another. There exists an urgent need to provide olfactory information where noise from poor control and measurement does not becloud signal.

Supported by Lyondell Chemical Co. and grant DC05602 from NIH.

Poster Session Fri AM

Gustatory Stimulation Inhibits Trigeminal Caudalis (Vc) Neuronal Responses to Noxious Electrical Stimulation of the Tongue in the Rat

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Objective: To test the hypothesis that chorda tympani (CT) activation suppresses nociceptive processing in Vc. **Methods:** 14 WDR nociceptive units with lingual receptive fields were isolated in the superficial layers of Vc in pentobarbital-anesthetized rats. Neuronal responses to 3 noxious electrical stimuli delivered to the tongue (trains of 0.2 ms pulses at 1 Hz, 45 mA for 10 s) were recorded before, during and after application of a taste mixture (sucrose 0.3 M, citric acid 0.03 M, NaCl 0.1 M) at a 15 min interstimulus interval. An automated system continuously perfused the tongue surface with either the taste mixture or water. Vc units were also characterized by their response to chemical (pentanoic acid 200 mM), thermal (55 °C), and mechanical (non noxious and noxious) stimuli. Neuronal responses were quantified as spikes/30 s stimulus condition and compared using

the Wilcoxon test. **Results:** Taste stimulation resulted in a 27% decrease in the mean electrically-evoked Vc response. The mean response during perfusion with water (173 spikes/30 s \pm 31 SEM) was significantly reduced ($P = 0.002$) during perfusion with the taste mixture (127 \pm 26 SEM) with recovery upon reapplication of water (176 \pm 39). All neurons also responded to mechanical, thermal and pentanoic acid stimuli. **Conclusion:** The acute depression of Vc responses by tastant stimulation suggests that the gustatory system exerts an inhibitory effect on trigeminal nociceptive processing.

Poster Session Fri AM

Capsaicin Avoidance following Chorda Tympani Transection

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Certain chronic oral pain conditions including Burning Mouth Syndrome may be associated with damage to the gustatory system. We investigated if transection of the chorda tympani (CT) increases oral sensitivity to capsaicin as a possible model of hyperalgesia due to release of the trigeminal system from tonic gustatory inhibition. Water-restricted female rats had 2-h access to 2 bottles, one containing capsaicin (0.1, 0.3, 0.5, 1 or 10 ppm) and the other water + ethanol vehicle. Each concentration was tested for 2 days with bottle positions switched daily. Two days of water-only interceded before testing the next concentration. We measured % volume of capsaicin consumed and lick counts. The concentration series was tested presurgery and at 0.5, 3, 6, 9 and 12 months postsurgery in 3 groups: bilateral CT transection, bilateral CT transection plus ovariectomy (CT-OVX), and sham-operated controls. Presurgery there was a concentration-dependent decrease in licks and volume of capsaicin consumed with a threshold between 0.1 and 0.5 ppm. The majority of drink licks occurred during the first 10 min of access. Two weeks postsurgery the CT-OVX group significantly (ANOVA, $P = 0.05$) avoided 0.3 ppm capsaicin. At later times there were few significant between-group differences; the CT-OVX group tended to avoid capsaicin more at 6 and 9 months, whereas the CT group exhibited reduced avoidance that was significant at 6 months (ANOVA, $P < 0.05$). Overall, transection of the CT did not result in marked symptoms of chronic hyperalgesia.

Funding: NIH, Calif. TRDRP.

Poster Session Fri AM

Peripheral Trigeminal Nerve Responses to Artificial Sweeteners, KCl, and NaCl

Vajini Atukorale, Matthew Greene and Wayne Silver

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The trigeminal nerve provides sensory information from the eyes, nose, and mouth. It is a multisensory nerve, responding to a variety of irritants in the environment. Artificial sweeteners, which are of obvious economic importance, are said to be irritating at high concentrations. The present study demonstrated that 3 artificial sweeteners, Na saccharin, acesulfame K, and Na cyclamate can elicit responses from the ethmoid branch of the trigeminal nerve in the nasal cavity when presented in solution. NaCl and KCl also elicited responses from the trigeminal nerve and therefore, Na⁺ and K⁺ may have played

a role in the response to the sweeteners tested. However, the thresholds for NaCl and KCl were higher than for the sweeteners, suggesting that the sweeteners must be stimulatory by themselves. Several receptor proteins associated with the trigeminal nerve mediate responses to irritants. In vitro studies demonstrate that artificial sweeteners stimulate TRPV1 receptors (capsaicin, irritant receptors) and T2R receptors (bitter receptors). Both of these receptor proteins are associated with trigeminal nerves. Either or both of these receptors may contribute to the trigeminal nerve responses to the sweeteners tested.

Poster Session Fri AM

Viral “Live-Cell” Tracing of the Trigeminal System—Comparative Analysis of Different Pseudorabies Virus Strains

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Somatosensory information from the mammalian head is mainly mediated by the trigeminal nerve. Trigeminal perception encompasses temperature, touch, and pain. However physiological features of individual trigeminal neurons depending on their somatosensory function and area of innervation remain largely unclear. We already demonstrated that the Bartha strain of the Pseudorabies virus (PrV) can be used as a “live-cell” tracer for physiological investigations of selectively labelled peripheral neurons. Since this approach was limited to primary sensory neurons we analysed the use of the bidirectional tracer PrV-Kaplan as a “live-cell”-tool for characterisation of defined neuronal populations within the trigeminal ganglion and synaptically connected higher order neurons in the brainstem. We used marker protein expressing variants of PrV to identify trigeminal neurons innervating the murine nasal cavity. Plating of ganglionic and brainstem tissue allowed physiological analysis of identified neurons in vitro. Our results indicate that basic electrophysiological properties of traced neurons were not altered by PrV infection compared to control neurons. These data show the benefit of PrV for “live-cell” tracing studies, making PrV-Kaplan eligible for functional analysis of somatotopically defined and synaptically connected neurons in vitro.

Poster Session Fri AM

Temporal Integration in Nasal Lateralization of Homologous Volatile Organic Compounds

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Monell Chemical Senses Center

In past studies of 3 model stimuli, temporal integration in detection of nasal irritation was imperfect: It required more than a 2-fold increase in stimulus-duration to compensate for a 2-fold decrease in concentration to maintain a fixed level of detection. Interestingly, degree of integration varied greatly, from nearly perfect integration for ammonia to highly imperfect integration for ethanol. How do such differences relate to molecular parameters? Our initial hypothesis is that molecules that more readily dissolve into the lipid-rich peri-receptor

environment will accumulate, and therefore integrate, better over time. Four model stimuli, including 2 homologous alcohols (ethyl and butyl alcohol), and esters (ethyl and butyl propionate), allowed an initial test of this hypothesis. Irritation threshold was measured using nasal lateralization. Concentration was fixed within experimental runs, and stimulus-duration varied to find the briefest stimulus subjects could reliably lateralize. Concentration varied between runs. For both the alcohols and esters, the more lipophilic molecule demonstrated more complete integration. Further, across all 4 compounds, there was an association between log octanol/water partition coefficient (related to lipophilicity) and degree of integration. That just one key molecular parameter can help predict degree of integration strongly suggests that it may prove possible to predict some aspects of perceptual dynamics based on molecular parameters.

Supported by the Kraft Foods Term Chair (P.W.) and NIEHS (RO3, P.W.).

Poster Session Fri AM

Olfactory Coding in *Anopheles gambiae*

Allison Carey¹, Guirong Wang², Zina Berman¹,
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¹Yale University and ²Vanderbilt University

Blood-feeding mosquito species such as *Anopheles gambiae* act as vectors for transmission of malaria, which remains a leading cause of death worldwide despite numerous public health initiatives and technological advances. It has been established that 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) of the peripheral nervous system, which express a small number of odor receptor (Or) genes which confer upon the neuron a unique odor sensitivity. A family of 79 candidate odor receptors has been identified in *A. gambiae*. Two of these receptors have been shown to respond to specific olfactory stimuli in an in vivo expression system in *Drosophila*. We are employing the same approach to systematically characterize the function of the *A. gambiae* odorant receptor family. We are testing each receptor against a panel of odorants selected for chemical diversity, volatility, and ecological and behavioral relevance. We find that each receptor possesses a distinct odor response profile and tuning breadth. A global, functional analysis of the *Anopheles* Or family will further our understanding of how olfactory information is encoded across populations of receptors, and may potentially prove useful in the control of malaria mosquitoes.

This work was supported by the NIGMS MSTP Training Grant and the Grand Challenges in Global Health program of the Bill and Melinda Gates Foundation and the Foundation for the NIH.

Poster Session Fri AM

Functional Characterization of an Anopheline Specific Component of the Odorant Receptor Repertoire in the Malaria Vector Mosquito *Anopheles gambiae*

Guirong Wang¹, Allison Carey², John Carlson² and Laurence J. Zwiebel¹

¹Vanderbilt University and ²Yale University

Malaria is a mosquito borne disease that afflicts more than 200 million people annually and causes as many as 3 million deaths. *Anopheles gambiae* mosquitoes are the principal Afrotropical malaria vector and use olfactory cues to manifest a profound attraction toward potential human hosts (anthropophily). In order to examine the molecular genetics of anthropophily in this system we have identified the complete odorant receptor (OR) repertoire of *An. gambiae* using molecular and bioinformatic approaches. We now investigate the receptive range of an anopheline specific subset of the AgOR gene family through functional characterization studies that examine odorant-induced currents in *Xenopus* oocytes. Data will be presented that suggest there is a large diversity of AgOR tuning responses: some AgORs are responsive to a small panel of odorants (specialists), while others are more broadly tuned (generalists). These results, together with other ongoing efforts, provide valuable insight into the olfactory coding mechanisms of *An. gambiae* as well as fostering the design and development of novel olfactory-based strategies for reducing the global transmission of malaria.

This work was supported by the Grand Challenges in Global Health program of the Bill and Melinda Gates Foundation and the Foundation for the NIH.

Poster Session Fri AM

Novel Ligands for Physiologically Characterized Olfactory Receptor Neurons of Female *Aedes aegypti*

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Antennae of adult mosquitoes are covered by a rich array of sensory organs, sensilla, which are vital for olfactory detection. Physiological characterization of ORNs housed in 4 distinct morphological types of *s. trichodea*, the principal types of antennal sensilla of female *Ae. aegypti*, revealed 18 different ORN types, housed in 8 sensillum types, which responded to behaviorally relevant olfactory cues. In addition, 4 ORNs housed in 3 sensillum types were non-responding. The ORN types were generally combined in stereotyped configurations and sent their axons to defined antennal lobe glomeruli. The temporal firing patterns of different functional types of ORNs were diverse (i.e., long lasting tonic responses or moderate phasic responses) and dependent on the odors tested. Through combined gas chromatographic (GC)-SSR analysis of headspace-associated extracts from different human body parts we are currently screening for novel ligands for ORNs housed in trichoid as well as other types of sensilla on the olfactory appendages. Preliminary results indicate that neurons of specific sensillum types respond differentially to the tested extracts. In general, GC-SSR active compounds elicit stronger responses compared to previously used compounds.

Poster Session Fri AM

A Pheromone Receptor Mediates VA-Induced Responses in *Drosophila*

Dean Smith and Tal Soo Ha

University of Texas Southwestern Medical Center

Insect pheromones elicit stereotypic behaviors that are critical for survival and reproduction. Defining the relevant molecular mechanisms mediating pheromone signaling is an important step to manipulate pheromone-induced behaviors in pathogenic or agriculturally important pests. The only volatile pheromone identified in *Drosophila* is 11-*cis* vaccenyl acetate (VA), a male-specific lipid that mediates aggregation behavior. VA activates a few dozen olfactory neurons located in T1 sensilla on the antenna of both male and female flies. Here we identify a neuronal receptor required for VA sensitivity. We identified 2 mutants lacking functional VA-sensitive neurons and show that the expression of the VA receptor is dramatically reduced or eliminated. Importantly we show mis-expression of this receptor in neurons normally insensitive to VA, confers pheromone sensitivity at physiologic concentrations. Responses of pheromone-sensitive neurons to VA require LUSH, an extracellular protein present in the sensillum lymph bathing trichoid olfactory neuron dendrites. LUSH is also required for neurons mis-expressing the VA receptor to respond to VA. These data provide clear evidence of a requirement for the OBP LUSH in VA detection and provides insight into the neuronal basis of VA pheromone perception.

Poster Session Fri AM

The Role of Vomeronasal Organ in Reception of Androstenone

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Genetic model of specific anosmia to androstenone (AND) exists in the form of inbred strains of mice: highly sensitive to AND CBA/J (CBA) mice and almost insensitive NZB/B1NJ (NZB) mice. In current study we investigated the role of vomeronasal organ (VNO) in reception of AND using CBA and NZB mice. We used 2 approaches: behavioral and immunohistochemical (*c-fos*). AND thresholds were established in both strains of mice using Y-maze paradigm. Thresholds to AND were re-established after surgical removal of VNO (VNX). VNX caused 4–16-fold decrease in sensitivity to AND in highly sensitive CBA mice ($n = 12$), but did not affect thresholds in NZB mice ($n = 12$). Fos positive cells were counted in sensory epithelium of VNO after 1.5 h of exposure to 0.1% AND in CBA ($n = 8$) and NZB ($n = 8$) mice. In CBA mice we observed activated cells in the basal and apical zone of VNO receptor tissue. Consistent pattern of activation in basal zone may indicate the existence of specific AND receptor belonging to V2R family. In NZB mice activated cells were observed only in apical zone. We observed individual variation in number and location of activated cells in apical zone of VNO of NZB mice. Behavioral and histochemical data indicate the involvement of both systems: main olfactory and VNO in reception of AND in CBA mice. In low sensitive to AND NZB mice involvement of VNO in reception of AND requires further investigation.

Supported by RFBR, RAS Program “Biological Resources” #3.1.5, FIRCA TW00495.

Poster Session Fri AM

Response Structure of Single Olfactory Receptor Neurons Correlates with Its Odorant Specificity

Alexandre Nikonov and John Caprio

PhD

We recorded *in vivo* responses to amino acid stimuli of single olfactory receptor neurons (ORNs) simultaneously with the electro-olfactogram (EOG) in the channel catfish to determine the relationship between the response structure and single unit specificity. Stimulus duration was 600 ms which overlaps the time frame required for quality discrimination in vertebrates. For these experiments, only excitatory responses were studied. The number of spikes recorded during the first and second 300 ms time bins following the onset of the EOG along with an additional ~600 ms of response time was analyzed. Two different response structures were evident. Type A ORNs ($n = 40$) were characterized by a response onset correlated with either the initial 300 ms of EOG onset ($n = 29$) or the second 300 ms time frame ($n = 11$) and a spike pattern with an inter-spike-interval (ISI) of ~30 ms and a coefficient of variation (cv) of ~0.25. Responses of type B ORNs ($n = 43$) started ≥ 600 ms subsequent to EOG onset, and the odorant-evoked spikes had an ISI of ~30 ms and a cv of ~0.6. ORNs with type A response structure were also characterized by having a narrow excitatory molecular response range (EMRR) to amino acids that correlate with the specificity determined for group I olfactory bulb (OB) and forebrain (FB) (submitted) units; however, ORNs with type B response structure correlate well with group II OB and FB units which have a broader EMRR.

Supported by NSF IBN-0314970.

Poster Session Fri AM

Odorant Response Properties of Septal Organ Neurons: Broad Tuning and High Sensitivity

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University of Pennsylvania

The septal organ is a distinct chemosensory organ with identified odorant receptors, but its function remains obscure. Using perforated patch clamp recordings, we investigated the response properties of the mouse septal organ neurons in the intact epithelium to a panel of 28 odorants or mixtures. Odor stimulation was delivered by pressure ejection through 7-barrel pipettes. The results revealed several intriguing features of these neurons. First, most of the septal organ neurons are relatively broadly-tuned. Approximately 70% ($n = 258$ tested) of the neurons responded to odor stimulation. Out of 56 neurons tested with structurally diverse odorants, 39 (69.6%) responded to at least one compound. Within the responsive neurons, 79.5% responded to multiple odorants. When stimulated by aliphatic acids with increasing carbon chains (C4 to C9), responsive neurons responded to all acids, suggesting these neurons are broadly tuned within this particular class of molecules. Second, the septal organ neurons are very sensitive to odorants with a broad dynamic range. Different concentrations of octanoic acid, (+) camphor or *n*-amyl acetate were applied to obtain dose–response relations in single neurons. The detection thresholds of individual neurons responding to these 3 odorants varied from the

subnanomolar to submicromolar range. The dynamic range from threshold to saturation often covered 3 to 4 log units of the concentration. This study suggests that the septal organ, situated in the airpath, may serve as a sensitive and broadly-tuned odor detector.

Supported by NIDCD/NIH and Penn IOA.

Poster Session Fri AM

Molecular Basis for Ligand Specificity in a Mouse Olfactory Receptor

Tatjana Abaffy and Charles Luetje

University of Miami

Sequence differences between members of the mouse olfactory receptor MOR42 subfamily (MOR42-3 and MOR42-1) are likely to be the basis for variation in ligand binding preference among these receptors. We investigated the specificity of MOR42-3 for a variety of dicarboxylic acids. We used site-directed mutagenesis, guided by homology modeling and ligand docking studies, in order to locate functionally important residues. Receptors were expressed in *Xenopus* oocytes and assayed using high throughput electrophysiology. The importance of the V113 residue, located deep within the receptor, was analyzed in the context of interhelical interactions. We also screened additional residues predicted to be involved in ligand binding site, based on comparison of ortholog/paralog pairs from the mouse and human olfactory receptor genomes. A network of 8 residues in transmembrane domains III, V and VI was identified. These residues form part of the ligand-binding pocket of MOR42-3. C12 dicarboxylic acid did not activate the receptor in our functional assay, yet our docking simulations predicted its binding site in MOR42-3. Binding without activation implied that C12 dicarboxylic acid might act as an antagonist. In our functional assay, C12 dicarboxylic acid did indeed act as an antagonist of MOR42-3, in agreement with molecular docking studies. Our results demonstrate a powerful approach based on the synergy between computational predictions and physiological assays.

Poster Session Fri AM

Mapping the Binding Pocket of a Mouse Odorant Receptor Using the Substituted Cysteine Accessibility Method

Sarah Repicky, Tatjana Abaffy and Charles Luetje

University of Miami

Modeling of odorant receptors (ORs), using a rhodopsin template, can provide insight into OR structure and function. However, low homology between ORs and rhodopsin makes precise alignment and orientation of transmembrane (TM) helices difficult. We are using the substituted cysteine accessibility method (SCAM) to map residues lining the binding pocket of a mouse OR (MOR), thus providing an orientation for TM helices. MOR23-1 (highly responsive to octanoic acid and octanal), G-alpha-olf, CFTR and RTP1 were expressed in *Xenopus* oocytes, allowing assay of receptor function by electrophysiology. Within TM5, each residue from D193 to L209 was mutated, in turn, to cysteine. Mutants were screened with 3 charged, hydrophilic, sulfhy-

dryl-specific reagents (small positively charged MTSEA, bulkier positively charged MTSET, bulkier negatively charged MTSES). All 3 reagents inhibited odorant responses of the N197C and A205C mutants, indicating that these positions face a relatively large aqueous cavity. V194C and A207C were sensitive to MTSEA, but not MTSET or MTSES, indicating a smaller aqueous space. Within a simple helical structure, V194, N197 and A205 are located on the same face of TM5, while A207 (near the cytoplasmic end of TM5) is oriented roughly 120° around the helix. The position of these residues within MOR23-1 is being investigated by homology modeling, using a SCAM-corrected alignment of TM5. The contribution of each residue to the function of the ligand-binding pocket is being investigated with conventional mutagenesis and functional analysis.

Support: MH66038 (C.L.).

Poster Session Fri AM

Odorant Receptor Expression Profiles in Human Sperm—Part I: From Gene to Function

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¹Ruhr-University, ²University of Arizona and ³University of California

In addition to odorant receptor (OR) expression in sensory neurons of the olfactory epithelium, some OR proteins are also found in ectopic tissues, for example, in testes. In mammals, such testicular ORs have been attributed a potential function as molecular mediators of sperm swimming behavior. Recently, we identified a human testicular OR, OR1D2, that triggers chemotactic and chemokinetic sperm responses *in vitro*. Here, we report functional characterization of 2 novel putative sperm ORs via recombinant expression in HEK293 cells. Comparative analysis of receptor-specific activation profiles reveals non-overlapping molecular receptive fields as well as distinct susceptibility to antagonistic odors. Using a combination of single cell calcium imaging, sperm accumulation assays, flagellar beating and video motion analysis, we show that activation of specific ORs in human sperm mediates different behavioral response patterns. Our findings thus indicate that OR-dependent signaling pathways could play a substantial role in various aspects of sperm physiology.

Supported by: DFG (T.V., M.S. and H.H.), Heinrich and Alma Vogelsang Foundation (A.T. and K.S.), University of Arizona Center for Insect Science, NIH training grant 1K126M00708 (J.A.R.), NSF awards IBN 01-32635/02-06775, California Sea Grant (Project R/F-197), NIH (2-K12-GM000708-06), and UCLA Council on Research (R.K.Z.).

Poster Session Fri AM

Functional Analysis of an Insect Odorant Receptor Using *Xenopus* Oocytes and Robotic Electrophysiology

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Many insect behaviors are controlled by olfaction, critical components of which are the odorant receptors (ORs) and their ability

to discriminate among many thousands of odorants. As a useful technique for receptor characterization, insect ORs can be expressed in *Xenopus laevis* oocytes and functionally analyzed by robotic 2-electrode voltage-clamp recording. Here, we report the expression and characterization of the *Drosophila* OR, DmOr35a, a receptor known to respond to hexanol. Hexanol-induced currents are only apparent when this receptor is coexpressed with DmOr83b, a general association partner for many *Drosophila* ORs. However, receptor function did not require the addition of any other heterologous proteins. The 35a/83b complex responds to 1-hexanol with an EC_{50} of 670 ± 70 nM, but is not activated by known ligands of other *Drosophila* ORs, such as geraniol and octanoic acid, thus exhibiting proper specificity in this expression system. A panel of 1-alcohols between 4 and 9 carbons in length all activate 35a/83b, albeit with varying degrees of responsiveness. This suggests a relatively broad tuning aspect of DmOr35a. In conclusion, we have successfully expressed an insect OR in *Xenopus* oocytes and used robotic electrophysiology to functionally characterize this receptor. The approach provides a convenient method for studying insect ORs and their structural/functional characteristics.

Support: MH66038 (C.W.L.) and AI56081 (H.M.R.).

Poster Session Fri AM

Participation of Kainate Receptors in Synaptic and Extrasynaptic Transmission

Laura J. Blakemore and Paul Q. Trombley

Florida State University

Glutamate mediates most, if not all, excitatory transmission in the olfactory bulb (OB). Whereas NMDA and AMPA receptors are clearly involved, the function of kainate receptors (KARs) is poorly understood. Recently published RT-PCR and ISH data from our lab indicate that each of the KAR subunits is expressed but heterogeneously among OB layers. Our prior immunocytochemical analysis suggests both synaptic and extrasynaptic loci for KARs, particularly within glomeruli. Electrophysiological analysis, in OB slices, indicates that most OB neuron subtypes express functional KARs; at least a subset of these contains the GluR5 subunit, which is activated by ATPA, a GluR5 selective agonist. Preliminary evidence suggests that olfactory nerve layer (ONL) stimulation activates postsynaptic KARs (isolated by blockade of NMDA and AMPA receptors) in PG, ET, and mitral cells. Furthermore, kainate had biphasic actions on ONL-to-OB neuron transmission: potentiation at low concentrations and inhibition at higher concentrations. By contrast, ATPA only inhibited ONL-to-OB transmission, suggesting that different subunit combinations may have different functions. The large population of extrasynaptic KARs in glomeruli suggests that KARs also may play a role in glutamate spillover. Stimulation of the lateral olfactory tract evoked a spillover event in mitral cells. This spillover event was inhibited by ATPA and enhanced by UBP 301 (GluR5 antagonist), suggesting that GluR5-containing KARs may influence transmitter release from M/T cell dendrites as well as the olfactory nerve.

Supported in part by NIH/NIDCD.

Poster Session Fri AM

mGluR1 Activation Enhances Nonselective Cation Currents and Rhythmic Bursting in External Tufted (ET) Cells

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Group I metabotropic receptor (mGluR) mediated depolarization has been linked to activation of nonselective cation currents in hippocampal and cerebellar cells. Olfactory bulb (OB) ET cells express high levels of the group I mGluR, mGluR1. The present study investigated the effects of the group I mGluR agonist DHPG on the excitability of ET cells in OB slices. Rhythmic bursting in ET cells was enhanced by DHPG and dampened by mGluR1 antagonists. Voltage clamp recordings performed in the presence of ionotropic glutamate and GABA receptor antagonists revealed that DHPG enhanced 2 distinct currents in ET cells: (1) a TTX-sensitive persistent Na^+ current previously described in these cells, and (2) a TTX-insensitive, putative nonselective cation current. The nonselective cation current exhibited a relatively linear I-V relationship that reversed in polarity near -10 mV. The current persisted in the presence of cadmium ($100 \mu M$) and nickel (1 mM), and therefore does not depend upon Ca^{2+} influx via voltage-gated Ca^{2+} channels. However, the current was reduced by blockers of Ca^{2+} -activated nonselective cation currents. The persistent Na^+ and nonselective cation currents enhanced by DHPG in ET cells are active at membrane potentials near the burst threshold (-60 mV). Thus, activation of mGluR1 may facilitate rhythmic bursting in ET cells tonically or in response to olfactory nerve input.

Support: PHS grants: DC06356, DC07123, DC03195, RR020146.

Poster Session Fri AM

L/T-Type Calcium Channel Regulation of ET Cell Bursting

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External tufted (ET) cells in olfactory bulb glomeruli are characterized by bursting activity, which occurs both spontaneously and in response to olfactory nerve (ON) stimulation. Bursting is generated by intrinsic ionic currents expressed in ET cells. We previously found that T-type Ca^{2+} channels are required for spontaneous and ON-evoked bursting. We now report that nimodipine ($20 \mu M$), an L-type Ca^{2+} channel blocker, significantly inhibits the low-voltage activated (LVA) Ca^{2+} current in ET cells while the L-type Ca^{2+} channel activator Bay K8644 ($5 \mu M$) significantly enhances I_{LVA} and shifts its activation threshold from -50 to -55 mV. In current clamp, evoked LVA Ca^{2+} spikes are blocked by nimodipine, and significantly broadened by BayK. In the presence of fast synaptic blockers, nimodipine reduces rhythmic bursting to single spiking; this is reversed upon nimodipine washout and addition of BayK. BayK, alone significantly increases burst duration and the number of spikes/burst; this is reversed by BayK washout and addition of nimodipine. In response to ON stimulation without synaptic blockers, nimodipine reduces bursting to single spikes whereas Bay K increases burst

duration as well as the number of spikes/burst. These results demonstrate that ET cells express Ca^{2+} conductance mediated by L- and/or T-type Ca^{2+} channels, which are essential for spontaneous and sensory-evoked bursting behavior. Both I_L and I_T are targets of modulatory transmitters. Modulation of these conductances should significantly regulate the transfer of olfactory input at the initial site of synaptic integration in the bulb.

Supported by NIH NIDCD DC005676.

Poster Session Fri AM

Activation of Postsynaptic GABA_B Receptors Directly Modulates the Bursting Pattern and Synaptic Activity of Olfactory Bulb Juxtglomerular Neurons

Abdallah Hayar and Nikolay Karpuk

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There is solid evidence that GABA released from olfactory bulb periglomerular (PG) neurons presynaptically inhibits glutamate release from olfactory nerve terminals via activation of GABA_B receptors (GABA_B-Rs). However, it is still unclear whether juxtglomerular cells have GABA_B-Rs. We have investigated whether juxtglomerular neurons have functional postsynaptic GABA_B-Rs using extracellular and whole-cell recordings in olfactory bulb slices. In the presence of fast synaptic blockers (CNQX, APV and gabazine), the GABA_B-R agonist, baclofen (10 μM), either inhibited completely the bursting, or reduced the bursting frequency and increased the burst duration and the number of spikes/burst in external tufted (ET) cells. In the presence of synaptic blockers and TTX, baclofen induced an outward current in ET cells suggesting a direct postsynaptic effect. Baclofen reduced the frequency and amplitude of spontaneous bursts of EPSCs in PG cells and reduced the frequency of spontaneous bursts of IPSCs in ET cells. We suggest that activation of GABA_B-Rs directly inhibits ET cell bursting, leading to a decrease in excitatory drive to PG cells and subsequently to a significant reduction in PG-to-ET cell feedback inhibition. All baclofen effects were reversed or prevented by application of the GABA_B-R antagonist, CGP55845 (10 μM). Thus, the postsynaptic GABA_B-Rs on ET cells may play an important role in shaping the activation pattern of the glomeruli during olfactory coding.

PHS grants: DC06356, DC07123, RR020146.

Poster Session Fri AM

Characterization of Inhibitory Gates in Mitral Cell Pairs

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It is generally thought that granule-mitral cell synapses in the olfactory bulb function to inhibit mitral cell firing, and that this inhibition can underlie such functionally important phenomena as lateral inhibition and synchronization. Our objective was to use a computational approach to survey the effect of dendrodendritic synapses on firing of pairs of mitral cells sharing a granule cell. Using the detailed biophysical mitral cell model of Bhalla and Bower we quantified the magnitude of inhibition needed along the dendrite to counteract various excitatory stimuli. We show that depending on the

magnitude and especially location of the dendrodendritic synapses along the mitral cell lateral dendrite, 3 types of inhibitory effects can be described between mitral cell pairs: 1) A *bidirectional gate* arises when the granule cell induces a discernible inhibitory response in both mitral cell somas. 2) A *unidirectional gate* occurs when the granule cell induces a discernible inhibitory response in only one mitral cell soma. 3) An *inconsequential gate* occurs when the granule cell does not induce a discernible inhibitory response in either mitral cell soma. Preliminary results indicate that most of the lateral dendrite contains unidirectional or inconsequential gates. This is important as most olfactory bulb models effectively treat the mitral-granule dendrodendritic synapse as a bidirectional gate and may need to account for these other types of gates.

This work was funded by NIH grants DC004657 and DC006070 (D.R.), 5R01-LM008111-03 (L.H.), DC006640 (N.S.).

Poster Session Fri AM

The Effect of Sniff Frequency on Presynaptic Inhibition of Receptor Input to the Olfactory Bulb

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Boston University

Olfactory receptor neurons (ORNs) converge onto olfactory bulb (OB) glomeruli corresponding to individual odorant receptors. ORN input to a glomerulus is modulated via feedback presynaptic inhibition. Slice experiments using paired olfactory nerve stimulation have shown that this inhibition peaks 100–200 ms after a conditioning pulse and decays with a time-constant of ~ 500 ms. Sniffing in rodents varies between 2 and 10 Hz (intersniff intervals from 100 to 500 ms); thus, changes in sniff frequency could change the level of presynaptic inhibition of ORN input. We asked how sniff frequency modulates the level of presynaptic inhibition at the ORN synapse in vivo by imaging odorant-evoked input to the OBs of anesthetized mice. ORNs were loaded with calcium-sensitive dye and sniff frequency was controlled using an artificial sniff protocol. As reported previously, blocking GABA_B-mediated presynaptic inhibition increased the amplitude of odorant-evoked calcium signals, but, contrary to that study, we observed no change in the relative amplitude of “surround” signals. Surprisingly, the increase in ORN input was independent of sniff frequency, and was seen even during the first sniff after odorant onset. It is possible that each sniff (150–200 ms in duration) evokes sufficiently prolonged input to the glomerulus that feedback presynaptic inhibition is evoked by all sniffs regardless of intersniff interval. Alternatively, presynaptic inhibition may occur tonically in the anesthetized mouse but not in slice preparations. Experiments testing these 2 hypotheses are currently underway.

Funded by NIH DC06441.

Poster Session Fri AM

Matrix Metalloproteinases in Olfactory Development

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Yale University School of Medicine

During olfactory pathway formation, growing olfactory sensory axons (OSN) must navigate a complex extracellular environment to reach their correct target in the olfactory bulb (OB). To form

their precise topographic connections, which in part underlie odor coding, we have hypothesized that OSN axons use extracellular guidance cues. Recently, we have become interested in matrix metalloproteinases (MMPs), a family of zinc-dependent proteolytic enzymes that have been shown elsewhere in the developing CNS to regulate axon guidance by degrading extracellular matrix (ECM) or by cleaving guidance cues and their receptors. We hypothesized that MMPs may play a role in guiding OSNs by sculpting the ECM and influencing axon interactions with the environment. To investigate this, we used RT-PCR to screen 18 members of the MMP family and their 4 endogenous inhibitors (tissue inhibitors of metalloproteinases, TIMPs) at different stages of olfactory development. We found a subset of MMPs and TIMPs were expressed, with many genes having differential age of onset and spatial patterns of expression. In addition, we used immunohistochemistry to localize some candidate MMP proteins and found different spatio-temporal patterns of expression that were consistent with their having a role in axon pathway formation. To assess where MMPs were present in their active form, we performed *in situ* zymography and found restricted patterns of activity within the olfactory nerve. Collectively, our data suggest that MMPs are active in the developing olfactory system and have a role in olfactory pathway formation.

Supported by NIH DC005706 and DC007600 to H.T.

Poster Session Fri AM

Modulation of Olfactory Receptor Axon Sorting and Targeting by Lipid Raft-Associated Signaling Molecules

Nicholas Gibson, Lynne Oland, Mark Higgins and Leslie Tolbert

University of Arizona

During their growth to the olfactory lobe, axons of receptor neurons (ORNs) in the moth *Manduca sexta* grow through a glia-rich domain where the axons sort and fasciculate according to their target glomerulus in a glia-dependent manner. In this sorting zone (SZ), we have shown that fibroblast growth factor receptors are activated on the glia, epidermal growth factor receptors (EGFRs) are activated on ORN axons, and axonal neuroglian becomes tightly anchored in the membrane. We are testing the hypotheses that (1) SZ glia alter axonal behavior by regulating neuroglian–neuroglian interactions that then affect activation of the GFRs, and (2) control of this signaling pathway is exerted by inclusion of these molecules in lipid rafts. EGFRs colocalize with a marker for lipid rafts, and we find that disruption of raft assembly blocks EGFR activation. Direct disruption of EGFR activation leads to axon stalling and abnormal fasciculation in the SZ. To test whether disruption of rafts and of EGFR signaling alters axon targeting, we are analyzing the growth of a small subset of ORN axons that sort under the influence of glial cells in the SZ and target a single dorsal glomerulus that is present in both sexes. These axons are unique in being recognized by an antibody against an epitope similar to a region of the *H. virescens* chemosensory receptor 13 (EMB CAG38114.1). This axonal labeling occurs during development but is lost in the adult, when only ORN cell bodies and dendrites are labeled.

Funded by NIH P01-NS28495 and DC004598.

Poster Session Fri AM

Knockdown of Olfactory Axon Guidance Molecules in *Manduca sexta*

Mark Higgins, Christine Pham, Maris Jameson and Alan Nighorn

University of Arizona

We are using the moth *Manduca sexta* as a model system in which to investigate olfactory receptor neuron (ORN) axon guidance. The developmental expression patterns of a variety of different molecules from different superfamilies suggest they may help to regulate this process. We have found that Eph receptors tyrosine kinases (EphR) and their ephrin ligands are expressed on ORN axons which innervate a subset of complimentary glomeruli. In addition, the Ig superfamily cell adhesion molecules fasciclin II (MFasII) and neuroglian (Nrg) are expressed on ORN axons at the right developmental times. To begin to test the hypothesis that these molecules play critical roles in olfactory axon guidance, we are using RNAi to knockdown their expression in the developing olfactory system of the moth *M. sexta*. Fragments of dsRNA from each candidate molecule were injected, individually or in combination, during the period when the first ORC axons are innervating the brain. Two days after injection, knockdown was measured using Q-PCR and results were compared to control animals injected with nonsense dsRNA constructs. Injections of Eph dsRNA gave knockdown of up to 88%. Injections of Nrg gave knockdown of up to 50%. Injections of MFasII gave knockdown of 60–70%. Injections of Eph/Nrg/ephrin consistently gave knockdown of 96% of both Eph and Nrg. We are now assaying the effects of this knockdown using immunohistochemistry and dye-filling experiments.

This work is supported by NIH–NIDCD DC04292.

Poster Session Fri AM

Extracellular Matrix Gene Profiling in the Developing Mouse Olfactory System

Arundhati Ray and Helen Treloar

Yale University

Precise targeting of olfactory sensory neuron (OSN) axons to their target glomeruli during development is critical for olfactory perception/coding. Despite the burgeoning evidence of the role of various growth promoting and inhibitory factors in olfactory axon development, the function of various extracellular matrix (ECM) molecules in this process remains largely unknown. Our current goal is to determine whether ECM molecules play a role in the navigation of olfactory axons. Initial screening of genes at different developmental stages was analyzed by hybridization to 2 different oligo arrays. These commercially available pathway-focused arrays allow screening of small sets (100–500) of relevant pathway-specific genes. Using these arrays we have identified several novel genes in the olfactory epithelium and bulb which are good candidate guidance cues. Further characterization of the expression of these identified genes is being undertaken using *in situ* hybridization, immunohistochemistry and quantitative RT-PCR. This expression profiling of ECM genes provides insight into their possible role as axon guidance cues and lays the foundation for further functional studies.

Poster Session Fri AM**Phagocytosis-Dependent Labeling of Presumptive Microglial Cells in the Deafferented Olfactory Bulb of Zebrafish**

Christine Byrd and Jamie Johnson

Western Michigan University

Peripheral deafferentation profoundly effects the morphology of the adult olfactory bulb. In zebrafish, we have shown that olfactory-organ ablation results in decreased bulb volume, increased cell death, and decreased immunoreactivity for a variety of proteins. The current study is part of a project designed to investigate the potential role of an immune response in these deafferentation effects. We previously used plant lectins to identify microglia, the phagocytic cells in the brain that respond to neuronal injury and death by removing cellular debris. We found that there were very few lectin-positive cells in normal olfactory bulbs and no evidence of proliferation of these cells following olfactory damage. It is possible that lectins label only a subset of microglia in zebrafish or that deafferentation does not cause activation of microglia in this fish. To examine further the potential injury-induced immune response of the zebrafish brain, we used an alternative technique to mark microglia: phagocytosis-dependent labeling. Olfactory axons were traced anterogradely by application of DiA to the olfactory organ. Olfactory deafferentation was then performed using a cautery iron to ablate the right olfactory organ. Following various survival times from 1 to 4 days, the brain was dissected, sectioned on a cryostat, and viewed with conventional fluorescence or confocal microscopy. As the brain responded to this damage, phagocytic removal of degenerating axons by presumptive microglial cells caused them to pick up the fluorescent dye.

Supported by NIH DC04262 to C.B.

Poster Session Fri AM**Differences in MMP-2 And MMP-9 Expression in Response to Olfactory Nerve Injury**

Richard Costanzo and Lisa Perrino

Virginia Commonwealth University School of Medicine

Matrix metalloproteinases (MMPs) are associated with extracellular remodeling that occurs in injury and repair processes in the CNS. The gelatinases, MMP-9 and MMP-2, have been linked to blood brain barrier disruption, inflammation, angiogenesis, scar formation and possibly neural degeneration and regeneration. We previously reported that MMP-9 levels are elevated early in the response to olfactory nerve injury and that these changes correspond to neuronal degeneration and increased glial activity. In this study we examined the role of MMP-2 in olfactory nerve injury and recovery in adult mice. MMP-2 expression in the olfactory bulbs was examined using Western blot and gelatin zymography over a 60 day recovery period following bilateral olfactory nerve transection. In control and sham animals, almost no MMP-2 was detected in the olfactory bulbs. Following olfactory nerve transection, MMP-2 levels increased above control levels within 5 h, and peaked at 7 days post-injury. MMP-2 levels decreased rapidly after day 7 and returned to control levels over the 60 day recovery period. The peak of MMP-2 expression occurred later (day 7) than that for MMP-9 (day 1) and the decline in

MMP-2 levels was more rapid than that for MMP-9. This is the first report of MMP-2 changes in the olfactory bulb following injury. While MMP-9 levels are elevated at the early stages of neuronal degeneration, MMP-2 levels peak later, at the onset of neuronal regeneration. These findings suggest that MMP-9 and MMP-2 may play different roles in the response to olfactory nerve injury.

Supported by NIDCD DC00165-21.

Poster Session Fri AM**Proliferation in the VNE Decreases with Age but Response to Injury Does Not**Jessica H. Brann¹ and Stuart Firestein^{1,2}¹*Department of Biological Science and* ²*Program in Neurobiology and Behavior*

The vomeronasal epithelium (VNE) detects volatile and nonvolatile socially relevant odorants, and exhibits neuronal turnover. This process is unique, in that the majority of neuronal populations do not exhibit growth beyond a short developmental period. Here, we examined the proliferative ability of the VNE at 2, 6, and 24 months of age in order to evaluate its regenerative potential. Since previous work has shown 2 types of proliferating cells, at the margin and center of the VNE, we measured BrdU incorporation in specific VNO regions. In initial studies, we show that BrdU labeling in basal cells in 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. OBX results in rapid death of mature sensory neurons within 5 days, followed by the massive proliferation of basal cells and reconstitution of ~70% 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 VNE of old animals, this rate increases when challenged with an injury.

J.H.B. supported by F32 DC008455.

Workshop: Odor Signaling in Humans**Human Pheromones and Behavior**

Karl Grammer

Ludwig-Boltzmann-Institute

Olfactory communication is very common amongst animals, and since the discovery of an accessory olfactory system in humans, possible human olfactory communication has gained considerable scientific interest. The importance of the human sense of smell has by far been underestimated in the past. Humans and other primates have been regarded as primarily "optical animals" with highly developed powers of vision but a relatively undeveloped sense of smell. In recent years this assumption has undergone major revision. Several studies indicate that humans indeed seem to use olfactory communication and are even able to produce and perceive certain pheromones; recent studies have found that pheromones may play an important role in the behavioural and reproduction biology of humans. The presented evidence on pheromones

suggests that the model of humans being only optical animals has to be revised. Human sociosexual interactions are influenced by pheromones, even if they cannot be detected consciously. Pheromones have the potential to influence human behaviour and physiology and so there has to be asked the question, in which way the modern striving for cleanliness and odourlessness affects our everyday social lives and human reproductive success in the future. The current state of knowledge, as many studies in the last few years have pointed out, is that humans, like other animals, use olfactory signals for the transmission of biologically relevant information.

Workshop: Odor Signaling in Humans

The Identification of Compounds in Human Sweat—Signals of Individuality, Gender and Genes

Elisabeth Oberzaucher¹, Karl Grammer¹, Katharina Zimmer¹, Gottfried Fischer², Helena A. Soini³, Milos V. Novotny³, Sarah J. Dixon⁴, Yun Xu⁴, Simeone Zomer⁴, Richard G. Brereton⁴ and Dustin J. Penn⁵

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Individuals are thought to have their own distinctive odour, analogous to a signature or fingerprint. To determine whether volatile components of human odour show sufficient variation among individuals or consistency over time necessary to provide fingerprints, we systematically sampled emanations from 196 HLA-typed adults in a population in the Austrian Alps. We chemically analyzed the samples, and analyzed the chromatograms using pattern recognition techniques. We found individually distinct and reproducible fingerprints in sweat, and some individuals were more distinctive than others. We identified compounds that are stable and individual in the chemical profile, and others that serve as gender markers. Our bioassays showed that HLA-genes indeed affect the chemical signature, and even to a greater extent than overall genetic relatedness, which supports the idea that body odor conveys information about genes that control immune resistance to disease.

Workshop: Odor Signaling in Humans

Odor-Independent Effects of Human Chemosignals and Pheromones on Ovulation, Sexuality and Cognition

Martha McClintock

Department of Psychology, University of Chicago, Chicago, IL, USA

Human pheromones, one type of social chemosignal, modulate neuroendocrine function by regulating the timing of the preovulatory surge of luteinizing hormone. Here, we demonstrate that natural compounds collected from lactating women and their breastfeeding infants not only modulated the timing of the menstrual cycle but also increased the sexual motivation of other women, measured as sexual desire and fantasies. Other social chemosignals modulate the adrenal axis, different types of psychological states and widely distributed yet specific areas in the brain. They also accelerate processing of social information, lightening both cognitive and emotional loads. In this newly emerging field, there appears to be directly conflicting results. Here I will argue that this apparent conflict arises, in many cases, from an exquisite sensitivity to social

context that enhances or precludes a response. Thus, human social chemosignals may serve a broad range of neuroendocrine and social functions.

Workshop: Odor Signaling in Humans

Perception of Chemosensory Anxiety Signals in Socially Anxious Subjects

Bettina Pause¹, Dirk Adolph¹, Alexander Prehn¹, Anne Ohrt¹, Joachim Laudien², Bernfried Sojka² and Roman Ferstl²

¹Department of Experimental Psychology, University of Duesseldorf, Germany and ²Department of Psychology, University of Kiel, Germany

Similar to most socially living phyla, also humans seem to communicate anxiety/stress via chemosensory signals. Here, we investigated whether socially anxious subjects (SAS) respond to chemosensory anxiety signals in a different way than non-anxious subjects. Axillary sweat samples were taken from 49 university students before an academic oral examination (anxiety condition) and during a sport situation (control condition). The sweat donors described themselves as more anxious ($P < 0.001$) and less dominant ($P < 0.001$) during the anxiety condition than during the control condition. The chemosensory stimuli deriving from the pooled sweat samples were presented to 16 SAS as well as to 32 non-anxious subjects via a constant-flow olfactometer. The impact of the chemosensory anxiety signal on the perceivers was investigated by analyzing chemosensory event-related potentials (CSERP), the skin conductance response (SCR) and the modulation of the startle reflex (in response to acoustic startle probes). In non-anxious subjects chemosensory anxiety signals are processed advantageously (early components of the CSERP) and increase the startle reflex. However, there is no effect on the SCR. The effect on the startle reflex was much more pronounced in SAS subjects than in the control group. It will be discussed whether chemosensory anxiety signals pre-attentively prime withdrawal related behaviour, especially in anxious subjects.

Workshop: Odor Signaling in Humans

Changes in Olfactory Threshold, Hedonics and Brain Activity in Response to Repetitive Exposure to Androstadienone

Tim Jacob¹, Liwei Wang^{1,2}, Nassima Boulkroune³, Amy March¹ and Natalie Walker¹

¹Cardiff University, ²Jinan University and ³Liverpool University

Background: Androstadienone is a steroid found in human secretions that has been widely proposed as a candidate for a human pheromone. **Methods:** Using threshold testing, psychometrics and electrophysiological techniques we investigated the effects of a repetitive exposure protocol on the perceptual and physiological response to androstadienone. **Results:** First, repetitive exposure caused up to a 1000-fold decrease in detection threshold and second, accompanying this sensitization process, there was a change in the perceived odour quality, from pleasant to unpleasant. Third, there was an increase in the amplitude of the peripheral response of the olfactory system, the electro-olfactogram (EOG), that was more pronounced in

women than in men, and finally, there was a gender dimorphic increase in the amplitude of early and late components of the olfactory event-related potential (OERP) that encodes both peripheral and central brain responses. None of these changes occurred with a control odorant, benzaldehyde. **Conclusions:** Repetitive exposure to androstadienone causes peripheral changes to the olfactory system; either additional expression of receptors, or increased number of receptor cells. This is associated with a change in odour perception. While the sensitivity and hedonic changes occur in both men and women, the changes in the later components of the evoked potentials are specific to women, suggesting gender-specific cognitive plasticity.

Workshop: Odor Signaling in Humans

Brain Response to Putative Pheromones in Humans

Ivanka Savic

Department of Clinical Neuroscience, Karolinska Institute, Stockholm, Sweden

The progesterone derivative 4,16-androstadien-3-one (AND), and the estrogen like steroid oestra-1,3,5(10),16-tetraen-3-ol (EST) are candidate compounds for male and female pheromones. Several psychophysical studies indicate that they have specific effects in humans of which some are sex differentiated. Smelling of these compounds is shown to activate the human brain in a sex differentiated manner, and recent data suggest that the pattern of activation in homosexual subjects deviates from controls of the same sex, raising the question as to whether this could be effect of sexual behavior or reflect a variant organization of the hypothalamic circuits. These issues will be discussed in relation to new data from male-to-female transsexuals, which offer some additive information. Furthermore, possible pathways for the differentiated processing of pheromones and ordinary odors will be addressed from old and new perspectives.

Olfaction: CNS

Slit-Robo Signaling Is Required for Zonal Segregation of Olfactory Sensory Neuron Axons in the Main Olfactory Bulb

Jean-François Cloutier, Manon Lépine and Jin Hyung Cho

Montreal Neurological Institute and McGill University

The formation of precise stereotypic connections in sensory systems is critical for the ability to detect and process signals from the environment. In the olfactory system, olfactory sensory neurons (OSNs) project axons to spatially defined glomeruli within the olfactory bulb (OB). A spatial relationship exists between the location of OSNs within the olfactory epithelium (OE) and their glomerular targets along the dorsal-ventral axis in the OB. However, the molecular mechanisms underlying the zonal segregation of OSN axons along the dorsal-ventral axis of the olfactory bulb are poorly understood. Using *robo-2*^{-/-} and *slit-1*^{-/-} mice, we examined the role of the Slit family of axon guidance cues in the targeting of OSN axons during development. We show that a subset of OSN axons that normally project to the dorsal region of the OB mistarget and form glomeruli in the ventral region in *robo-2*^{-/-} and *slit-1*^{-/-} mice. In addition, we show that the Slit receptor, Robo-2, is expressed in OSNs in a high dorso-medial to low ventro-lateral gradient across

the OE, and that Slit-1 and Slit-3 are expressed in the ventral region of the OB. These results indicate that the dorsal-to-ventral segregation of olfactory sensory neuron axons within the OB are not solely defined by the location of olfactory sensory neurons within the OE but also relies on axon guidance cues of the Slit family.

This research is funded by grants from the Canadian Institutes of Health Research and FQRNT to J.-F.C.

Olfaction: CNS

Regulation of Olfactory Bulb Laminal Organization and Periglomerular Interneuron Phenotypes by ER81

John Cave^{1,2}, Yosuke Akiba², RoseAnn Berlin² and Harriet Baker^{1,2}

¹Weill Medical College of Cornell University and ²Burke Medical Research Institute

The laminar organization and the generation of neuronal diversity in the olfactory bulb (OB) may be regulated by common molecular and genetic pathways. Previous studies have identified an ETS transcription factor, ER81, as a marker for both progenitor and mature glomerular layer interneurons. In the present study, we have examined the contribution of ER81 to the establishment of interneuron diversity in the glomerular layer of the OB. ER81 null-mutant mice displayed both a disruption of the laminar organization of the OB as well as a dramatic loss of immunoreactivity for calbindin and dopaminergic (as indicated by tyrosine hydroxylase, TH) interneuron phenotypes. Analysis of upstream gene regulatory regions revealed 2 conserved ER81 consensus binding sites in the rodent TH promoter, but none with calbindin. ChIP and EMSA experiments demonstrated that ER81 can directly bind these conserved sites in the TH promoter. In the naris closed mouse model of odor deprivation, ER81 expression levels, like calbindin and TH, are modulated by afferent olfactory sensory input. The findings suggest that the molecular mechanism by which ER81 regulates the dopaminergic phenotype involves direct regulation of TH expression, whereas the regulation of calbindin is indirect. Together these results indicate that ER81 is important for laminar organization of the olfactory bulb and the generation of sensory-dependent calbindin and dopaminergic interneuron phenotypes in the periglomerular layer.

Funded by NIH AG09686 and the BRMI.

Olfaction: CNS

Disruption of Voltage-Gated Activity in Mitral Cell Neurons Causes Supernumerary and Heterogeneous Glomeruli While Decreasing the Number of OSNs Peripherally

D.A. Fadool, D.R. Marks and K.C. Biju

Florida State University

While odorant receptor (OR) sequence may act as one determinant in creating a stereotypic olfactory sensory map, the extent by which voltage-dependent activity of projection neurons in the OB influences the development of the sensory map is not known. We generated OR-tagged mice in a Kv1.3-null background by breeding mice with targeted M72- or P2-IRES-taulacZ mutations with mice that have a gene-targeted deletion in the Kv1.3 ion channel (called KvP2,

KvM72). Absence of Kv1.3 voltage-gated activity in mitral neurons caused the formation of small, supernumerary glomeruli in the KvM72 but not KvP2 mice. Sixty-eight KvM72 mice were examined from P14 to 2 years of age to confirm that the supernumerary synaptic connections were not developmentally delayed but rather the result of altered neural pruning. Two photon imaging in KvM72 mice demonstrated that axons coalesced into heterogeneous glomeruli suggesting that processes that eliminate axons with dissimilar identities are being perturbed. Interestingly, absence of Kv1.3 voltage-gated activity also significantly decreased the number of P2, M72 and MOR28 expressing OSNs without changing epithelial expression patterns. Immunocytochemical localization of MOR28 showed increased subcellular expression in the cilia in Kv1.3-null mice compared with that in WT. Moreover, when combined with odor-sensory deprivation, unilateral-naris occlusion revealed that deletion of Kv1.3 protected OSNs from sensory deprivation-induced degeneration.

Supported by NIH DC03387 (NIDCD).

Olfaction: CNS

Olfactory Bulb Odor Response Dynamics Enhanced by Odor Enrichment

Christiane Linster¹, Nathalie Mandairon¹, Emily Wyatt² and Leslie Kay²

¹Cornell University and ²University of Chicago

Electrophysiological experiments have shown that odor exposure alone, unaccompanied by behavioral training, changes response patterns of olfactory bulb (OB) neurons. In agreement with these studies, we have shown that exposure alone improves odor discrimination in rats, and that changes in the olfactory bulb are at least partially responsible for the observed changes in perception. Daily activation of the OBs by local NMDA infusion affected rats' discrimination of chemically similar odorants similarly to daily exposure to enrichment odors. We show here that activation of granule cells in response to odorant stimulation, as measured by immediate early gene mapping (*Zif286*), is modulated by daily odor exposure. The number of labeled granule cells is significantly increased in enriched rats as compared to non-enriched control rats, and labeled cells are more widely distributed throughout the OB. Computational modeling of activity dependent processes in the OB predicts that an increase in inhibitory neuron responses after enrichment could lead to an increase in the power of stimulus evoked oscillations in the OB. This in turn translates into increased population synchrony and improved odor discrimination at the level of OB mitral cells. Using field potential recordings in behaving animals, we show that indeed the power of stimulus evoked OB gamma oscillations is significantly increased in enriched animals compared to non-enriched controls.

Olfaction: CNS

Timing of Granule Cell Activity in the Main Olfactory Bulb

Nathaniel Urban and Vikrant Kapoor

Carnegie Mellon University

Considerable evidence points to the timing of mitral cell action potentials playing an important role in the coding of information about odor stimuli. Granule cells, via reciprocal dendrodendritic synapses made with mitral cells play a key role in regulating spiking in mitral cells. Thus, we investigated the timing of granule cell activity in vitro. We imaged granule cell population activity using olfactory bulb slices bulk loaded with calcium dye. We found that extracellular stimulation of single glomeruli resulted in asynchronous recruitment of granule cells with different granule cells in the same slice having response latencies ranging between 0 and 900 ms. These latencies are reliable across trials (average standard deviation of latencies was ~50 ms) and are glomerulus-specific. This delayed activity in granule cells following mitral cell stimulation suggests that granule cells integrate information about mitral cell activity over periods of hundreds of milliseconds. We further imaged activity in populations of granule cells following paired pulse stimulation of single and pairs of glomeruli with interstimulus intervals of 100–600 ms. We saw maximal granule cell population activity (>300% recruitment in some cases) when 2 stimuli were delivered with a 200 ms interstimulus interval. This specificity for 200 ms delay between stimuli suggests that granule cells have the ability to integrate activity across multiple sniff cycles. This ability may be essential for generation of slow temporal patterns in the olfactory bulb.

Supported by NIDCD (RO1DC005798).

Olfaction: CNS

Olfactory Bulb Gamma Oscillations Are Dynamically Altered to Adjust to Task Demands

J. Beshel and L.M. Kay

University of Chicago

In the 60 years since discovery of olfactory bulb gamma oscillations, fast oscillations of neural assemblies have been proposed as a possible mechanism to facilitate stimulus representation in a variety of sensory systems across species. Previous studies in extremely disrupted olfactory systems suggest that enhancing (in mice) or suppressing (in honeybees) these high frequency oscillations increases or decreases fine odor discrimination, respectively. However, it has never been shown that animals dynamically change population synchrony to adjust to task demands. We provide the first direct evidence that gamma oscillatory power in the rat olfactory bulb is modulated online in the intact system in a task-demand dependent fashion. We paired a 2-alternative choice odor discrimination task with simultaneous local field potential recording, which previous studies lacked, as an essential test for a functional role of fast oscillatory population activity in the resolution of odorant stimuli during normal functioning. Comparisons between trials from criterion sessions (>70% correct, 200-trial sessions) show that rats increase the power of gamma oscillations during fine but not coarse odor discrimination [$t(3112) = 19.757$, $P = 0.0001$], indicating large scale population cooperativity with fine temporal precision. The enhancement in gamma power differs from oscillatory responses previously linked to familiarization and learning and may reflect a switch in the dynamics of the system to a strategy that optimizes stimulus resolution when the input signal is ambiguous.

Supported by NIDCD F31DC008467 (J.B.) and DC00795 (L.M.K.).

Poster Session Fri PM**Hindbrain Orexin-A Increases Licking for Sucrose But Not Water**

Angela Choe, Jasmine Loveland and John-Paul Baird

Amherst College

Orexin-A (ORA) is a neuropeptide produced in perikarya limited to the lateral hypothalamus that stimulates feeding when centrally administered. The motivational bases of this ORA effect remain unclear: ORA may augment intake through a variety of effects, including enhanced taste processing or diminished satiety. We used lick microstructure analysis to evaluate the effects of hindbrain ORA application on feeding in more detail. Twelve rats fitted with cannulas aimed at the 4th ventricle received either vehicle (2 μ l artificial CSF) or ORA (1 nM/2 μ l) injections 15 min prior to 90 min access to either 0.0, 0.1, and 1.0 M sucrose solutions, in counterbalanced order. The effects of ORA were concentration specific as ORA increased intake for 0.1 M sucrose only. The lack of increased water consumption rules out non-specific activational effects of the peptide, but it also contrasts findings reported after forebrain ORA delivery and suggests that ORA has separate brain sites of action on drinking and feeding. The increase of 0.1 M sucrose intake was achieved by prolonging the meal with little effect on ingestion rate in early phases, or burst size, suggesting that hindbrain ORA reduced inhibitory post-ingestive feedback rather than enhanced gustatory evaluation. The results draw attention to direct hypothalamic ORA projections to hindbrain sites implicated in feeding control, particularly the parabrachial nucleus, nucleus of the solitary tract and area postrema.

Supported by Amherst College, HHMI and NIH DC-05326.

Poster Session Fri PM**Neural Circuits Mediating Nursing Analgesia in Neonatal Rats**

Teresa Bell, Matthew Ennis and Yi-Hong Zhang

University of Tennessee Health Science Center

Analgesia in human and rat neonates elicited by nursing is mediated by 2 distinct mechanisms: 1) a gustatory or taste component engaged by sugars in mother's milk, and 2) an orotactile or somatosensory component engaged by suckling. Taste analgesia is opiate receptor-dependent whereas orotactile analgesia is not. The circuits mediating nursing analgesia are unknown. Here, Fos immunocytochemistry and tract tracing were used to identify pathways mediating nursing analgesia in neonatal (P10–13) rats. Taste analgesia produced by intraoral sucrose infusion elicited Fos expression in the first 2 relays in the ascending gustatory system, the nucleus of the solitary tract (NST) and the parabrachial nucleus (PBN), and in 2 sites implicated in opioid analgesia, the periaqueductal gray (PAG) and rostroventromedial medulla (RVM). Fos positive (+) NST and PBN neurons projected to PAG but not RVM, and Fos+ PAG neurons projected to RVM. These findings disclose a candidate circuit underlying sucrose-induced, opiate receptor-dependent analgesia: 1) sucrose-responsive NST and PBN neurons project to PAG, and 2) PAG neurons activated by sucrose project to RVM. Fos staining was also used to identify brainstem neurons activated

by orotactile stimulation during non-nutritive suckling from the anesthetized dam. Compared to non-suckling controls, suckling elicited Fos expression in the spinal trigeminal nucleus, PAG, nucleus cuneiformis, locus coeruleus and RVM. Thus, suckling in neonatal pups induces Fos expression in several brainstem sites involved in pain processing and descending nociceptive modulation.

Support: PHS grants DC03895 and NS41384.

Poster Session Fri PM**Hippocampal Coding of the Behavioral Relevance of Taste Stimuli**

Bethany Reville and Donald Katz

Brandeis University

Several behavioral experiments have indicated a role for the hippocampus (HP) in taste processing. According to this research, lesions of the HP disrupt proper behavioral adjustments to experimenter-induced changes in the palatability or incentive value of taste stimuli. To better understand these effects, I began looking at the electrophysiological responses to taste stimuli in the dorsal HP. Multielectrodes were used to record ensemble neural responses from the HP of behaving rats during the passive delivery of tastants. Time-averaging techniques as well as temporal analyses revealed stimulus-triggered responses to tastants of varying qualities (sweet, sour, bitter, and salty). Consistent with the results of the behavioral research, the response patterns for similarly palatable tastants are more strongly correlated than tastants with opposite palatabilities. Preliminary evidence also suggests more diverse coding for stimuli with inherent motivational properties. This research emphasizes the distributed nature of taste processing while also demonstrating the extensive influence of the HP.

Poster Session Fri PM**Amygdalar and Cortical Processing of Taste and Conditioned Taste Aversion**

Stephen Grossman and Donald Katz

Brandeis University

We performed simultaneous extracellular recordings from gustatory cortical (GC) and basolateral amygdalar (BLA) neural ensembles in awake, behaving rats as they sampled various taste qualities. To one particular taste, we induced a conditioned taste aversion (CTA), whereby the taste becomes associated with nausea resulting in a sharp decline in its perceived hedonic value. Changes in neural coding were observed in GC and BLA as the taste transitioned from palatable to unpalatable following CTA. These changes emerged relatively late in the response in both regions indicating taste-related information is absent in the earliest BLA responses (as has been shown previously in GC). There were important differences in the temporal character of the changes in the 2 areas, however: many changes in BLA appeared earlier than those found in GC, supporting the hypothesis that the passage of information from BLA to GC is a vital component of CTA memory formation. Sham-conditioned rats, that is, physiological saline was substituted in place of the malaise-inducing injection of LiCl, demonstrated stable taste responses in both GC and BLA. Examining the time-course of

learning-related changes in neuronal responding offers insights into the correlates of taste hedonic processing and CTA memory formation.

Poster Session Fri PM

Gustatory Cortex Response Dynamics and Tastant Concentration

Brian Sadacca and Donald Katz

Brandeis University

The palatability of a solution on the tongue is a function of both the chemical identity and the concentration of that solution: rodents at normal salt and water balance readily ingest low concentration NaCl solutions and avoid high concentration NaCl solutions. The mechanism underlying palatability processing involves both brainstem and forebrain, and it has been suggested that temporal progression of forebrain activity in gustatory cortex (GC) reflects the processing of both a stimulus's chemical identity and that stimulus's palatability. To test the validity of this characterization, we presented awake, freely moving rats with intraoral infusions of a tastant array while recording isolated single-unit responses in GC. Our array included both a reliably palatable (0.03 M) and reliably unpalatable (0.5 M) concentration of NaCl, as well as prototypical sweet, highly palatable sucrose (0.1 M) and sour, less palatable citric acid (0.2 M) to manipulate identity while providing palatability comparison for each of the 2 NaCl concentrations. Consistent with other recent results, our findings demonstrate that certain early components of the GC responses reliably represent chemical identity, while later components reliably track palatability, independent of stimulus identity.

Poster Session Fri PM

Effects of Age on the Association between Hunger and fMRI Hypothalamic and Orbitofrontal Activity in Response to a Taste Stimulus

Erin Green¹, Lori Haase^{1,2}, Aaron Jacobson¹, Barbara Cerf-Ducastel¹, Nobuko Kemmotsu^{1,2} and Claire Murphy^{1,2}

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While it is generally accepted that reduced appetite in the elderly can lead to weight loss and nutritional deficits, the mechanisms responsible are largely unknown. The objective of the present analysis was to investigate associations between central processing of taste stimuli and the subjective experience of hunger in young and older adults. As part of a larger study, participants were given 0.3 ml of various taste stimuli separated by water rinses over 2 fMRI sessions; once after fasting and once after ingesting a preload. Participants rated their hunger before and after receiving the preload prior to entering the scanner. For this study, analysis was run on the fMRI activation to sucrose. Resulting fit coefficients from a region of interest analysis were correlated with hunger ratings. In young adults, differences in hunger ratings from the hungry to satiated condition were significantly associated with cortical activation in the hunger minus satiety contrast for the hypothalamus, right orbitofrontal cortex (BA 11), and right insula. In older adults, associations were found only for left BA 11. Findings suggest that central modulation

of hunger and satiety and associated perceptions of hunger decrease with aging. More specifically, associations between hunger perception and brain regions integral in maintaining energy homeostasis such as the hypothalamus may weaken with age and could be a factor in nutritional problems associated with aging.

Supported by NIH grant #1 R01 AG04085 to C.M.

Poster Session Fri PM

Correlations between BMI and BOLD in Medial and Lateral Orbitofrontal Cortex during Selective Attention to Taste

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Attentional manipulations have been shown to affect feeding. Here we investigated the possibility that neural encoding of attention to taste varies as a function of body mass index (BMI). Previous research has demonstrated a role for the insula and orbitofrontal cortex (OFC) in taste and feeding and differential neural responses have been observed in these regions in lean and obese subjects. In the present study we reasoned that there would be differential activity in the insula and OFC during attention to taste as a function of BMI. fMRI was used to scan 14 Ss while they received one of 4 solutions (weak sweet, sour, salty, or tasteless). In the attention condition Ss were instructed to probe the solution for the presence of a taste and respond by pressing a button. In the baseline condition Ss passively tasted and randomly pressed a button. Differential responses from attention-baseline were regressed against subject BMI. BMI was positively correlated with activity in the medial OFC (mOFC) and negatively correlated with activity in the lateral OFC (lOFC). Activity in mOFC is implicated in hunger and motivation to eat and activity in lOFC with satiety and meal termination. Our results suggest that response in these regions is differentially correlated with BMI when subjects attend to taste. One implication is that higher body weight is associated with a higher motivation to eat and a lower motivation to stop eating when attention is focused on taste.

Supported by R01DC006706.

Poster Session Fri PM

Neural Correlates of Umami and Salt Qualities during Hunger and Satiety

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Salt and umami are taste qualities that are nutritionally relevant for food intake. Umami is characterized by a protein taste. fMRI experiments have demonstrated brain activation within the primary and secondary taste cortices in response to these qualities. However, it remains unclear if activation to umami and salt is modulated by hunger and satiety. The present study employed fMRI to investigate cortical activation in response to guanosine 5-monophosphate (GMP) and NaCl, when subjects were hungry or sated. During scanning, subjects evaluated either the perceived pleasantness or intensity of the stimuli. Activation when rating pleasantness of GMP

and NaCl in the hunger condition was found in the dorsal and ventral insula, and orbitofrontal cortex (BA 47) for GMP alone. However, activation in these regions did not reach statistical significance when rating pleasantness in the sated condition. Activation when rating intensity of GMP and NaCl in the hunger condition was found in the dorsal insula and anterior cingulate gyrus (ACG), and ventral insula for NaCl alone. Evaluating intensity for GMP in the sated condition resulted in activation in the dorsal insula and ACG; however, activation in these regions did not reach statistical significance for NaCl. These results suggest that rating pleasantness and intensity of GMP and NaCl activates primary and secondary taste cortices and that activation is differentially modulated by hunger and satiety.

Supported by NIH grants R01AG04085 to C.M. and R03DC05134 to B.C.-D.

Poster Session Fri PM

Trying to Taste in the Absence of Taste: Neural Correlates of Selective Attention to Taste

Maria Veldhuizen and Dana Small

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Trying to see, hear, or smell in the absence of a sight, sound, or odor results in an increase in baseline activity in the respective primary sensory cortex. This is thought to signify a higher sensitivity to incoming sensory signals relevant to current goal-directed behaviour. Demonstration of increased sensitivity for attended compared to unattended weak taste stimuli suggests a similar mechanism exists in taste. We hypothesized that trying to taste in the absence of a taste should result in an increase in activity in the primary gustatory cortex (PGC). To test our hypothesis we used fMRI to evaluate brain response in 14 subjects who were trying to determine whether a solution was tasteless or contained a taste. Brain response to trying to detect a taste in the tasteless solutions was then compared to passive perception of the same tasteless solution. As predicted, we found enhanced activity in the left mid-dorsal insula and overlying operculum at the level of the precentral gyrus. Since such baseline shifts are characteristic of primary cortex this finding supports the designation of this insula/opercular region as PGC. In contrast, differential responses were not observed in the orbitofrontal cortex, consistent with its designation as a higher-order gustatory region. To our knowledge this is the first study to demonstrate the neural correlates of selective attention to taste in any species.

Supported by NIDCD grant R01 DC006706.

Poster Session Fri PM

p27^{Kip1} and Cyclin D2 in Taste Cell Turnover in Mice

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We are studying the role of cell cycle regulatory proteins in the ongoing generation of cells in adult mouse taste buds. In these taste buds, p27^{Kip1}, which promotes cell cycle exit and differentiation, is the major cyclin dependent kinase (CDK) inhibitor expressed, while cyclin D2, which promotes transit through G₁, is the only

D cyclin expressed. We recently reported that, although p27^{Kip1} “knock-out” mice show increased size and/or cell numbers in many organs including the tongue, their taste buds are normal in size, cell number and morphology. However, mutant taste buds contain 3 times as many BrdU-labeled cells 3 days after injection as do wild-type. Recent studies show that mutant taste buds have increased numbers of apoptotic profiles (196 ± 21.7 vs 133 ± 2.1, $P < 0.005$), suggesting that increased cell generation is balanced by increased cell death in these mutants. Immunohistochemistry (IHC) in wild-type mice for both p27^{Kip1} and cyclin D2, unexpectedly resulted in a large proportion of double-labeled cells. Preliminary IHC results in mutants indicate no major changes in cyclin D2 expression in the absence of p27^{Kip1} protein. Experiments are underway to examine possible up-regulation of p21 or p57, also members of the Cip/Kip family of CDK inhibitors, to support normal taste bud cell number in p27^{Kip1} knock-outs.

Poster Session Fri PM

Apoptosis in Rat Circumvallate Papillae; New Theory for Cell Lineage

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Traditionally, taste cells are categorized at least 3 types, dark (type I), light (type II and III), and basal (type IV) cells. There are 2 hypotheses about cell lineage, that is, one cell-line theory and multiple cell-line theory. It is well known that the life span of taste cells is about 10 days and they die by apoptosis. But it is not clear which type(s) of cells undergo apoptosis. In this study we performed double labeling of single-stranded DNA (ssDNA) and markers for type II, III and IV cells to reveal which type(s) of cells die by apoptosis. We used G α -gustuducin (Gust) and phospholipase C β 2 (PLC β 2) as markers for type II cells, neural cell adhesion molecule (NCAM) as a marker for type III cells and Jacalin as a marker for type IV cells. We found some ssDNA-immunoreactive (IR) nuclei in Gust or PLC β 2-IR cells, but never in NCAM-IR or Jacalin-labeled cells. These results suggest that type II cells are the conceivable cells that will die by apoptosis, and that type III cells possibly differentiate into type II cells. We also observed that majority of ssDNA-IR cells were not labeled with Gust, PLC β 2, NCAM or Jacalin. This result suggests that dark cells have a different growth pathway from light cells. Therefore we propose a new theory for cell lineage named 2 cell-line theory that type IV cells differentiate into dark cells and light cells and light cells grow up into type II cells through the process of type III cells.

This study was supported by a Grant-in Aid for 21st century COE program from MEXT.

Poster Session Fri PM

Identification of Taste Cell Progenitors and Lineage Analysis in the Adult Tongue

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Cells of the mammalian taste bud are remarkable in their capacity to regenerate throughout adult life. Taste cells are estimated to turn

over every 10–14 days under normal conditions and are also able to recover following their initial disintegration under stress conditions. Thus, identification of adult stem cells of the tongue presents an intriguing challenge. Interestingly, lineage studies have suggested that, unlike most other sensory cells that are derived from neurogenic ectoderm, taste bud cells arise entirely from local epithelium during embryogenesis and throughout adult life. Still, longstanding questions regarding their origins and lineage remain unanswered: Where do progenitors of adult taste bud cells reside? Do the different cell types arise from a common or from multiple progenitor pool(s)? Do the defined cell types of taste buds represent progressive stages of differentiation or distinct lineages? We address questions of relationships between cells in and around adult mouse taste buds first by identifying a putative taste cell progenitor population and then by characterizing progeny generated by this population using a Cre-loxP fate-mapping approach.

Poster Session Fri PM

BMP4 Expression Differs in Circumvallate and Fungiform Taste Buds of Mice

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Bone Morphogenetic Protein 4 (BMP4) is a diffusible factor which is expressed in developing taste papillae and regulates embryonic taste organ development. BMP4 is also expressed in adult taste buds, and thus may regulate taste cell turnover. Taste cells comprise 4 types: I, II, III, and proliferative basal cells, the latter assumed to generate cell types I–III. To determine if BMP4 is expressed in specific cell types within fungiform and circumvallate taste buds, we utilized transgenic mice with lacZ under the BMP4 promoter. We then combined immunolabeling for β -galactosidase, to reveal BMP4 expression, with specific taste cell type immunomarkers. In circumvallate taste buds, BMP4 is present in basal cells and some intragemmal fusiform cells. Specifically, 1.7% of serotonin- and 5.2% of NCAM-IR type III cells, 5.9% of PGP9.5-IR type II/III cells, 4.3% of PLC β 2- and 0% of gustducin-IR type II cells, and 7.8% of NTPDase2-IR type I cells were also β -galactosidase-IR. In contrast, in fungiform taste buds BMP4 is expressed only in basal cells and edge cells, and never co-localizes with immunomarkers of differentiated taste cells. That its expression differs in fungiform and circumvallate taste buds suggests different functions for BMP4 in these 2 locations. In fungiform taste buds, BMP4 may influence stem and/or transit amplifying cells located outside taste buds, whereas in the circumvallate, BMP4 may regulate cell fate decisions among proliferating basal cells and immature taste cells.

Supported by NIDCD DC003947 to L.B. and a VEF fellowship to H.M.N.

Poster Session Fri PM

Epithelial BDNF Is Required for Initial Gustatory Targeting But Not for Long-Term Fungiform or Palatal Taste Bud Maintenance

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Brain-derived neurotrophic factor (BDNF) is required for initial targeting of gustatory neurons to fungiform papillae. BDNF is produced in epithelia, the CNS, and gustatory ganglia. BDNF in any of these locations could allow gustatory neurons to differentiate and successfully invade their peripheral target. To determine if epithelial BDNF is required for targeting, mice with a floxed *Bdnf* allele were bred with K14-Cre mice to generate K14-*Bdnf*^{-/-} mice that lack BDNF in gustatory epithelia. In K14-*Bdnf*^{-/-} mice, gustatory axons failed to innervate fungiform papillae in the tongue tip indicating that epithelial BDNF is specifically important for targeting. However, most papillae on the dorsal tongue surface were innervated normally. K14-Cre expression begins at the tongue tip and proceeds caudally as the tongue develops. K14-Cre does not appear to eliminate BDNF from the dorsal tongue surface early enough to disrupt targeting, indicating that there is a critical period for this effect. Adult K14-*Bdnf*^{-/-} mice have 28% fewer fungiform papillae and 29% fewer geniculate neurons than Cre-negative littermates; papillae losses were concentrated at the tongue tip where targeting was also disrupted. Taste buds on the soft palate were unaffected in K14-*Bdnf*^{-/-} mice. These findings indicate that removal of BDNF from epithelium after initial target innervation has little effect on fungiform and palatal taste buds. In contrast, there was a 49% loss in the number of circumvallate taste buds in K14-*Bdnf*^{-/-} mice, even though BDNF removal followed the initial innervation of this region.

Support contributed by DC005252.

Poster Session Fri PM

BDNF Regulates Taste Bud Development at Late Embryonic Ages

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Brain-derived neurotrophic factor (BDNF) is required for taste buds to develop normally by birth. We sought to determine specifically when during development BDNF null mutant mice (*Bdnf*^{-/-}) first have fewer/smaller fungiform taste buds. Taste buds were identified as cell clusters that were labeled for anti-keratin 8. Taste buds were counted in serial sections, and taste bud volumes were measured from confocal images in *Bdnf*^{-/-} and wild type mice at E16.5, E18.5, and birth. *Bdnf*^{-/-} mice had fewer taste buds than wild type mice at birth (37 \pm 13 vs 142 \pm 6.0; $P < 0.0001$), but not at E16.5 (7.3 \pm 3 vs 10 \pm 7) or E18.5 (29 \pm 19 vs 54 \pm 10). The taste buds of *Bdnf*^{-/-} mice were not smaller than those of wild type mice at E16.5 (288 \pm 37 vs 197 \pm 81) or E18.5 (868 \pm 279 vs 1730 \pm 389), but they were smaller at birth (1261 \pm 152 vs 2595 \pm 258; $P < 0.005$). Thus, fungiform taste buds initially develop similarly in *Bdnf*^{-/-} and wild type mice. Unlike wild type taste buds, *Bdnf*^{-/-} taste buds do not increase dramatically in number and size between E18.5 and birth. Similar to wild type mice, the remaining taste buds in *Bdnf*^{-/-} mice are innervated by a dense syntaxin-positive nerve plexus. To determine whether the remaining taste buds in *Bdnf*^{-/-} mice contain sensory cells, we labeled for anti- α -gustducin. Both *Bdnf*^{-/-} and wild type mice show the same percentage of taste buds containing α -gustducin (10% vs 12.6%). We are currently determining whether the taste buds remaining in *Bdnf*^{-/-} mice express SNAP-25.

Support contributed by DC005252.

Poster Session Fri PM

Identification of the Source of BDNF in Human Saliva

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Brain-derived neurotrophic factor (BDNF) is a member of the growth factor family, polypeptides necessary for the survival, maintenance, and death of many types of central and peripheral inner-ating neurons. These proteins function mainly as target-derived factors, but may additionally display autocrine or paracrine trophic effects. Several growth factors, including nerve growth factor (NGF) and epidermal growth factor (EGF), are also produced and released in an exocrine manner by the salivary glands. In humans, the submandibular gland releases the majority of NGF, while the parotid gland is the main source of EGF. Recently, it was demonstrated that BDNF is also present in human saliva, though the source remains unknown. This study was therefore undertaken to determine the source of BDNF in human saliva. Stimulated whole (W), parotid (PG), and submandibular saliva (SMG), as well as oral mucosal transudate (OMT) samples, were obtained from 4 healthy subjects. The amount of BDNF in each sample was measured by sandwich ELISA. The median concentrations of BDNF were 279 pg/ml in W saliva, 14.8 pg/ml in PG saliva, 441.6 pg/ml in SMG saliva, and 130.1 pg/ml in OMT samples. Non-parametric analysis indicated that SMG BDNF concentrations were significantly greater than PG and OMT. The role of BDNF and other growth factors in saliva is still unknown, but previous findings in rodents suggest that the salivary glands may be releasing the proteins to promote survival, differentiation, and/or death of lingual epithelial cells.

This work was supported by an NIH training grant.

Poster Session Fri PM

Perinatal Development of Taste Buds and von Ebner's and Weber's Glands in the Rat

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von Ebner's glands (EGs) and Weber's glands (WGs) are lingual salivary glands whose secretions may affect taste perception. In this study, we observed fetal and early postnatal development of these glands, as well as lingual and palatal taste buds, using lectin histochemistry. Our results showed that taste buds in the soft palate first reacted positively to *Griffonia simplicifolia*-II (GSL-II), *Ulex europaeus* agglutinin-I (UEA-I), and *Sophora japonica* agglutinin (SJA) in fetuses at 18/19 days of gestation (E18/19), and those to DBA *Dolichos biflorus* agglutinin (DBA) in P2 neonates; while EGs and WGs became identifiable only by hematoxylin and eosin staining, but not by the lectin reactions at E18/19. GSL-II was positive for EGs but negative for WGs in adults; it was negative for both glands in fetuses, yet weakly positive for EGs in P5 neonates. UEA-I was negative for EGs in adults. Co-localization of UEA-I-positive and negative cells for WGs was observed in fetuses at E21

and in adults. SJA was intensely positive for EGs and moderately positive for WGs in adults. Positive reactions to SJA were observed for WGs at E21, but those for EGs were not identified until P5. DBA was positive for both EGs and WGs in E21 as in adults. Our results suggest that the maturation of the salivary glands does not necessarily correlate to the maturation of taste buds.

Poster Session Fri PM

BDNF Is Mildly Trophic and Tropic for Postnatal Geniculate Neurites

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We previously demonstrated that BDNF is both trophic and tropic for rat geniculate neurites in collagen gels at embryonic stages when axons are contacting their BDNF-positive lingual targets in vivo. BDNF continues to be expressed in a subset of taste cells in adults, so we are investigating if it continues to exert a trophic or tropic influence on geniculate neurites after birth. Bath applied BDNF (50 ng/ml) stimulated longer and more fasciculated neurite outgrowth from postnatal days 3–8 (PN) geniculate ganglia than control media (8/8 comparisons) indicating that BDNF exerted a trophic influence. Gradients of BDNF established by slow release beads exerted a subtle tropic effect: neurite fascicles turned toward BDNF beads in 11/16 cultures, but neurites turned toward control beads in only 2/15 cultures, a significant difference ($P < 0.05$, chi-square). Neurite length, however, was not affected: the ratio of neurite outgrowth length toward the BDNF bead to that away from the bead ($n = 16$) was not significantly different from the ratio observed in cultures containing a control bead and BDNF in the bath ($n = 15$) ($P < 0.2$, ANOVA). We have begun to characterize the response of ganglia from more mature rats. Geniculate ganglia dissected from P29–33 (juvenile) or P55 (adult) rats required 2 weeks to extend neurites in collagen I, but exhibited considerable growth in 4–5 days in Matrigel. BDNF did not influence the length or morphology of juvenile (8 control, 7 BDNF-treated) or adult (4 control, 3 BDNF-treated) neurites in Matrigel cultures. Growth factors in Matrigel may underlie the apparent decrease in responsiveness to BDNF.

NIH R15 DC08103-01.

Poster Session Fri PM

Apoptosis in Embryonic Geniculate and Trigeminal Neurons Cultured with BMP4 and Noggin

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Bone morphogenetic protein 4 (BMP), and its antagonist, noggin, regulate neuron survival and differentiation, and dramatically influence taste papilla development. Both are expressed in rat tongue by E13, when nerve fibers from geniculate and trigeminal ganglia are within the tongue base and, are intense in fungiform papillae by E16, when these ganglion cells innervate papillae. BMP and noggin each cause a substantial decrease in neuron number and neurite outgrowth from cultured E16 geniculate ganglion. Low concentration of BMP or noggin sustains a large population of E16 trigeminal neurons; high concentrations result in reduced neurons and neurite

extension. To learn whether apoptosis contributes to these effects, cultured E16 ganglia were exposed to high concentrations of BMP, noggin, or to brain derived neurotrophic factor (BDNF), and processed with a TUNEL assay. Too few geniculate neurons survive exposure to BMP to quantify apoptosis; however, compared to BDNF, there is a significant increase in apoptotic neurons exposed to noggin or standard medium. Apoptosis is not increased when BDNF is combined with BMP and/or noggin. In trigeminal neurons, apoptosis is significantly increased with either BMP or noggin, compared to BDNF exposure alone, and when BMP and noggin are combined with BDNF. Thus, BMP and noggin are potent apoptotic factors for geniculate and trigeminal neurons. Only for geniculate neurons are the apoptotic effects counteracted in the presence BDNF, indicating the necessity of BDNF for survival and a divergence from effects on trigeminal neurons.

Supported by NIDCD NIH grants DC00456 (C.M.) and T32DC00011 (O.M.).

Poster Session Fri PM

BDNF Dependent Geniculate Ganglion Neurons Are Rescued in *Bax* Knockout Mice

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Removal of brain-derived neurotrophic factor (BDNF) results in a loss of 50% geniculate (taste) ganglion neurons by birth. We demonstrated that neurons are first lost in *Bdnf*^{-/-} mice during targeting (E12.5–E14.5), and continue to be lost through E18.5 of development. This timing makes it likely that BDNF functions by preventing geniculate cell death and not by increasing proliferation. To test this hypothesis, we quantified geniculate ganglion neurons in mice carrying null mutations in both the *Bdnf* gene and the pro-apoptotic *Bax* gene. Functional deletion of BAX typically blocks apoptosis in sensory neurons. We counted geniculate ganglion neurons in wild-type, *Bdnf*^{-/-}, *Bax*^{-/-} and *Bdnf*^{-/-}/*Bax*^{-/-} hybrid mice at birth using the optical dissector method. Compared to wild-type mice, *Bdnf*^{-/-} mice lost a considerable number of geniculate neurons (840 ± 66 vs 526 ± 97, *P* = 0.03). In *Bax*^{-/-} mice, the number of geniculate neurons doubled compared to wild-type mice (840 ± 66 vs 1676 ± 208, *P* = 0.009), indicating that developmental cell death in the geniculate ganglion is regulated by BAX signaling. *Bdnf*^{-/-}/*Bax*^{-/-} hybrid mice did not have fewer neurons than *Bax*^{-/-} mice (1580 ± 112 vs 1676 ± 208, *P* = 0.35). These findings indicate that *Bax* null mutations completely rescued survival of BDNF-dependent geniculate neurons. We are currently trying to determine exactly when BDNF first regulates geniculate neuron number and plan to determine whether the dying cells in *Bdnf*^{-/-} mice are all fully-differentiated neurons or also include neuronal precursors.

Support contributed by DC005252.

Poster Session Fri PM

Are We Mixing Odorants or Odors?

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We know that an olfactory stimulus that is gradually changed from odorant A to odorant B over mixtures of A and B, will yield a grad-

ual change in perception of the quality A to B with a maximal probability of reporting both A and B around the middle of this physical continuum. We investigated if it would be possible to learn participants to recognize the mixture AB as being odor “X” and mixture BC as being odor “Y” and then to gradually change odor “X” to “Y” over a the single odorant B, and to observe the same response patterns as in the former case. The odors used were amyl acetate (A), *n*-butanol (B), and pyridine (C). The results showed that participants were as likely to report both A and B in response to a balanced mixture of A and B, as they were to report both “X” and “Y” in response to stimulus B. This suggests that participants cannot differentiate between a single odorant and a mixture of 2 odorants in terms of perceived complexity. Moreover, what we typically have regarded as perceptual mixture phenomena may have little to do with actual physical mixtures.

Supported by the Swedish Research Council.

Poster Session Fri PM

Evidence for Blending in Odor Mixtures

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Zou and Buck recently demonstrated that binary odorant mixtures can activate cortical neurons that are not activated by their individual component odorants. They suggested that this could explain the perception of a novel odor in a mixture containing several odorants. Here we reported a series of experiments, relying on psychophysical measurements, which account for this idea. In Exp. 1, 20 subjects were asked to sniff 10 odorants eliciting pineapple odor (7 single compounds, 1 binary mixture and 1 ternary mixture) and to perform a qualitative ranking from what they consider to be the most typical of a pineapple odor. Results showed that single components such as ethyl isobutyrate and/or ethyl maltol were not considered as good representative for pineapple odor when sniffed separately, but obtained a better rank when they are mixed together in a specific ratio. In Exp. 2 and 3, 30 subjects were asked to rate typicality of 6 mixtures and their components (2–6). Results showed that mixtures of definite proportions of the components were judged as more typical of a specific novel odor as compared to their individual component odorants. From these results, it is concluded that mixing odorants that do not evoke a specific target odor could elicit the perception of a novel odor through a perceptual odor blending process.

Supported by ANR grant 2005, Région Bourgogne and Fondation Roudnitska.

Poster Session Fri PM

Adaptation Study of 2-Methylisoborneol Odors

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2-Methylisoborneol (MIB) exhibits a camphorous odor at high concentration and musty geosmine like odor at low concentration. Data gathered using GCO on pure samples of camphor and geosmine indicated a threshold range for geosmine at about 100 ng/injection (ng/in) and the threshold range for camphor is 1000 ng/in.

The point at which a subject perceived a distinct odor change for MIB occurred at 333 ng/in. In this study we examine whether the occurrence of odor duality is dependent upon detection thresholds or mixture suppression. Because musty geosmine has a much lower threshold than camphor, it is possible that at high concentrations of MIB the musty odor is not detected due to adaptation and/or the camphor odor is no longer suppressed by musty-ness. In an adaptation study using odor quality contrast, we examined whether the odors perceived at low concentration are due to mixture suppression or a detection threshold. If at low concentration the phenomenon is due to mixture suppression, we hypothesized that after adaptation to geosmine a subject that sniffs MIB would perceive camphor. However, if at low concentrations MIB's camphor odor is below the detection threshold, the subject would detect no camphor odor. Furthermore, when adapted to geosmine, upon sniffing MIB at high concentration, a subject should perceive camphor due to release from suppression. Additionally, when a subject is adapted to camphor we expect MIB at low concentrations to still have a musty odor.

Poster Session Fri PM

Continuous Intensity Evaluation for Odorants and Quantitative Characterization of Adaptation

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It is well known that the odor intensity is decreased when persons are exposed to odorants for a long time, which phenomenon is generally called "adaptation." No previous reports, however, described about temporal attribution of adaptation for various odorants. A few studies report temporal change of subjective intensity, by participants' evaluation every several seconds. The accuracy will not be enough to calculate temporal character for adaptation. We first, therefore, developed new evaluating system by which participants can rate odor intensity continuously. Participants were exposed to one odorant for several minutes, and were asked to evaluate subjective intensity continuously by sliding lever, and this rating was recorded by a PC. We decided odorant concentration so as maximum ratings during one experiment should be between "easy detectable" to "strong." We investigated 2 methods for classification, "adaptation was occurred in this experiment" or not. One method was to use ratio of temporal integration of intensity value for former and latter half of one experiment. The other method was to match temporal pattern of intensity according to a previous reported study, by 3 experimenter inspection. All data were evaluated by these 2 methods, high correlation was found between 2 methods.

Poster Session Fri PM

Proper Times for Odor Detections

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Our behavior for odors is variable and changeable depending on the quality, intensity, situation and previous experiences. It has been still unclear, however, the cognitive and neural mechanisms for the olfactory cognition. Sensitivity for various odorants is also different. This variety may reflect the complex and dynamic neural mechanism of human olfaction system. Reaction time is one of the important indexes to estimate the neural temporal aspect of olfactory cognition. In the previous established olfactometer, however, neither temporal delay from signal onset nor dispersion of changing time from air into odor has been considered, because of lacking techniques high-speed gas monitoring. This means it has not been measured the real reaction times in previous olfactory studies. To measure reaction time properly, we monitored the odor material at the end of tube of the olfactometer (near the nose of subject) by using a high-speed gas sensor which was developed by Toda et al. Our procedure allowed us to detect proper time from the odor material arrived to the subject's nose to the subject pressed the button. We measured reaction times for 4 odors, and compared the reaction times between these different types of odor and different intensities.

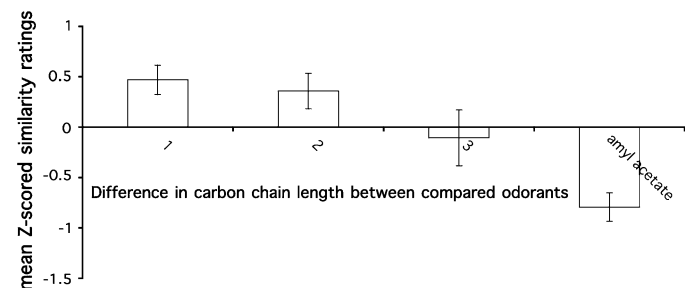
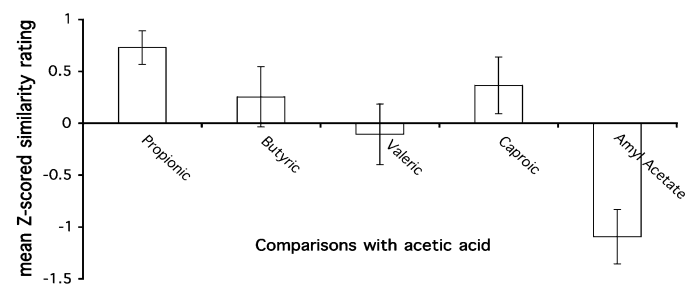
Poster Session Fri PM

Human Similarity Judgments Predict Rodent Olfactory Performance

Jessica Porter, Christina Zelano, Rehan Khan and Noam Sobel

UC

Carbon chain length has been suggested as a possible axis of odor space determining similarity. Indeed, behavioral results in rodents have shown that inferred similarity varies with carbon chain length. As carbon number should have no privileged connection with rodents, one would expect perceived similarity in other animals to follow this pattern. To address this, we asked 10 human volunteers to rate the pairwise similarity of a homologous series of aliphatic acids (acetic, propionic, butyric, valeric and caproic), and one control odorant. Acetic acid was rated as significantly more similar to the series odorants than to the control, amyl acetate (all $P < 0.02$, Figure 1 top). The similarity of acetic acid to the other



odorants followed the predicted order, with the exception of caproic acid. When caproic acid was removed, all pairwise comparisons in the series were rated as significantly more similar than comparisons to the control (all $P < 0.04$, Figure 1 bottom), with the predicted ordering. Thus, some aspects of similarity are consistent across species, and may reflect physico-chemical structure. However, that caproic acid appears to be perceived differently, suggests that other mechanisms, such as learning, and differences in olfactory receptors may contribute to variability in perception across species.

Poster Session Fri PM

Optimal One Odorant Choice Method and Its Application to the Simple Version of the Odor Stick Identification Test (OSIT)

Hideki Toda¹, Nao Goto¹, Tateki Miwa², Sachiko Saito¹ and Tatsu Kobayakawa¹

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A variety of olfactory tests have been proposed and developed in recent years. Since these tests consist of many odors, it will take more than 10 min for measuring subjects' ability of odor identification. This time consumption will become critical when quick diagnosis is required. Reduction of diagnosis for olfactory function will lead prevalence this olfactory tests. In order to realize the shrink diagnosis time, we need to reduce the number of odorants with keeping detection ability. We tried, therefore, to investigate which combinations of 3 odorants represent whole odor stick identification test (OSIT) examination's result. And we also tried to find which the best one is. In order to investigate the best combination of 3 odorants from 12, we used Spearman's correlation coefficient. In the case of selection of best one odorant, we used 2 mathematical different ways—Spearman's correlation coefficient and inner product operation based on discriminant analysis, which method is newly proposed. This result would be reliable, when the results derived by these 2 methods are consistent. And the results from these analyses were actually consistent. Our proposed methods for selection of one odor from many odor items would be applicable for other olfactory examinations.

Poster Session Fri PM

Working Memory across Nostrils

Yaara Yeshurun, Rehan Khan, Yadin Dudai and Noam Sobel

Weizmann Institute of Science

Olfactory working memory (OWM) maintains information over short time frames. Whether this involves semantic representations, or neural images of odor per se, remains unknown. Also, the contribution of primary olfactory structures versus higher-order brain system is unclear. To address these issues, 14 subjects participated in a button-press delayed-match-to-sample task (trials = 20, ISI = 3 s, ITI = 30 s), with 10 nameable and 10 unnameable odorants. Critically, on every trial, odorant was delivered to one nostril only. In half of the trials, "sample" and "match" were presented in the same nostril, and in half "sample" was in one nostril, and "match" in the

other. Odorant order was randomized, and test side was counterbalanced. There was a strong trend towards an effect of nostril-order [$F(1,13) = 4.1$, $P = 0.06$], reflecting more mistakes when "sample" and "match" were presented to different nostrils. However, a nameability by nostril-order by test-side interaction [$F(1,13) = 5.2$, $P < 0.04$] revealed that this effect was attenuated when subjects were tested in the right nostril for nameable odors that were presented to the left [$t(13) = 2.5$, $P < 0.03$]. Considering the predominantly ipsilateral nature of olfactory projections, poor performance in the unexposed nostril suggests that OWM may rely on a neural image of odor within primary olfactory areas. However, the same-nostril-advantage (better performance when "sample" and "match" were in the same nostril) dissipated when "target" was a nameable odor presented to the left nostril and tested on the right. This implies involvement of higher-order OWM mechanisms when odor semantic information is available.

Poster Session Fri PM

Are We Mixing Odorants or Odors?

Mats Olsson

Uppsala University

How odorants blend together has been one of the key questions in human olfactory psychophysics for decades. With the emerging knowledge of receptor codes, neural pathways and cortical firing patterns in different species, it is increasingly possible to see how perception and neural processing fit together. For instance, receptor codes do not differentiate between single odorants and mixtures. In parallel, perceived complexity does not differ between single odorants and mixtures. Moreover, how we perceive a mixture has quite simple and consistent relations to the perception of its components, rather than to properties of the component stimuli. These and other observations suggest that a stimulus response model is not sufficient in explaining olfactory mixture perception. Alternative views are discussed.

Supported by the Swedish Research Council.

Poster Session Fri PM

Hormonal Changes Induced by Smelling the Human Chemosignal Androstadienone

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Androstadienone (AND) is a steroid derivative of testosterone present in male body secretions. AND has been labeled a putative human pheromone, because it induces changes in mood and autonomic physiology in women. Here we set out to ask whether smelling AND influences levels of salivary cortisol (CORT) and testosterone (TESTO). Twenty-one women (22.5 ± 3.46 years old) participated in two 2-h long sessions, conducted at the same time of day. In one session subjects smelled AND, and in the other a control odor (CONT), matched for pleasantness [$t(20) = 0.6899$; $P < 0.49$] and intensity [$t(20) = -0.6519$, $P < 0.52$]. Session order was counterbalanced, and subjects and experimenters were blind to

the condition. As shown previously, AND increased physiological measures of arousal [$F(1,248) = 19.11, P < 2.10 \times 10^{-5}$], while maintaining higher positive mood [$F(1,167) = 13.61, P < 0.0005$], and sexual arousal [$F(1,167) = 10.3857, P < 0.002$], as compared to CONT. Levels of CORT and TESTO at baseline were not significantly different between sessions [$t(\text{CORT}(19)) = -0.7209, P < 0.4793, t(\text{TESTO}(19)) = 0.0838, P < 0.9341$]. However, smelling AND maintained significantly higher levels of CORT [$F(1,152) = 11.88, P < 0.0011$] and increased levels of TESTO [$F(1,163) = 29.3372, P < 0.000001$] compared to CONT. Our results demonstrate that smelling a single component of male sweat modifies endocrine balance in women.

Work supported by the Marie Curie OIF, the David Foundation, NIH NIDCD DC006915, DC005958.

Poster Session Fri PM

Inhibitors of Nasal Enzymes Influence the Perceived Quality of Odorants

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Odorants are recognized via a combinatorial process in the olfactory system, where a single odorant is recognized by multiple receptors and multiple odorants are recognized by the same receptor. The role of perireceptor events such as the presence of OBPs, or enzymes which are located in the olf. epithelium has not been elucidated yet. Enzymatic reactions in nasal tissue have been described for rodents, and little information is available on human biotransformation enzymes. We looked at the type of enzymes which occur in the human olf. mucosa, and started their characterization. In particular the cytochrome P450 CYP2A13 is dominantly expressed and a considerable number of substrates and metabolites have been identified. Biochemical and sensorial studies reveal that metabolites can have an odor themselves which can be distinct from the odor of the substrate. Analytical means allow to demonstrate the formation of metabolites in vivo by analyzing exhaled air. Selected results were used to design fragrance accords where an odorless inhibitor is boosting the fruity note of the accord. The results indicate that in-nose biotransformation of odorants can modify the quality and quantity of compounds reaching the olf. mucosa, and those events may have to be considered when comparing receptor-odorant interaction patterns with olfactive descriptors of odorants. The intentional use of molecules modifying the activity of biotransformation enzymes in perfume accords is an interesting approach to validate their use in fragrance creation.

Poster Session Fri PM

The Psychophysical Assessment of Odor Valence: Does an Anchor-Stimulus Influence the Hedonic Evaluation of Odors?

Marion Schultheiss, Andrea Gossler, Udo Reulbach and Norbert Thuerauf

University of Erlangen-Nuremberg

The hedonic categorization in olfaction is probably the most important criterion for odor grouping and olfactory stimuli are experienced primarily in terms of their hedonic tone. However the assessment of hedonic estimates is poorly standardized and the influence of anchor-stimuli on the hedonic evaluation of odors is less investigated. Thus, we investigated in 19 healthy volunteers, 13 females and 6 males (mean age: 30.00 years SD 8.83 years, minimum age: 21 years, maximum age: 51 years), the effects of different anchor-stimuli on the hedonic evaluation of odors. We assessed the hedonic estimates for the 16 standard odors of the Sniffin' Stick Test for 4 psychophysical measuring conditions using a bipolar analog rating scale. Subjects rated the perceived odor in comparison A) with "orange" as a pleasant standard odor, B) with "licorice" as a neutral standard odor, C) with "fish" as an unpleasant standard odor, and 0) without a standard odor (non-anchor condition). Our parametric statistical analysis (General Linear Model) revealed significant correlations between the measuring conditions A and 0, B and 0, C and 0, A and B, B and C. The measuring conditions A and C did not correlate in a statistical significant manner but a statistical tendency could be observed. We interpret our results in the way that—under the condition of presenting a single anchor stimulus at the beginning of a hedonic measuring series—anchor and non-anchor conditions produce similar results.

Poster Session Fri PM

fMRI of Olfactory Working-Memory in Primary Olfactory Cortex

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To probe the role of primary olfactory cortex (POC) in olfactory working memory (OWM), 10 subjects performed a button-press delayed-match-to-sample task in the scanner (trials = 80, ISI = either 5 or 10 s, ITI = 30 s), with 10 nameable and 10 unnameable odorants. There was significantly more activity in POC in response to odorants that were difficult to name [$F(1,9) = 6.18, P < 0.03$]. In addition, sustained activity during the delay was only present when subjects were remembering odorants that were unnameable [$F(1,9) = 14.78, P < 0.003$]. To ask whether the sustained activity reflected memory for odor-related information other than odor percept, we conducted an additional experiment where subjects remembered odor intensity estimates. We found no sustained activity in POC under these conditions. To ask whether the sustained activity was olfactory-specific, we conducted an additional experiment where subjects remembered the pitch of an auditory tone that was presented along with the odorant. We found no sustained activity in POC under these conditions. In conclusion, OWM in POC was specific to odors that were unnameable. To ask if language-associated brain regions exhibited sustained activity when subjects remembered nameable odors, we examined the activity in these areas. We found increased [$F(1,9) = 7.4, P < 0.02$], and sustained activity in response to nameable odors in these regions. These results indicate that POC is involved in OWM for unnameable odorants, and higher-order areas are involved in OWM for nameable odorants.

Poster Session Fri PM

A Study on Olfactory Lateralization: The Perception of Olfactory Intensity But Not the Hedonic Estimation Is Highly Lateralized

Norbert Thuerauf, Udo Reulbach, Agabi Vassiliadu, Jens Lunkenheimer, Birgit Lunkenheimer and Katrin Markovic

University of Erlangen-Nuremberg

An earlier study in humans comparing the olfactory sensitivity of both nostrils revealed a small but significant advantage of the right nostril for olfactory sensitivity. However lateralization was not evaluated for the perception of odor intensity and hedonic evaluation. Thus we investigated lateralization of olfactory intensity and hedonic evaluation in right handed healthy volunteers ($n = 186$) from the HeDoS-F database (Hedonic Database of Smell–Franconia). For olfactory evaluation the Sniffin' Stick Test was employed with the parameters threshold, discrimination, identification and extended by analog hedonic and intensity rating scales. Over all odors subjects rated the perceived intensity significantly higher following stimulation of the right compared to the left nostril. The analysis of the single odors of the Sniffin' Stick Test consistently confirmed higher intensity ratings for the right compared to the left nostril reaching a statistical significant difference for 10 out of 16 odors. In contrast we found no significant differences between the nostrils for the hedonic estimates over all odors. Differences in odor threshold, discrimination and identification did not reach a statistical significant level, but for all these parameters the scores of the right nostril were slightly higher compared to the left nostril. Based on our results we concluded that olfactory intensity estimates represent the most sensitive parameter of olfactory lateralization.

Poster Session Fri PM

OMP Deletion Alters Odorant Transduction Currents of Single Olfactory Sensory Neurons Revealed by Patch Clamp Recordings

Anderson Lee and Minghong Ma

University of Pennsylvania

Olfactory marker protein (OMP) is expressed at high levels in mature olfactory sensory neurons (OSNs) of vertebrates. OMP-null mice exhibit a higher odor detection threshold than wild-type mice. In electro-olfactogram (EOG) recordings, OMP-null mice display a slower onset and smaller response to odors, as well as a slower decay and reduced recovery from adaptation, suggesting that OMP plays a role in olfactory signal transduction. However, the effects of OMP deletion in single OSNs have not been investigated. In this study, we have recorded odor-induced transduction currents of single OSNs in the intact epithelium and compared the response properties (delay, 0–90% rise time, amplitude, half width, and paired-pulse amplitude ratio) of OMP-null mice to those of wild-type. In the OMP-null neurons, activation deficiencies include a prolonged delay (0.73 ± 0.20 s vs 0.16 ± 0.02 s, $P < 0.01$), a slower rise time (0.45 ± 0.71 s vs 0.29 ± 0.04 s, $P < 0.05$), and a decreased peak amplitude (65.6 ± 10.0 pA vs 299.9 ± 103.2 pA, $P < 0.05$). There is also a slower deactivation phase with an increased half width (3.35 ± 0.78 s vs 1.19 ± 0.20 s, $P < 0.01$). Recovery from adapta-

tion is not significantly different (the ratio of response2/response1, 0.40 ± 0.08 vs 0.26 ± 0.20). Further work targeting key proteins in the olfactory signalling pathway need to be done. Nevertheless, this single cell characterization of OMP deletion provides insight into the specific role OMP plays in the olfactory responses.

Supported by NIDCD/NIH.

Poster Session Fri PM

OMP Controls the Kinetics of the Odor-Induced Response in Mouse Olfactory Sensory NeuronsJohannes Reisert¹, King-Wai Yau² and Frank L. Margolis³¹Monell Chemical Senses Center, ²Johns Hopkins University School of Medicine and ³University of Maryland School of Medicine

The role of OMP, a small cytoplasmic protein found almost exclusively in mature olfactory sensory neurons (OSNs), has been elusive since its discovery. Phenomenologically, OMP-KO mice show a slowing of the odor-induced EOG response; reduced odor responsiveness; and slowed rate of Ca^{2+} recovery. We used suction pipette recordings from single OSNs of OMP-KO mice to investigate how OMP controls the kinetics of olfactory signal transduction. The response latency, the time to reach the response maximum and the time needed for the response to terminate are all prolonged 2–8 \times . Also the oscillatory response pattern observed during prolonged odor stimulation was slowed, with the oscillation frequency decreasing 10-fold. By contrast, when the phosphodiesterase inhibitor IBMX, instead of odorants, was used to raise cAMP levels no difference in the response kinetics was observed between controls and OMP-KO mice. These data indicate that signal transduction components, that are downstream of cAMP production (Ca^{2+} activated Cl^- channel, $\text{Na}^+/\text{Ca}^{2+}$ exchanger) are not targets for OMP regulation. Hence, OMP might act prior to cAMP production and early in olfactory signal transduction (e.g., on the olfactory G protein or adenylyl cyclase) to greatly accelerate the response to insure fidelity of an odor signal.

Poster Session Fri PM

Regulation of Olfactory Transduction in Lobster Olfactory Receptor Neurons by Phosphoinositides

Yuriy Bobkov, David Price and Barry Ache

University of Florida

Using a number of pharmacological probes affecting key components of phosphoinositide metabolism (phosphoinositide 3-kinase, phospholipase C, and phosphoinositide 4-kinase), we demonstrate that accurate turnover of phosphoinositides is essential for regulating the odor-evoked output of lobster olfactory receptor neurons (ORNs). In particular, PIP2 pool depletion causes a reduction in transduction current and odor-evoked ORN discharge. The ORNs express a transient receptor potential (TRP)-related, divalent cation permeable, non-selective cation channel. The channel contributes to the generation of a substantial part of the depolarizing receptor potential. We report that phosphoinositides are essential to maintain the function of the channel, with the calcium-activated form of the channel that is predominantly expressed in the transduction zone being more sensitive to exogenously applied phosphoinositides

(PIP2, PIP3) than the calcium insensitive form of the channel. Collectively, these data further our understanding that the channel is a major downstream target of phosphoinositide turnover in these cells.

Supported by the NIDCD (DC 001655).

Poster Session Fri PM

The Role of Phosphodiesterase 1C in Shaping Olfactory Sensory Neuron Responses

Katherine Cygnar and Haiqing Zhao

Johns Hopkins University

Cyclic nucleotide second messengers are used by many systems to convert external stimuli into cellular responses. The regulation of 2 enzymes—the cyclase for production of cyclic nucleotides, and the phosphodiesterase (PDE) for their degradation—controls the strength and duration of the response. In the nose, odor exposure stimulates an adenylyl cyclase on the cilia of olfactory sensory neurons (OSNs) and elevates cAMP levels, which open ion channels and generate an electrical signal. When odor is removed, the electrical responses of OSNs terminate rapidly. This rapid termination of the OSN response has long been hypothesized to occur mainly through degradation of cAMP by PDEs. To date, only one PDE, PDE1C, is known to localize to the OSN cilia, making it the principal PDE candidate responsible for rapid termination. To determine the role of PDE1C in olfactory transduction, we have knocked out the *pde1c* gene in mice and conducted electroolfactogram analysis. Surprisingly, *pde1c*^{-/-} OSNs still exhibit rapid termination of the response to odors. More interestingly, the termination kinetics are affected in a dose-dependent manner; *pde1c*^{-/-} OSN responses terminate faster than wild type after high odor concentrations. Also unexpectedly, *pde1c*^{-/-} OSNs exhibit slower activation kinetics than wild type OSNs. These results suggest that other cellular mechanisms are sufficient for rapid termination of the OSN electrical responses. In addition, this line of research is beginning to reveal unanticipated roles of PDE1C in shaping OSN responses.

Supported by NIH DC006178.

Poster Session Fri PM

The Role of Calcium/Calmodulin-Mediated CNG Channel Inhibition in Regulation of Olfactory Neuron Response

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Previous reports suggest that olfactory adaptation is largely attributed to negative-feedback inhibition of the olfactory cyclic nucleotide-gated (CNG) channel by Ca²⁺/calmodulin. We have developed a mouse model, CNGB1^{ΔCam}, in which a specific deletion of a calmodulin-binding site in the CNGB1b channel subunit renders the channel insensitive to Ca²⁺/calmodulin. In this mouse, the mutated olfactory CNG channel maintains normal OSN ciliary localization and normal sensitivity to cAMP. Electrophysiological analysis with electroolfactogram and single-cell suction pipette recording revealed that mutant OSNs exhibited normal initial sensitivity, but slower termination kinetics. Surprisingly, mutant OSNs showed

no adaptation deficit under repeated stimulation paradigms. Additionally, mutant OSNs still preserved a significant response reduction during prolonged stimulation, although the degree of the response decline was less. These results question the essential role of Ca²⁺/calmodulin-mediated CNG channel inhibition in olfactory adaptation, but implicate its role in rapid termination of OSN response. The CNGB1^{ΔCam} mice further provide an animal model to evaluate the behavioral significance of Ca²⁺/Calmodulin-mediated CNG Channel inhibition.

Supported by NIH DC006178 (R03) and Whitehall Foundation.

Poster Session Fri PM

Individual Olfactory Sensory Neurons Exhibit Mechanical Sensitivity

Lindsey Ciali-Santarelli, Xavier Grosmaître and Minghong Ma

University of Pennsylvania

Within the nasal mucosal layer, the binding of odor molecules by odorant receptors on the cilia of olfactory sensory neurons (OSNs) sets off an intracellular second-messenger cascade involving adenylyl cyclase III-synthesis of cAMP, which opens olfactory-specific cyclic nucleotide-gated (CNG) channels, that ultimately depolarize the neuron enabling it to fire action potentials. In addition to this well-established odorant response pathway, we have recently shown that OSNs within the main olfactory epithelium and septal organ of the intact epithelial preparation also respond electrically to a mechanical stimulus delivered by pressure ejection of odor-free Ringer solution. The current generated by Ringer puffs is similar to that caused by odorant application in response kinetics, reversal potential (at 0 mV) and inhibition by an adenylyl cyclase blocker. Using the perforated patch-clamp technique, we demonstrate here that native dissociated OSNs maintain the mechanical response properties similar to those of OSNs within the intact preparation, indicating that the neurons themselves contain the constituents necessary to generate the response. Whether the exact second-messenger pathway used during odorant transduction is activated in response to the mechanical stimulus remains to be investigated. Nevertheless, the mechanical sensitivity of the OSNs may serve a role in increasing the response to less-concentrated odorants to ensure these signals reliably reach the brain and synchronizing the bulb activity with respiration.

Supported by NIDCD/NIH.

Poster Session Fri PM

Physiological Fingerprints of Genetically-Labeled Vomeronasal Neurons: Maintained Firing Requires Interplay between BK_{Ca} and L-Type Ca_v Channels

Kyrill Ukhanov¹, Trese Leinders-Zufall² and Frank Zufall²

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The sensory epithelium of the vomeronasal organ (VNO) is segregated into apical and basal zones both of which express a unique set of transduction related molecules. Recent evidence has indicated important differences between the functional properties of vomeronasal sensory neurons (VSNs) located in each zone, including the detection of chemosignals, the signal transduction mechanisms, and

the strategies underlying stimulus encoding. We, therefore, have begun to systematically compare the properties of VSNs in each zone by performing patch clamp recordings from identifiable, GFP-expressing VSNs, using an acute mouse VNO slice preparation. Here, we replace sensory input by current injection and focus on the role of downstream voltage- and Ca^{2+} -dependent channels in the generation of sustained action potential firing. We provide evidence that VSNs generate Ca^{2+} spikes. We analyze in detail the voltage-activated Ca^{2+} (Ca_v) currents underlying such oscillations and show that a complex interplay between L-type Ca_v channels and large conductance Ca^{2+} -activated K^+ (BK_{Ca}) channels is required for maintaining prolonged, stable firing in VSNs of both zones. We propose that these functional properties of VSNs are critical to produce long-lasting, low-frequency firing in response to the detection of vomeronasal stimuli.

Poster Session Fri PM

Activity-Dependent Regulation of Connexin Expression in the Olfactory Epithelium

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Connexins are gap junction-forming proteins. Gap junctions play important roles in maintaining various aspects of normal physiological activity. We have shown spatial distribution and expression of multiple connexins in the olfactory epithelium. Expression of connexin 43 (Cx43) was localized to some sustentacular cells, mature olfactory neurons, immature olfactory neurons and basal cells. We have further demonstrated that disruption of Cx43 related gap junctions in the olfactory epithelium alters olfactory responses to certain odors. Here we show that expression of the Cx43 protein in the olfactory epithelium is regulated by olfactory activity. Mice that were exposed to strong nature complex odors emitted by urine and feces exhibited high expression of Cx43 proteins in various regions of the olfactory epithelium. A mixture of authentic odors was also capable to induce upregulation of Cx43. The upregulation was observed in layers where sustentacular cells and olfactory neurons reside. Expression of Cx43 was lower if the naris was occluded. An in situ experiment indicated that olfactory turbinates incubated in an odor mixture or in a cell-permeable cAMP analogue had higher levels of Cx43 expression compared to untreated controls. The connection between olfactory activity and connexin expression suggests a possible mechanism of gap junctions in modulating olfactory transduction at the peripheral level.

This work was supported by grants DC00566, DC04657 and DC04952 from the NIDCD.

Poster Session Fri PM

Olfactory Epithelial and Bulb Recordings in the Rat Indicate That Retronasal Olfaction Is Influenced by Odorant Solubility

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Responses of the rat olfactory system were studied while odorants were presented to the external nares with an artificial sniff (orthonasal) or to the internal nares by positive pressure. Electroolfactograms (EOGs) were recorded from both dorsal and lateral epithelium to assure sensitivity to both polar and non-polar odorants. We used an odorant series that varied from very polar, hydrophilic odorants to very non-polar, hydrophobic. While polar odorants evoked substantial responses with orthonasal presentation, they were not effective when forced through the nasal cavity from the internal nares (retronasal presentation). However, the non-polar odorants were effective in both stimulus modes. These results were independent of stimulus concentration. The results were reproduced when carrier air humidity was shifted from 85% to 25%, showing that a difference in humidity of orthonasal versus retronasal airflow was not the determining factor. Similar results were obtained with multiunit recording in dorsal and lateral positions on the olfactory bulbs of rats anesthetized with urethane (1.5 g/kg). These results help explain why human investigations often report differences in the sensation or ability to discriminate odorants presented orthonasally versus retronasally. The results also strongly support the importance of odorant sorption in normal olfactory processes.

Supported by NIH grant number DC00113. We thank H.P. Acevedo for technical assistance.

Poster Session Fri PM

Movement of Pheromone into Insect Olfactory Sensillae

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Insect sensillae are widely known to be the detectors of insect pheromones. Virtually all olfactory sensillae contain tiny pores ranging from 10 to 50 nm. Pheromone molecules pass through the tiny pores and then enter the sensilla lymph. In terms of surface area, the sensilla are not likely to allow a significant concentration of pheromone to directly enter them. Instead, most of the pheromone will adhere to the waxy surface of the external cuticle first. Pheromone transport through the wax layer then becomes necessary in order to reach these pores. Rapid pheromone movement through the insect wax layer has not been demonstrated in the literature; it has been assumed, but not demonstrated. Furthermore, viscosity of the wax layer is dependent on temperature. Pheromones will travel slower through a more viscous substance. Ample electrophysiological evidence demonstrates pheromone detection occurs in about 1 ms. If pheromone does not pass through the wax layer in less than 1 ms, pheromone detection cannot occur according to the current theory. Using published thicknesses of the wax layer and standard insect pheromones, it is calculated that pheromone detection cannot occur via this mode. An alternate mode of pheromone transport is necessary to help explain the well-established electrophysiological evidence.

Poster Session Fri PM

In Vivo Study of Anosmia Rat Models Using Manganese Enhanced MRI

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Objectives: To image and visualize the functional status and neuronal connections of temporary and permanent olfactory dysfunction. **Experimental Methods:** Female SD rats were used. To investigate the distribution of Mn^{2+} along the olfactory neural network in normal rats ($n = 3$), a series of MRI was performed along the planned time course after intranasal administration of Mn^{2+} . The reversibly damaged model ($n = 6$) was made by injecting 3-methylindole intraperitoneally and a series of MRI was performed. For irreversibly damaged model ($n = 6$), axotomy was done and then serial MRI was performed. The delivered odor was vanillin (4-hydroxy-3-methoxybenzaldehyde). MRI was carried on a 4.7 T MRI. **Results:** The nasal turbinates showed signal enhancement at 1 h after injection, and the layers of the olfactory bulb could be detected at 3 h after injection. The reversibly damaged anosmia model showed enhancement only in the nasal turbinates at 1 week after injection of 3-methylindole and no enhancement in the olfactory bulb. The follow-up MRI at 4 weeks showed normal enhancement pattern. In the irreversibly damaged anosmia model, MRI performed 1 week after axotomy showed enhancement only in the nasal turbinates, no enhancement in the disconnected olfactory bulb. In this irreversibly damaged model, no enhancement in the disconnected olfactory bulb continued for 4 weeks. **Conclusion:** We have succeeded in imaging of olfactory neural network in vivo in temporary and permanent anosmia rat models using manganese enhanced MRI.

Poster Session Fri PM

Use of Sudan Black to Block Lipofuchsin Autofluorescence in Olfactory Epithelium Immunofluorescent Preparations

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Background autofluorescence of lipofuchsin deposits can significantly complicate analysis of olfactory epithelium (OE) immunofluorescent preparations. This is especially true of histological sections through axotomized or bulbecomized OE, in which lipofuchsin deposition can be pronounced. Lipofuchsin has a broad emission spectrum (430–670 nm); however, distinguishing histologically specific immunofluorescence in the red range (580–620 nm) from lipofuchsin autofluorescence can be particularly difficult visually. Additionally, in double-label immunofluorescent studies using both red and green (520–540 nm) emitting fluorophore-tagged secondary antibodies, lipofuchsin autofluorescence in the yellow range (570–590 nm) can severely interfere with detection of any yellow due to specific co-localization of the 2 fluorophores in image overlays. Sudan Black has long been used as a label for compound lipids such as lipofuchsin; recently it has also proven to be a useful blocker of lipofuchsin autofluorescence in a variety of tissues. We describe a convenient application of Sudan Black methodology to OE immunofluorescent preparations. Our procedure significantly reduces OE lipofuchsin autofluorescence and works equally well with frozen or paraffin-embedded sections from both normal OE and axotomized or bulbecomized OE using both epifluorescent and confocal microscopy.

Supported by internal funding, Departmentt of Otolaryngology, Northwestern University.

Poster Session Fri PM

Establishing a Toolkit to Unravel Odorant Receptor-Mediated Signaling in Male Germ Cells

Katharina Klasen, Thomas Veitinger, Christian Wetzel, Marc Spehr and Hanns Hatt

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In addition to their conventional nasal expression, odorant receptors (ORs) are also found ectopically expressed in both developing and mature sperm. In both human and mice, these receptors could play important roles in development and fertilization. However, a comprehensive toolkit for the analysis of OR-induced signaling cascades in this context is still lacking. Here, we introduce several methods to examine the role of OR-mediated signaling pathways in germ cells. We established a number of in vitro approaches to transduce different testicular cells at various developmental stages. We designed culture systems of *tubuli seminiferi* as well as dissociated testicular cells. Using acute slice preparations of seminiferous tubules, we are investigating germ cell physiology via Ca^{2+} -imaging and electrophysiology. In addition to these in vitro models, we also perform in vivo experiments on OR-dependent germ cell signaling in male mice. Here, various reporter constructs such as green fluorescent protein (GFP) or $PH_{PLC}GFP$, a marker indicating PIP_2 hydrolysis, are expressed in germ cells after cDNA electroporation or infection with adenoviral vectors. This toolbox will lay a solid basis for future experiments aimed to provide new insight into the pathways underlying OR signaling in mammalian sperm.

Supported by the Studienstiftung des Deutschen Volkes (K.K.) and the Deutsche Forschungsgemeinschaft (T.V., M.S., H.H.).

Poster Session Fri PM

Spatial Distribution of Transduction System with Nano-scale Resolution in Living Olfactory Cilia

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Osaka University

Olfactory signal transduction starts at the sensory cilia. Up to this point, knowledge about the spatial distribution of this system is limited, because of technical limitations accompanying the fine ciliary structure (100 nm diameters). To overcome such difficulties, we employed a combined technique of the patch clamp and photolysis of caged compounds under the fine visualization of sub-micron structure with the LSM. Cilia were loaded with both caged cNMP for photolysis and Lucifer Yellow for fluorescent visualization. To obtain a small spot (D) for photolysis, based on the relation, $D \propto \lambda/NA$, the laser light was collected at the focus plane with the objective lens having a large NA (1.4) and with the short wavelength ($\lambda = 364$ nm). When the intensity of the UV spot was assumed to express spatially a 2-dimensional Gaussian distribution, δ was estimated to be <300 nm. The on-off and position of the UV spot were regulated by AOTF device and Galvano-mirror. When the local part (ca. 1 μ m length) of cilium was illuminated (therefore, when local cAMP was jumped) under the whole-cell voltage clamp ($V_h = -50$ mV), cells showed an inward current response of ~ 10 pA. The current was observed at any part of the cilia, but the amplitude gradually became smaller when the position was moved to the apical part. This

may be consistent with the fact that the apical part becomes thinner than the proximal part. We conclude that signal transduction channels are present along entire cilia, presumably depending on the membrane surface area.

Poster Session Fri PM

Optical Recorded Responses from the Human Nasal Mucosa to Chemosensory Stimuli

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The distribution of the olfactory epithelium in the human is not entirely clear. The olfactory epithelium is usually thought to be located on the olfactory cleft and superior turbinate. To investigate the distribution of olfactory epithelium, using intrinsic optical signal recording responses from the nasal mucosa were obtained. Recording equipment included an endoscope, a CCD camera, a light source of 617 nm, and a PC. Two concentrations of H₂S (high: 5.56 ppm; low: 2.78 ppm), generated by a computer controlled olfactometer were used for olfactory stimulation. Eight healthy normosmic volunteers joined as subjects. In a typical subject, using high H₂S stimuli responses were recorded from the olfactory cleft, middle turbinate, and middle meatus while responses were less pronounced for low H₂S stimuli. Differences in response area between H₂S low and high was significant ($n = 6$). In a typical subject, concentration-related changes were found in certain areas at the middle turbinate. In conclusion, intrinsic optical recording appears to be applicable to record human response from nasal cavity to specific olfactory stimulation.

Poster Session Fri PM

Administration of Drugs to the Olfactory Cleft

Mandy Scheibe and Christoph Bethge

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Treatment of sinusal olfactory dysfunction is problematic. Few patients benefit from intranasal drug application which may partly be due to access of the intranasal spray to the olfactory epithelium. Aim of the present study was to investigate how nasally applied substances distribute within the nose depending on the form of application. A total of 15 young, healthy volunteers participated. The application forms were 1) nasal drops applied with a pipette, 2) nasal spray, and 3) a system producing squirts. Blue food dye was used to visualize the intranasal distribution of the liquid. The investigation was carried out by nasal endoscopy by an ENT specialist; the distribution of the dye was judged by 2 independent observers blinded with regard to the applicator system that had been used. Using the squirt system the olfactory cleft was reached in most cases. Nasal drops reached the nasal floor and the lower turbinate. The nasal spray was widely distributed in the nasal mucosa; however, most of it was deposited on the middle turbinate and did not reach the olfactory cleft. The present data suggest that previous failure of therapy with locally applied drugs in case of sinusal smell disorders is due to the fact that the substances did not reach the olfactory cleft when using the traditional forms of applicators. Using a new

applicator system, a squirt system, it seems likely to present the drug more effectively the olfactory receptor neurons.

Poster Session Fri PM

Representation of the Nose in the Human Somatosensory Cortex: A Functional Magnetic Resonance Imaging Study

Veronika Schoepf, Johanna May, Rainer Kopietz, Jessica Albrecht, Anna Maria Kleemann, Andrea Anzinger, Tatjana Schreder, Maria Demmel, Gunther Fesl and Martin Wiesmann

University of Munich

The tactile sensation of the nose is involved in the regulation of both breathing and olfaction. Using the non-invasive method of functional magnetic resonance imaging, the cortical sensory representation of the right nasal skin and mucosa in the primary somatosensory cortex were investigated. Twenty-four healthy, right-handed subjects were studied using a 1.5 T MR scanner. Tactile stimuli were applied to 2 parts of the nose: the alar wing of the nose and the lateral nasal mucosa. Images were analyzed using SPM2. The postcentral activation coordinates from the random effects group study were used to define search volumes of 16 mm³ radius. Local maxima in the postcentral gyrus within these search volumes were found in 18 of 24 subjects when stimulating the alar wing of the nose, and in 23 of the 24 subjects when stimulating the lateral nasal mucosa. Our results regarding the alar wing of the nose were consistent with the classic "sensory homunculus" proposed by Penfield. The nasal skin was represented on the postcentral gyrus beneath the thumb and the forehead. In comparison, the nasal mucosa was represented more inferiorly on the postcentral gyrus near the coordinates for the tongue and the pharynx. These results may facilitate the interpretation of fMRI studies of human olfaction.

Poster Session Fri PM

A Physico-chemical Metric for Olfaction

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Weizmann Institute of Science

The rules linking olfactory receptor neuron (RN) or glomeruli (GLO) responses to the structure of odorants remain unknown. Several studies have linked response patterns to restricted features, such as number of carbons, type and position of functional group, etc. These features, however, account for the variability of the response in only a limited set of RNs/GLOs/odorants. To address this, we used DRAGON software to obtain 1664 molecular descriptors for each molecule. We represented each molecule as a vector of descriptor values. This allowed us to calculate distances between any 2 odorants. We find that this metric is better at accounting for neural responses than previously used metrics. For example, whereas carbon chain length explains ~12% of the variability in the RN responses measured by Carlson et al., and ~3% of the variability in the GLO responses measured by Galizia, our metric explains ~22% in both cases ($r = 0.46$, $P < 1e-55$; $r = 0.46$, $P < 1e-18$). This improvement, that was evident across several additional data sets, is statistically significant ($P < 1e-7$ in both cases). Finally, our metric successfully accounted for previously unexplained reports. For example, Imamura et al. reported

that many neurons responded to acids and aldehydes, fewer to both acids and alcohols, and none to acids and alkanes. Using our metric we find that these groups are ordered in a manner that predicts the results obtained by Imamura. To conclude, we suggest that our metric provides a unified account for several independently obtained results, and provides a quantitative tool for odorant comparison.

Poster Session Fri PM

Discrimination of Carvone and Terpinen-4-ol Enantiomers Indexed by Odor Sample Time

Burton Slotnick

University of South Florida

The failure to find that enantiomers that produce nearly identical olfactory bulb (OB) odotopic maps would be more difficult for rats discriminate than those that produce different patterns of bulb activation was re-assessed using mice and both response accuracy and odor sampling time as dependent measures. Acquisition functions for mice trained to detect 1% (+)-carvone and to discriminate 1% (+)-carvone from (–)-carvone did not differ from those trained in a similar manner to detect 1% (+)-terpinen-4-ol and discriminate enantiomers of terpinen-4-ol. Mice odor sampled longer on the initial carvone than on the initial terpinen-4-ol detection task but there were no between group differences for odor sample time on the discrimination problems. These results confirm and extend those of McBride and Slotnick and provide further evidence that differences in patterns of odor-induced OB activation obtained using 2-deoxyglucose do not necessarily predict outcomes of behavioral tests.

Supported in part by NIH grant DC04671.

Olfaction: Periphery

Hypomorphic CEP290/NPHP6 Mutations Result in Anosmia Due to the Loss of G Proteins in Cilia

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Cilia regulate diverse functions such as motility, fluid balance, and sensory perception. Genetic mutations of proteins known to localize to cilia and/or the basal body, such as the nephronophthisis protein (NPHP) family, result in human disease. Mutations in NPHP6/CEP290, which localizes to the connecting cilium of rods and cones in the retina, cause retinitis pigmentosa, Leber congenital amaurosis, and Joubert syndrome. Here, we establish a role for CEP290 in olfactory sensory function. Patients that are homozygous for a CEP290 mutation, Cys998X, exhibit severely abnormal olfactory function (compared to age-matched controls). In mice, CEP290 localizes to the ciliary base in the dendritic knob of olfactory sensory neurons but is absent from the ciliary axoneme. Despite the complete loss of olfactory function in CEP290 mutant mice, cilia remain intact. The anosmia is due to the mislocalization of olfactory G-proteins resulting in loss of ciliary expression. Interestingly, other proteins in the signaling cascade, such as adenylyl cyclase type III, are unaffected suggesting that olfactory signaling molecules are not transported into cilia as preassembled complexes. Our data link defects in olfactory function to other ciliary diseases and indicate

that smell identification tests may be useful as a diagnostic tool in pre-genetic screening for ciliopathies.

Olfaction: Periphery

β-Arrestin2 Mediated Internalization of Mammalian Odorant Receptors

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Ruhr-University Bochum

While the endocytic mechanisms of G-protein-coupled receptors have been characterized extensively, almost nothing is known about the intracellular trafficking of odorant receptors (ORs). We describe the endocytic pathway of mammalian odorant receptors, which bind β-arrestin2 with high affinity and are internalized via a clathrin-dependent mechanism. Odorant-induced OR desensitization is promoted by PKA phosphorylation and is dependent on serine and threonine residues within the third intracellular loop of the receptor. Moreover, β-arrestin2 is redistributed into the dendritic knobs of mouse olfactory neurons after treatment with a complex odorant mixture. Adaptation of olfactory neurons to odorants can be abolished by the inhibition of clathrin mediated endocytosis, showing the physiological relevance of the here described mechanism of OR desensitization. Prolonged odorant exposure resulted in accumulation of β-arrestin2 in intracellular vesicles in the olfactory neurons, concomitant with the phosphorylation of cytosolic MAPK, which may then induce changes in the protein expression of the sensory neurons. A better understanding of OR trafficking and further insight into the molecular determinants underlying the interactions of ORs with β-arrestin2 and other trafficking proteins will therefore be important to fully understand the mechanisms of adaptation and sensitization in the olfactory epithelium.

Olfaction: Periphery

Ultra-sensitive Chemosensory Responses to Carbon Dioxide and 1-Octen-3-ol on the Maxillary Palp of Female *Anopheles gambiae*

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²Equal contributions

Many species of mosquitoes, including the Afrotropical malaria vector *Anopheles gambiae*, perceive CO₂ and 1-octen-3-ol as crucial olfactory cues in their host-seeking behaviors that play critical roles in their ability to transmit diseases. Here we report that the maxillary palp, an accessory olfactory organ of *An. gambiae*, mediates ultrasensitive detection of these 2 mosquito kairomones. Using physiological recordings, fluorescent in situ hybridization and functional analysis, we define an olfactory map of the maxillary palp. We identify 3 types of olfactory receptor neurons (ORNs) within its capitate peg sensillum. Of these, one ORN coexpresses 3 putative gustatory receptors and exhibits a strong and specific response to CO₂. Two other ORNs which link together to form stereotypic triads with the CO₂-responsive neuron within each capitate peg express *An. gambiae* odorant receptors. One ORN is a specialist receptor displaying high selectivity and sensitivity to 1-octen-3-ol while the other ORN exhibits a broader response spectrum. Our results

facilitate understanding of how *An. gambiae* mosquitoes utilize both generalist and specialist chemosensory receptors to perceive potential long-range olfactory cues that are important in host location behaviors. Targeting this step could compromise this mosquito's ability to transmit deadly infectious diseases such as malaria.

Supported by NIH and the Grand Challenges in Global Health program of the Gates Foundation.

Olfaction: Periphery

Cannabinoid Action in the Olfactory Epithelium

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The perception of odors is influenced by a variety of neuromodulators and there is growing evidence that modulation already takes place in the olfactory epithelium. In the present study we report on cannabinergic actions in the olfactory epithelium of larval *Xenopus laevis*. First, we show that CB1 receptor-specific antagonists AM251, AM281 and LY320135 modulate odor-evoked calcium changes in olfactory receptor neurons, suggesting a tonic release of endocannabinergic substances in the olfactory epithelium. The fact that the highly specific CB1 agonist HU210 drastically accelerates the recovery from the effects of the antagonists substantiates the above results. Second, we localize CB1-like immunoreactivity on dendrites of olfactory receptor neurons. Finally, we describe the cannabinergic influence upon odor-induced spike-associated currents in individual olfactory receptor neurons. Taken together we demonstrate for the first time that the cannabinergic system has a profound impact on peripheral odor processing and discuss its possible function.

This work was supported by grants of the "Research program, Faculty of Medicine, Georg-August-Universität Göttingen" and by Deutsche Forschungsgemeinschaft through the DFG-Research Center for Molecular Physiology of the Brain.

Olfaction: Periphery

Octopamine Modulates Pheromone Responses Daytime-Dependently via Stimulation of an Adenylyl Cyclase in the Antenna of the Hawkmoth *Manduca sexta*

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Philipps-Universität Marburg

The biogenic monoamine octopamine (OA) functions as a neurohormone, -modulator, and -transmitter, and controls circadian rhythms in invertebrate nervous systems. With antibodies against tyramine and OA we found 2 immunoreactive neurons projecting via the antennal nerve into the antennal sensory epithelium of *Manduca sexta*. To determine whether OA modulates bombykal transduction daytime-dependently in situ tip recordings of single pheromone-sensitive trichoid sensilla were performed. During the 3 hrs long recordings OA was perfused into the trichoid sensillum while applying bombykal stimuli (10 ms, 10 µg BAL) every 5 min. Only at Zeitgeberzeit (ZT) 8–11 (the middle of the day), but not at ZT 22–1 and ZT 1–4 (the end of the night and the beginning of the day when the nocturnal moths are still active) OA increased the amplitude of the pheromone-

dependent sensilla potential and the initial action potential (AP) response and rendered the AP response more phasic. In a competitive biochemical assay we showed that OA stimulates the adenylyl cyclase in antennal homogenates daytime-dependently. In addition, the application of cAMP during tip recordings increased the pheromone-dependent sensillar potential amplitude and rendered the AP response more phasic. Thus, we hypothesize that daytime-dependent release of OA sensitizes the antenna and improves its ability to follow pheromone pulses at the late night when the male moths are seeking their mates.

Supported by DFG STE531/13-1.

Olfaction: Periphery

Olfactory Sensory Neurons: Plasma Membrane Calcium Pump-2 in Calcium Clearance and Odor Detection

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University of Vermont

Calcium rises in olfactory sensory neurons (OSNs) after odorants bind to receptors on the cilia and subsequently cyclic nucleotide gated and voltage gated calcium channels open. To explore whether plasma membrane calcium pumps (PMCA) play a role in removing free calcium from the dendritic knob and cell body following stimulation, we compared OSNs from wild type and mice with no PMCA-2, one of 4 PMCA (PMCA-2 KO mice the gift of G. Shull). We analyzed kinetics of decay of the fluorescence signal from fura-2 loaded OSNs following stimulation by high K⁺ or IBMX, to mimic cyclic AMP mediated odorants by increasing cAMP and opening the cyclic nucleotide gated channel. PMCA-2 KO mice reduced the calcium from the influx significantly more slowly than the wild type OSNs from cell body and knobs whether stimulated by high K⁺ or IBMX (by at least 30%). Carboxyeosin, a PMCA inhibitor, slows the rate of calcium removal from the cell body by about 40%. To test whether the PMCA-2 KO mice detect odors as well as wild type, we initiated behavioral tests that pair odor with shock to determine whether the animals reduce their activity in response to the odor. KO animals learn to reduce their activity when the odor is amyl acetate, but do not learn when the odor is geraniol, while the wild type animals learn with both odors. We are exploring the thresholds of and discrimination by the wild type and KO, and the effect of inter-trial interval the KO's ability to learn with amyl acetate.

NIH R21 DC 006643 and NIH R01 DC 00721.

Olfaction: Periphery

Functional Evolution of Odorant Binding Proteins in *Drosophila melanogaster*

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Odorant binding proteins (Obps) in *Drosophila melanogaster* are diverse polypeptides encoded by a multigene family of about 50 genes. We used cDNA expression microarrays that represent all *Drosophila* chemoreceptors to examine Obp expression levels in a standard laboratory strain. There was excellent concordance between

fluorescent signal intensity on the array and abundance of transcript detected by RT-PCR. Expression levels of *Obp* genes within clusters varied greatly in adults and larvae and in some cases were sexually dimorphic, suggesting that sex-specific and stage-specific functions of Obps can be subject to different selection pressures. We capitalized on naturally occurring mutations (polymorphisms) to gain insights into the evolution and functions of Obps. Analyses of the sequences of 13 *Obp* genes contained in 2 chromosomal clusters in a population of wild-derived inbred lines revealed different signatures of natural selection. Association studies in the population of wild-derived inbred lines identified 4 polymorphisms in 3 *Obp* genes associated with responsiveness to benzaldehyde, indicating that recognition of this odorant is combinatorial. Thus, functional population genetics presents a powerful strategy for gaining insights into the functions of Obps in chemosensation.

Contact Chemosensory Perception: From Receptor to Behavior

Taste Reception in *Drosophila*

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Yale University

The fruit fly detects soluble ligands in the environment via gustatory neurons that are housed in chemosensory sensilla. In the adult, the labellum is the primary taste organ and possesses about 60 sensilla. A family of 60 gustatory receptor (*Gr*) genes has been identified and a ligand for one receptor, Gr5a, has been found. In order to identify potential roles for individual gustatory receptors as well as to provide insight into the molecular and functional organization of taste perception, we are creating a map of gustatory receptor expression and physiological responses in the labellum. We have undertaken a systematic expression analysis of the *Gr* family using a reporter transgene system to map *Gr* gene expression in the periphery in adults as well as in larvae, and to determine how neurons expressing these genes project to the brain. We have found that most *Gr* genes are expressed in subsets of bitter-sensitive neurons, and a few are expressed in sugar-sensitive neurons. Using the tip-recording method, we are examining the electrophysiological responses of each labellar sensillum to a number of sugars, amino acids and bitter compounds. Consistent with results from the expression study, in most cases we find no qualitative differences in sugar responses among different sensilla; however, bitter compounds induce action potentials only in specific subsets of sensilla. Based on these data, we have identified candidate receptors for some ligands and are conducting a genetic analysis of these *Gr* genes to examine their function.

Contact Chemosensory Perception: From Receptor to Behavior

Sex Pheromone Discrimination and Taste Receptor Neurons in *Drosophila* Males

Jean-Francois Ferveur, Fabien Lacaille and Claude Everaerts

CNRS-Universite de Bourgogne

Sexual behavior requires animals to distinguish between the sexes and to respond appropriately to each of them. In *Drosophila melanogaster* males, several hydrocarbons found on the fly cuticle are

thought to be involved in sex recognition and in mating behavior: they tend either to stimulate or to inhibit hetero- and/or homosexual activity, and they can elicit a dose-dependent electrophysiological response in specific taste receptor neurons. We have used transgenes to target specific subsets of taste receptor neurons to determine their involvement in pheromonal discrimination. These transgenes were used either alone or combined with a second reporter transgene that genetically alters the function of the targeted neurons. Behavioral tests, performed with 2 different target flies simultaneously tested, indicate that the discrimination of their pheromones by tester males changes with the targeted neurons and with the type of genetic modification. Moreover, the discrimination response cannot be extrapolated from the responses directed towards each individual target.

This work was partly funded by the Centre National pour la Recherche Scientifique (CNRS) and by the Burgundy Regional Council.

Contact Chemosensory Perception: From Receptor to Behavior

Gr Genes and Their Role in Taste and Pheromone Perception of *Drosophila melanogaster*

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Duke University

In *Drosophila*, 2 subsets of gustatory receptor neurons (GRNs) express distinct members of the gustatory receptor (*Gr*) gene family encoding sweet and bitter taste receptors, respectively. A third group of GRNs appears to function as pheromone sensors. Gr68a, which is expressed in GRNs associated with male-specific bristles on forelegs, was previously shown to be necessary for high male courtship intensity and mating success. We investigated the possible role of several other *Gr* genes related to Gr68a and found that Gr32a encodes a second pheromone receptor. In simple courtship assays, Gr32a mutant (Gr32a⁻) males show normal courtship towards virgin females. However, in competitive situations (2 males competing for a single female), Gr32a⁻ males perform poorly against control males. Interestingly, Gr32a⁻ males court both the female and the competing male, suggesting an inhibitory role for GR32a in male-male courtship. To test this, Gr32a⁻ males were presented with decapitated male courtship objects and found to exhibit high courtship intensity (CI = 0.4), whereas control males showed little male-directed courtship (CI = 0.1). This suggests that GR32a is a (inhibitory) pheromone receptor for a male pheromone. All labellar Gr32a expressing neurons also express Gr66a, encoding a receptor for caffeine. In the forelegs, however, Gr32a expressing neurons project to brain structures previously not associated with any other set of GRNs. We propose that male courtship repression is mediated through 2 interacting neural circuits, one mediating general feeding avoidance and one repressing sexual behaviors.

Contact Chemosensory Perception: From Receptor to Behavior

Identification of Candidate Sour Taste Receptors in Mammals

Hiroaki Matsunami

Duke University Medical Center

Animals use their gustatory systems to evaluate the nutritious value, toxicity, sodium content, and acidity of food. In vertebrates, taste reception occurs at the apical tip of taste cells that form taste buds. Each taste bud has an onion-like shape and is composed of 50–100 cells. In mammals taste is classified into 5 distinct taste modalities: bitter, sweet, umami (taste of some *L*-amino acids), salty, and sour. Among them, the molecular basis of sour taste transduction is poorly understood, although a confusing variety of different putative receptors and transduction mechanisms have been proposed. We recently showed that 2 TRP family members and homologs of polycystic kidney disease genes, PKD1L3 and PKD2L1, are co-expressed in a subset of taste receptor cells in specific taste areas. Cells expressing these molecules are distinct from taste cells having receptors for bitter, sweet or umami tastants. The PKD2L1 proteins are accumulated at the taste pore region at the apical tip of taste cells, where taste chemicals are detected. Co-expression of the PKD1L3 and PKD2L1 is necessary for their functional cell surface expression. Finally, PKD1L3 and PKD2L1 are activated by various acids when co-expressed in heterologous cells, but not by other classes of tastants. Our results, together with reports from 2 other groups, suggest that PKD1L3 and PKD2L1 heteromers may function as sour taste receptors.

Supported by NIDCD.

Contact Chemosensory Perception: From Receptor to Behavior

Identification of the Pheromone Ligands and Sensory Neurons That Mediate Male–Male Aggression in the Mouse

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The Scripps Research Institute

In mice, pheromones serve as signals between individuals to regulate their social behavior. The identity of pheromones that are sufficient to regulate individual behaviors are largely unknown. Isolation of these chemical ligands and their target sensory neurons is essential to elucidate the logic of the underlying neural circuit. We have chosen to define the components that are sufficient to generate male–male territorial aggression. Previous behavioral analysis has revealed that activating pheromones are excreted in adult male urine. We have assayed fractionated urine through biobehavioral analysis to identify the aggression pheromone. Calcium imaging experiments on primary neurons reveals that the isolated compound is sufficient to robustly activate a subset of vomeronasal neurons. We are using single cell analysis of cDNA from these activated neurons to elucidate the molecular identity, including the pheromone receptor, of the aggression-mediating neurons. We expect that the identification of both the stimulating ligand and the responsive sensory neurons will provide the tools to manipulate and therefore study the molecular and neuronal pathway that regulates male–male aggression.

Poster Session Sat AM

L-Alanine CTA and Threshold Studies with T1R3 Knockout Mice

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University of Vermont

Several studies suggest G-protein-coupled receptors are responsible for detecting *L*-amino acids. Li et al. reported that the heterodimer T1R1/T1R3 receptor is responsive to monosodium glutamate (MSG), the prototypical umami substance, and *L*-amino acids but there is disagreement regarding mice lacking the T1R3 receptor. One study reported that T1R3 knockout (KO) mice show no preference for *L*-amino acids including *L*-alanine and MSG, suggesting T1R1/T1R3 is the sole receptor able to recognize the tastes of *L*-amino acids. However, a study with independently developed T1R3 KO mice found that these mice show only a reduced preference for MSG + IMP, suggesting additional *L*-amino acid taste receptors are present. To examine this further, we tested T1R3 KO mice (obtained from Damak et al.) and their wild-type counterparts (C57BL) for their ability to detect *L*-alanine and to develop a conditioned taste aversion (CTA) to *L*-alanine. Gustometer tests using water reinforcement/shock avoidance methods indicate that both mouse types have similar detection thresholds for *L*-alanine. For CTA, both types of mice were presented with *L*-alanine followed by an injection of LiCl or NaCl. Amiloride (Na channel blocker) was added during conditioning and testing. Both mouse types learned a CTA to *L*-alanine, but KO mice showed weaker conditioning and primarily at higher concentrations of *L*-alanine compared to wild type mice. These data suggest that the T1R3 receptor is important for detecting *L*-alanine, but is not the sole receptor for detecting the taste of this amino acid.

Supported by NIH DC007617 (E.D.).

Poster Session Sat AM

Laryngeal Taste Buds and Airway Chemoreceptors Express Little T1R3

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Taste buds and solitary chemosensory cells (SCCs) in the oral cavity, larynx and nasopharynx must both monitor potential foods as well as guard against intake of toxic material. All of these sensory cells rely on taste receptors ultimately impacting TrpM5 as a requisite downstream element. For sweet and umami, the receptor complex involves T1R3 as a required element. Thus T1R3 is a good marker of chemoreceptors responsive to these appetitive qualities. We utilized *in situ* hybridization, RT-PCR and 2 lines of transgenic mice to assess the distribution within the oropharynx and respiratory tract, of chemoreceptor cells expressing T1R3, TrpM5 and related markers. TrpM5+ receptor cells occur within all taste buds, including those on the epiglottis and larynx. In addition, numerous, scattered TrpM5+ SCCs lie within the nasopharynx, larynx and trachea. In contrast, T1R3+ cells occur predominantly in lingual and palatal taste buds. Comparatively few T1R3+ cells are present in laryngeal taste buds and essentially no T1R3+ SCCs are present within the airways. Thus laryngeal taste buds and SCCs do not display molecular features associated with detection of sweet or umami compounds. These findings are consistent with electrophysiological studies showing minimal laryngeal responsiveness to sweet and umami substances.

Grant support from NIH to T.E.F. and R.F.M.

Poster Session Sat AM

TAS2R38 Genotype, Fungiform Papillae and Suprathreshold Taste Response

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We continue our examination of TAS2R38 genotype in relationship to oral sensory phenotype in 170 adults. DNA from whole bloods was analyzed for genotypes of the 3 polymorphic sites (AVI/AVI, heterozygote, PAV/PAV); rare alleles were excluded (<3%). As previously, there was incomplete relationship between the psychophysical function for perceived bitterness of propylthiouracil (PROP) and genotype. Genotype clearly identified those with shallower functions (i.e., nontasters) but produced limited separation between those with steeper functions (medium vs supertasters). When classified by PROP threshold ($n = 137$), 9 of 40 AVI homozygotes were misclassified as tasters (78% sensitive, 96% specific). Fungiform papillae (FP) density and quinine bitterness represent additional phenotypes previously associated with diet and health outcomes. Quinine had a distribution independent from TAS2R38 genotype while FP density was not. Comparing AVI to PAV homozygotes, AVIs tended to have fewer than 25 FP/6 mm², while PAVs tended to have more. The net impact of FP density on intensity differed across genotype: in AVI homozygotes, FP density predicted gains in taste intensity for NaCl, sucrose, citric acid and PROP, but not quinine. We conclude that multiple phenotypic and genotypic markers are needed to adequately describe variation in oral sensation and quinine is a distinct phenotype from FP density, PROP bitterness, and TAS2R38 genotype.

Funded by NRICGP/USDA, NIH, NIAAA.

Poster Session Sat AM

Interactions of Bitter Tastants with Their TAS2R Receptors

Anne Brockhoff¹, Maik Behrens¹, Giovanni Appendino², Christina Kuhn¹, Bernd Bufe¹ and Wolfgang Meyerhof¹

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The perception of bitter substances in humans is mediated by 25 GPCRs of the hTAS2R family. Despite the recent identification of several agonist-receptor combinations, the structural basis for the ability of humans to detect the huge number of chemically distinct bitter tastants with a small set of receptors still remains unclear. Therefore, we investigate the structural requirements for the broad tuning of TAS2Rs using mutagenesis and calcium imaging of heterologously expressed receptors. To this end we use TAS2R46, a receptor for numerous sesquiterpene lactones, diterpenoids, denatonium, and strychnine, and TAS2R44, a receptor for aristolochia acid and saccharin. The analysis of hTAS2R46/44 chimeras indicated that amino acid residues in both, the extracellular loops and transmembrane domains (TM), mediate receptor-agonist interactions. Furthermore, these chimeras revealed that the selective activation of TAS2R44 and TAS2R46 by aristolochia acid and strychnine or denatonium, respectively, is mediated by the part of the receptor encompassing TM6 and TM7. However, this part is not sufficient for selective receptor activation by sesquiterpene

lactones or saccharin. Subsequently generated point mutations identified 2 amino acid residues in TM7 that determine the selective activation of TAS2R46 and TAS2R44 by strychnine and aristolochia acid, respectively, and one residue in TM7 of TAS2R46 required for selective activation by denatonium.

Poster Session Sat AM

Derivatives of Denatonium Benzoate—Bitter Taste of Humans versus Monkeys

Tiffany Otto, Alexey Koposov, Yiwen Wang, Viktoria Danilova and Göran Hellekant

University of Minnesota Medical School

Denatonium benzoate (DB) is one of the most bitter compounds ever known to humans. The DB is typically used as a strong taste modifier in food and personal care industries to prevent accidental poisoning. DB is detectable by humans at 10 ppb, and 50 ppb is already extremely bitter. In order to investigate structure-activity relationship in the case of the bitter taste we have prepared a number of DB analogs to answer the question which parts of the molecule are particularly active to the bitter receptor and to assess the usefulness of the monkey model for human taste by correlating data from a human sensory panel with monkey nerve recordings.

Supported by NIH R01 DC006016.

Poster Session Sat AM

Glycosylation of Human Bitter Taste Receptors

Maik Behrens, Claudia Reichling and Wolfgang Meyerhof

German Institute of Human Nutrition

Human bitter taste is mediated by 25 hTAS2R genes. To investigate the function of hTAS2Rs it is necessary to identify their agonist spectrum. One way to deorphanize receptors is to utilize heterologous expression. However, like other chemoreceptors, hTAS2Rs are difficult to express in heterologous cells. For many GPCRs Asn-linked glycosylation (N-glycosylation) was shown to affect functional expression. We therefore investigated the presence of consensus sites for N-glycosylation in hTAS2Rs, as well as their utilization and functional significance in a mammalian cell line. We expressed hTAS2Rs or non-glycosylated mutants in HEK 293 cells and performed Western blot experiments to determine protein glycosylation. By expression of these constructs in the presence of the inhibitor tunicamycin we studied the functional significance of N-glycosylation. We show that all hTAS2Rs contain a conserved N-glycosylation site in the second extracellular loop. For hTAS2R16 and -46 we demonstrated that this consensus site is utilized in mammalian cells. The almost complete loss of function caused by mutation or tunicamycin treatment of these receptors demonstrates the significance of glycosylation. The function of a mutant hTAS2R16 is partially restored by the addition of a glycosylated sst3 export-tag, or the coexpression of the auxiliary protein RTP3. The agonist profile of mutant hTAS2R14 and -46, however, is unchanged indicating a role in biosynthesis rather than agonist binding. We conclude that N-glycosylation during biosynthesis of hTAS2Rs is a prerequisite for their function.

Poster Session Sat AM**Response Characteristics of the Rat Chorda Tympani Nerve to Static and Dynamic Lingual Thermal Stimulation**

Jessica Lee and Robert Bradley

University of Michigan

Much of the research on the response properties of the chorda tympani nerve (CT), which innervates taste buds on the anterior tongue, has focused on responses to chemical stimuli. However, the CT also responds to lingual thermal stimulation. Previous investigations of the thermal characteristics of the CT have used a limited range of thermal stimuli. We have investigated the static and dynamic response properties of the CT to thermal stimulation of the dorsal tongue epithelium. Integrated responses were recorded from the CT of 27 rats. Thermal stimuli were delivered via a circulating water thermode. Temperature stimuli ranged from 10 °C to 30 °C. No responses were recorded from temperatures categorized as warm but robust responses were recorded to stimuli classified as cool. Responses to static temperatures ranged from 10 °C to 30 °C with a bell shaped function. The maximum response occurred at 20 °C. Increasing the rate of cooling resulted in increases in the integrated response. Recently thermal receptors have been shown to be TRP ion channels. Taste receptors cells express TRPM5 channels and TRP channels have been implicated in sour taste transduction. The TRP channels that respond to temperatures used in the current study are TRPA1 and TRPM8. The results of this study suggest that taste cells also express one or both of these ion channels.

Supported by NIH grant DE007057.

Poster Session Sat AM**Single Fiber Responses of the Chorda Tympani Nerve to Umami Taste Compounds in Wild Type, T1R3-KO and TRPM5-KO Mice**Keiko Yasumatsu¹, Ryusuke Yoshida¹, Yoshihiro Murata¹, Sami Damak², Robert F. Margolske³ and Yuzo Ninomiya¹¹Graduate School Kyushu University, ²Nestlé Research Center and ³Mount Sinai School of Medicine

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. Apparently contradictory data was obtained from 2 T1R3 knock-out (KO) mouse models: Zhao et al. showed that umami detection and perception was abolished in their T1R3-KO, while we found that responses to umami compounds were diminished in our T1R3-KO mice. In the present study, we further examined responses to umami compounds at single nerve fiber levels in wild type (WT), T1R3- and TRPM5-KO mice. The results indicated that umami-responsive single fibers of the chorda tympani nerve in WT mice could be classified into Sucrose-best (S-type) and MPG-best (M-type) fibers. Furthermore, each of these fiber types could be classified into 2 subgroups, one type showing synergistic effect between MPG and IMP (S1, M1) and the other type showing no synergism (S2, M2). In the KO mice, S1-type

was absent, but S2, M1 and M2 types still remained. This provides additional evidence for existence of multiple receptors, transduction pathways and fiber types underlining umami taste in mice.

Poster Session Sat AM**Triphenylphosphine Oxide (TPPO) Is a Potent, Selective Inhibitor of the Human Transient Receptor Potential M5 (hTRPM5) Monovalent Cation Channel**

Robert Bryant, Paul Lee, Tulu Buber, Karnail Atwal, Ivana Bakaj, Heather Devantier, Cynthia Hendrix, Dennis Sprou, Rok Cerne, Rosa Cortes and Kyle Palmer

Redpoint Bio

The TRPM5 cation channel has been established as a critical component of the bitter, sweet, and umami taste signal transduction pathway. TRPM5 conductance is gated by intracellular calcium and is additionally regulated by local membrane phosphatidylinositol, temperature, and pH. To date, no inhibitors of practical use have been described for investigation of TRPM5 function and its precise role in taste signaling. In order to identify potent, selective inhibitors of TRPM5, we developed a high throughput FLIPR-based assay using recombinant human TRPM5-expressing HEK293 cells (hTRPM5/HEK) and a membrane potential (MP)-sensitive fluorescent dye. ATP-activation of endogenous P2Y receptors elicited strong calcium signals in both hTRPM5/HEK and non-recombinant parental HEK293 cells. However, ATP elicited MP responses only in hTRPM5/HEK cells. A random library of >100 000 compounds was screened for TRPM5 inhibitors. Triphenylphosphine oxide (TPPO) was found as a selective and potent TRPM5 inhibitor (IC₅₀ = 6 μM) of ATP-elicited MP responses. TPPO also inhibited MP responses of murine TRPM5 (IC₅₀ = 20 μM) but had no effect on TRPA1, TRPV1 or TRPM4b. Related compounds, such as triphenylphosphine, and other TPPO analogs, were inactive on TRPM5 or other TRP channels. We conclude that TPPO is the first high potency, selective inhibitor of hTRPM5. TPPO should prove a useful pharmacologic tool for probing the function of TRPM5.

Poster Session Sat AM**Gurmarin Inhibition of the Chorda Tympani Nerve Responses to Sweeteners and Its Temperature Dependency in Mice**

Tadahiro Ohkuri, Keiko Yasumatsu, Ryusuke Yoshida, Noriatsu Shigemura and Yuzo Ninomiya

Kyushu University

Our previous studies demonstrated that responses of the mouse chorda tympani (CT) nerve to sweet compounds were classified into 2 components; one is inhibited by gurmarin (Gur-sensitive [GS]) and the other is not (Gur-insensitive [GI]). The C57BL strain possesses both components, whereas BALB mice have only the GI component. Recently, TRPM5 is found to be a temperature-sensitive channel through which temperature-dependent increase (TDI) in response to sweet compounds may be generated. In the current study, we examined temperature dependency of the GS and GI components of responses to sweeteners in the GS C57BL and GI BALB mice. In both strains of mice, the CT nerve responses to

all sweeteners tested increased significantly with increasing the temperature from 15 °C to 35 °C, suggesting that both GS and GI components showed TDI. In C57BL mice, we found that TDI of the GI components to sucrose and glucose were significantly larger than that of the GS component. However, in response to SC45647, which is reported to be only a sweet compound whose response was totally abolished in TRPM5-KO mice, TDI in the GS component was as large as that of the GI component. These results suggest that both GS and GI components exhibited the TDI, and the magnitude of TDI differed between the GS and GI components to sugars. Smaller TDI of the GS than GI component to sugars may be due to involvement of temperature-insensitive subcomponent of the GS which may occur through TRPM5-independent pathways.

Poster Session Sat AM

Polyose and Starch Preferences in *Trpm5*, *Gustducin* and P2X Knockout Mice

A. Sclafani¹, J.I. Glendinning² and R.M. Margolskee³

¹Brooklyn College, City University of New York, ²Barnard College and ³Mount Sinai School of Medicine

In addition to their well-studied preference for sweet taste, rats and mice are attracted to the taste of starch and polysaccharides (e.g., Polyose). The present study determined if taste cell signaling proteins involved in sweet taste detection also mediate Polyose and starch preference using 48-h 2-bottle tests. In an initial sweetener test, *gustducin* (*Gus*) and *Trpm5* knockout (KO) mice showed reduced or no preference for saccharin. The *Gus* and *Trpm5* KO mice showed no preference (47–60%) for Polyose at low concentrations (0.5–2%) that were strongly preferred (74–96%) by wildtype C57BL/6J (B6) mice. At higher concentrations *Gus* and *Trpm5* KO mice developed significant Polyose preferences which were likely due, in part, to post-oral effects. *Trpm5* KO mice were also indifferent to corn starch (0.5–4% in gum suspensions) whereas *Gus* KO and B6 mice showed similar preferences (68–95%; 73–97%). In a starch versus Polyose test, *Gus* KO mice preferred starch (87%) whereas B6 mice preferred Polyose (96%); *Trpm5* KO mice were indifferent. P2X KO mice, which have taste nerves insensitive to prototypical taste stimuli, also failed to prefer Polyose and starch at low concentrations. Together these results suggest that taste cells and nerves mediate the preference for dilute Polyose and starch, as well as for sugar. The differential response of *Gus* KO mice to Polyose and starch indicate that these carbohydrates activate different signaling pathways.

Supported by NIH grants DK031135 (A.S.), DC03055 and DC03155 (R.M.M.).

Poster Session Sat AM

Thymol and Related Phenols Are Potent Activators of the Transient Receptor Potential Channel, TRPA1

S. Paul Lee, Tulu Buber, Heather Devantier, Daniel Long, R. Kyle Palmer, Rosa Cortes, Rok Cerne, Ray Salemme and Robert Bryant

Redpoint Bio

Thymol is a major component of the spice thyme and is often present in oral care products among other medicinal uses. It has a distinctive pungent flavor mediated by receptor mechanisms in the nose and tongue in addition to having aversive properties. Using a voltage-sensitive fluorescent dye, we show that thymol specifically activates the human transient receptor potential channel A1 (TRPA1), EC₅₀ 12 mM, but not other taste sensory TRP channels (TRPV1 and TRPM5). Electrophysiology measurements further demonstrated that thymol activation was concentration-dependent, reversible, and rapidly desensitized TRPA1. A number of related phenols including 3-*tert*-butyl-4-hydroxyanisole (BHT), a food preservative, and propofol, an intravenous anesthetic, also selectively activated TRPA1 (EC₅₀ 3 and 13 mM, respectively). Phenols with less bulky carbon substitutions adjacent to the phenolic group, such as 2,6-dimethylphenol and 2-methylphenol (*o*-cresol), were less potent. TRPA1-specific siRNA inhibited thymol activation, confirming the specificity of the response. In addition, thymol activation was blocked by camphor (IC₅₀ 200–500 mM) whereas other camphor analogs, for example, camphorquinone, camphoric acid and limonene were TRPA1 agonists (EC₅₀ 0.3, 2, 3 mM, respectively). Our results suggest a role of TRPA1 activation in the reported pungent and aversive properties of some of these pharmaceutically (medically) important phenols.

Poster Session Sat AM

Preferences for Basic Tastes in 6- and 12-Month-Old Infants

C. Schwartz, S. Issanchou and S. Nicklaus

INRA

As part of the OPALINE longitudinal study, which aims are to determine the origins and evolution of sensory and food preferences from birth to the age of 2 years, taste preferences were investigated at 6, 12 and 20 months. Here are reported cross-sectional preferences for the 5 basic tastes: sweet (lactose), salty (NaCl), bitter (urea), sour (citric acid) and umami (monosodium glutamate) at the ages of 6 (*n* = 40) and 12 months (*n* = 54). The same method, adapted from previous studies, was applied at each age. It was approved by the local ethical committee and informed consent was obtained from both parents. Mothers and their infant participated in 2 videotaped sessions in a temperature-controlled room designed for infant testing, during which the 5 tastes were assessed in a balanced order. For each taste, 4 bottles (water, tastant, tastant and water) were presented by the experimenter who was blind to the tastant. Two indices were calculated to represent acceptability of the tastant relatively to water: one is based on ingested volumes, the other on mimics and behaviors. Preliminary results based on relative ingested volumes show that at 6 months, salty taste was as liked as sweet taste, but more liked than sour, umami and bitter tastes (*P* < 0.01); at 12 months, the same result was observed, but in addition bitter taste was less liked than any other taste (*P* < 0.001). Preferences for the 5 basic tastes differed at 6 months and even more at 12 months. Further investigation will determine the weight of food experiences in the establishment of these differences.

Funded by Regional Council of Burgundy, PRNH, ANR 2006-PNRA.

Poster Session Sat AM**Early Milk-Feeding History Influences Infants' Taste Preferences**

Catherine Forestell, Lindsay Morgan, Lauren Yourshaw, Gary Beauchamp and Julie Mennella

Monell Chemical Senses Center

We have identified a model system to experimentally investigate how early experiences contribute to individual differences in taste preferences. This system exploits the variation between different classes of infant formula and human milk. These sources of infant nutrients contain variable amounts of compounds that have specific taste qualities. Of specific interest is the pronounced bitter, sour and savory taste of protein hydrolysate formulas. To determine whether exposure to these different milks influence later taste preferences, we studied 3 groups of infants, who differed in milk feeding regimen (i.e., hydrolysate formula, milk-based formula, breast milk), on 6 separate occasions. In counterbalanced order, infants were videotaped while feeding either sweetened (lactose), salty (NaCl), bitter (urea), savory (MSG), sour (citric acid) or plain cereal. Infants who fed hydrolysate formula ate significantly more of the savory- and sour-flavored cereals than those fed milk-based formula, but only if they were not yet eating table food. Among infants who were breastfed, those whose mothers more frequently ate meat and vegetables high in free glutamate (e.g., peas, tomatoes) ate significantly more of the savory cereal. These findings add to the growing body of research that early experiences with milk and solid foods affect infants' taste acceptance patterns.

Supported by a grant from the International Glutamate Technical Committee, NIH grant HD37119 and a CIHR Postdoctoral Fellowship.

Poster Session Sat AM**Smoking and Breastfeeding**

Julie Mennella, Lauren Yourshaw and Lindsay Morgan

Monell Chemical Senses Center

According to the WHO, about 250 million women inhale tobacco, one of the most heavily addicted drugs, on a daily basis. Prior research in our laboratory revealed that cigarette smoking by lactating women results in a pronounced flavor change in human milk. To determine the short-term effects of breastfeeding from a mother who smokes, we monitored infants' feeding and sleep behaviors for 3.5 h on 2 separate days. After refraining from smoking for at least 12 h, the mother, shielded with a disposable lab coat and wearing gloves, went into a 700 ft³ stainless steel, environmental chamber alone (without the baby) for a 20-min period. In counterbalanced order, mothers smoked during one test day or refrained from smoking during the other. We found no significant difference in the patterning of breast feeding on the 2 testing days, suggesting that the flavor was familiar and not aversive to infants. However, infants spent significantly less time sleeping on the days their mothers smoked when compared to when mothers refrained from smoking ($P = 0.0004$). This decrease in total sleep was observed in 12 of the 15 infants and sleep was reduced by, on average, 36.6%. Infants spent less time in both active and quiet sleep and there was a significant shortening

of the longest sleep bout after breastfeeding from mothers who recently smoked. These findings reveal that short-term exposure to mother's milk flavored with nicotine significantly alters sleep-wake patterning in the infant. Whether infants are associating the flavor cue with such alterations is an important area for future research.

This research was supported in part by NIH grant AA09523.

Poster Session Sat AM**Cigarette Smoking, Family History of Alcoholism and Sweet Taste in Women**

M. Yanina Pepino

Monell Chemical Senses Center

The goal of the present study was to determine whether cigarette smoking and family history of alcoholism (FH) was associated with altered sweet taste preferences and thresholds in women. Each subject participated in a 2-day study separated by 1 week after having-refrained from smoking for approximately 12 h. In counterbalanced order, women smoked a cigarette with nicotine on one testing day and a nicotine-free cigarette on the other. Sucrose taste thresholds and preferences were assessed within 40–70 min after smoking. To allow for comparisons, we also determined sucrose preference and thresholds in a group of non smoker women. Procedures were identical except that the women did not smoke. We find that both FH and smoking status had differential effects on sucrose taste. Although smoking a nicotine cigarette did not alter sucrose taste thresholds among women who smoke, smokers had significantly higher sucrose thresholds when compared to non smokers. Sucrose preferences were significantly affected by FH but not by the habit of smoking. Women with an FH preferred higher sucrose concentrations than women with no FH. Because of the high association between alcohol and cigarette use, the present results may explain inconsistencies in studies of sweet preference of smokers when family history of alcoholism is not taken into account.

This project was funded by a grant from the Pennsylvania Department of Health. The Pennsylvania Department of Health specifically disclaims responsibility for any analyses, interpretations, or conclusions.

Poster Session Sat AM**Correlations among Supertasters—A Possible Link to the Freshman 15**

Chrissie Faust

Millsaps College

Research on taste sensitivity and subsequent studies aimed at identifying Supertasters and their characteristics have led to numerous reports on taste physiology and human taste behaviors. Reports examining the relationship between Supertasters and food choice are now becoming available, yet one area of potential interest has not been examined. Given that Supertasters may exhibit specific food preferences and thus avoid certain foods while gravitating to others it is possible that such enhanced taste sensitivity may lead to predictable eating behaviors. More precisely, the identification of a Supertaster may preclude or exclude persons from experiencing exaggerated weight gain once their dietary choices become less restrictive. Such a situation often occurs in college settings where the

anecdotal Freshman 15 has been linked to unrestricted access to fried foods, buffets, late night binge eating, and over-consumption of high calorie beverages. Our study used specific physiological correlates, food portion assessments, and sensitivity to PROP to identify Supertasters among college freshmen. Correlations between these measures were then made for Supertasters and non-Supertasters. Results indicate that Supertasters consume portions 15% larger than non-tasters, have 19% more body fat, and are 12% less fit than non-Supertasters. Additional testing of subject will allow us to determine the extent to which such measures can predict who is likely to experience excess weight gain during the first few years of college and possible interventions to prevent such weight gain.

Funding: Millsaps College Ford Fellowship.

Poster Session Sat AM

Damage to Taste (Otitis Media) Is Associated with Dysgeusia, Intensified Pain Experience and Increased Body Mass Index

Linda Bartoshuk¹, Frank Catalanotto¹, Valerie Duffy², Howard Hoffman³, Henrietta Logan¹, Vicki Mayo¹ and Derek Snyder^{1,4}

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The chorda tympani taste nerve traverses the middle ear and may be damaged by infections (otitis media). Lecture attendees ($N = 6984$) answered questions (Have you ever suffered from middle ear infections? Do you ever have persistent salty, sweet, sour, or bitter tastes in your mouth?) and reported height and weight. Analyses were done with multiple regression (controlling for sex and age). Those with histories of otitis media reported more dysgeusia and had higher BMIs (weight corrected for height). A subset ($n = 1599$) rated everyday sensations (e.g., strongest pain of any kind; strongest oral pain ever experienced) using the gLMS. Those with a history of otitis media reported the most intense pains. Chorda tympani input may inhibit a variety of central functions. Damage releases that inhibition with a variety of possible consequences including: intensification of non-taste oral sensations (e.g., creaminess of fats, oral pain) and increased oral phantoms (dysgeusia, burning mouth syndrome). The finding of increased non-oral pain generalizes taste inhibition of pain to non-oral locations. The impact of otitis media on body weight may reflect both the alteration in sensory properties of foods (e.g., increased palatability of energy-dense foods) and eating to reduce pain.

Funded by NIDCD.

Poster Session Sat AM

Assessment of Taste Changes in Human Patients and Rats following Weight-Reduction Surgery

A. Rebecca Glatt¹, David Tichansky², Atul Madan², Jason Harper² and John Boughter¹

¹University of Tennessee Health Science Center and ²University of Tennessee

Following laparoscopic Roux-en-Y gastric bypass surgery (LRYGB), patients report changes in taste sensitivity and preference. Our objectives were to assess possible taste changes following weight reduction surgery using a psychophysical assess-

ment of patients, as well as by developing a behavioral model with rats. We tested patients at a weight management clinic using a modified labeled magnitude scale (LMS) to gauge perceived taste intensity, and a 9-point scale to gauge perceived pleasantness, of concentration series of glucose, sucrose and NaCl. Test groups included non-obese controls, pre-surgical, post LRYGB, and post laparoscopic adjustable gastric banding (LAGB). We found no significant group differences in perceived intensity or pleasantness of these particular stimuli. For the rat model, we performed RYGB surgery on male Sprague-Dawley rats fed a high-fat diet. RYGB rats maintained weight loss at 80–86% of their original body weight over 4 weeks as compared to their sham counterparts, which lost no weight. Rats were tested with sucrose and NaCl (brief-access taste tests) 2 weeks after surgery. RYGB rats displayed a decrease in taste responsiveness to sucrose, but only at intermediate concentrations. RYGB rats also initiated significantly less trials and thus consumed less sucrose during the tests than shams. No differences were found in taste-based responses to NaCl.

Poster Session Sat AM

Effects of Chocolate Consumption on Cognition, Mood and Workload

Rosanna Drake, Daniel Felbaum, Chris Huntley, Alex Reed, Lauren Matthews and Bryan Raudenbush

Wheeling Jesuit University

Previous research has found that the nutrient content of foods aids in glucose release and increased blood flow. These increases have subsequently been implicated in augmenting cognitive performance. The present study assessed the effects of various chocolate types on cognitive performance, mood, and task workload. In a within-subjects design, participants completed the protocol under 4 conditions: 85 g milk chocolate (total fat 26 g, saturated fat 18 g, carbohydrates 50 g, fiber 2 g, sugar 44 g, protein 6 g), 85 g dark chocolate (total fat 34 g, saturated fat 20 g, carbohydrates 46 g, fiber 6 g, sugar 34 g, protein 4 g), 85 g carob (total fat 20 g, saturated fat 14 g, carbohydrates 45 g, fiber 11 g, sugar 40 g, protein 11 g), and a non-consumption control condition. After a 15 min digestive period, participants completed a variety of computer-based neuropsychological tests assessing word discrimination, verbal memory, design memory, attention span, reaction time, problem solving, and response variability. Mood and task workload were assessed via the Profile of Mood States (POMS) and the NASA-Task Load Index (NASA-TLX). Gender and age served as co-variants for the analyses. Composite scores for verbal and visual memory were significantly higher for milk chocolate than the other conditions. Consumption of milk or dark chocolate showed improved impulse control and reaction time. These findings provide support for nutrient release via chocolate consumption to enhance cognitive performance.

Poster Session Sat AM

Hunger Ratings among Restrained Eaters with High and Low Disinhibition

Nobuko Kemmotsu^{1,2}, Lori Haase^{1,2}, Marla Yidonoy¹, Margaret Chen¹, Erin Green¹, Aaron Jacobson¹ and Claire Murphy^{1,2}

¹San Diego State University and ²University of California

Previously we reported that individuals with high and low cognitive restraint in eating (defined by the Three Factor Eating Questionnaire: TFEQ) responded differently to certain psychophysical measurements when satiated and hungry. The current study examined additional effects of the Factor 2 of the TFEQ, disinhibition. Sixteen female restrained eaters and 14 non-restrained eaters participated. The study consisted of 2 sessions for both of which participants fasted for 12 h in advance. One of the sessions was deemed the "Hunger" condition in which participants received a nutritional preload. The other session was deemed the "Satiated" condition in which no preload was given. Participants gave hunger ratings upon arriving at the laboratory, after consuming the preload, and after 2 h. The analysis revealed that among restrained eaters, Factor 2 scores differentiated how they responded to hunger ratings. Specifically, in the satiated condition after the preload, restrained eaters with high disinhibition gave significantly lower hunger ratings (less hungry) than those with low disinhibition did. Additionally, those with high disinhibition showed a slight increase in the rating after 2 h, whereas those with low disinhibition became less hungry. These effects were specific to restrained eaters. Future studies are needed to examine whether the differences were due to cognitive bias or to physiological processes.

Supported by NIH grant AG04085 to C.M.

Poster Session Sat AM

Experience Induced Increases in Discrimination for the Familiar Taste of a Sugar Require Very Brief Experience and Reverse within 22–34 Days

K.M. Gonzalez, C. Peo, A. Whalen, V. Mike, T.P. Livdahl and L.M. Kennedy

Clark University

Experience induced changes are known in taste identification of monosodium glutamate, sensory detection of glutaraldehyde, and psychophysical and fMRI responses to novel taste stimuli. Yet whether such plasticity occurs for the highly familiar taste of sugar remained unconfirmed. We tested the taste induction hypothesis for sugar, using a counterbalanced design, consisting of 2 treatment conditions and 3 pre-treatment test conditions. First, 150 subjects were either not tested or tested. Those tested tasted paired glucose solutions and water (glucose pre-test), or water and water (sham pre-test), and indicated "the sugar" of each pair. All then briefly treated their tongues with a fructose solution or water once each day for 10 days. On day 11 or 12, all were tested as before, with glucose and water. Glucose pre-test, fructose treated subjects either continued or stopped treatment for another 10 days and were tested again at 22-3 and 33-4 days. The results show that 1) experience with fructose increases discrimination for the taste of the sugar, glucose, 2) there are no differences in the sugar discrimination between groups of randomly assigned subjects before treatments, 3) one session of 5 brief tastings has an effect on the discrimination when tested 11–12 days later, and 4) without continued treatment, the increased discrimination reverses after 22–34 days.

Supported by a NSF Graduate Fellowship to K.M.G. We thank Bio 040 2004 students for assistance.

Poster Session Sat AM

Effects of Video Game Play on Snacking Behavior

Trevor Cessna, Alex Reed, Ryan Hunker and Bryan Raudenbush

Wheeling Jesuit University

Past research notes children with higher BMI played moderate amounts of electronic games, while children with lower BMI played low amounts of electronic games. To investigate the possibility of video games being a distraction to food consumption in a controlled environment, 90 Ps entered a testing room in which 10 ounces of Hershey's M&M's® was placed. Ps were casually asked to indicate their level of hunger and instructed that if they were hungry there were welcome to consume the M&M's®. In a between-subjects design, Ps completed one of the following 30 min conditions: 1) fighting game (Capcom vs SNK 2 EO), 2) puzzle-based game (Tetris Worlds), and 3) a control condition in which no game was played. Upon completion, Ps rated their level of hunger, their level of liking M&M's® and estimated the number of M&M's® they had eaten. Prior to exiting the study Ps completed questionnaires related to task workload, mood, and normal/disordered eating habits. Actual consumption was recorded. Results indicated a trend for males to eat more in the fighting condition in comparison to females; however, there was no difference in the males between the control and puzzle condition. Females ate the most in the control condition, with no difference between the fighting and puzzle conditions. Playing either video game decreased Ps' ability to adequately estimate how much was consumed. Thus, video games of any type may lead to underestimating food consumption, and may contribute to decreased healthiness and increased weight gain.

Poster Session Sat AM

Mitral/Tufted Cell Odor Responses in Freely Moving Mice

Wilder Doucette and Diego Restrepo

University of Colorado at Denver and Health Sciences Center

In the awake freely moving rodent previous investigators rarely found Mitral/Tufted (M/T) cell responses to odors, and when they found responses, they were mostly inhibitory. We postulate that pairing the correct odor stimulus with electrode location is key to obtaining reliable M/T cell responses in vivo. Presumably odor matching in vivo is critical because of the increased background activity and smaller fraction of responding M/T cells due to increased inhibition by granule cells in the absence of anesthetics. We tested the hypothesis that the use of odors known to elicit responses in the area of the olfactory bulb (OB) targeted by our electrodes would result in an improved yield of odor responses in OB recordings in vivo. Odors were chosen among those known to stimulate the ventral areas of the OB in rats and mice. Mice were implanted in the ventral mitral cell layer with a total of 16 platinum/iridium electrodes coated with the biocompatible polymer paralyneC (8 in each bulb). Implanted animals were placed in a chamber and recorded from while they were exposed to an odor for 2 s with an intertrial interval of 60 s. Our results show that we can reliably find excitatory odor responses in awake freely moving mice provided these animals were tested with a large quantity of odors known to stimulate the ventral OB. These

data are amassing into a database of the odor responsiveness of single units recorded from electrodes within the context of an OB map.

Supported by grants: DC00566, DC04657, DC006070 and DC008066.

Poster Session Sat AM

Optical Imaging of Postsynaptic Odorant Representations in the Olfactory Bulb

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The anatomical organization of olfactory receptor afferent input into the olfactory bulb (OB) suggests that odorants can be encoded as spatial patterns of glomerular activity. This transformation of receptor activation into a topographical map of glomerular activation may serve as the initial basis for odor representation within the olfactory system. To date, functional imaging methods have mostly focused on mapping odor representation based on patterns of receptor input onto the OB glomeruli. Thus, the glomerular patterns seen only reflect pre-synaptic input and cannot reveal any post-synaptic odor processing within the bulb. Here we report visualization of post-synaptic odor representation within the OB using a transgenic mice line expressing the fluorescent Ca²⁺ indicator protein G-CaMP2. This indicator is only expressed in OB neurons postsynaptic to receptor input, including mitral/tufted cells and a subset of periglomerular cells. G-CaMP2 odor-evoked signals could be detected across the dorsal surface and displayed sensitivities spanning a broad odorant concentration range. Different odorants evoked distinct, but overlapping odor-evoked response patterns that were conserved across different animals. Binary mixture analysis revealed that postsynaptic response maps of binary mixtures cannot always be predicted by the sum of the component maps.

Poster Session Sat AM

Glomerular Response Mapping Using Virtual Projection Neuron Populations: A Step towards Representing Whole Antennal Lobe Activity in Realtime

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Our understanding of odor processing in the primary olfactory neuropil has been greatly enhanced by population recordings with imaging and multi-unit methods. However, inherent limitations constrain the directness of these measures, their resolution and comprehensiveness and hence the strength of the conclusions that can be made by their use. To complement these methods, we have begun to build a virtual antennal lobe and to characterize its response to a restricted range of odors. This will result in spatio-temporal activity maps at the level of identifiable glomeruli and output neurons. Individual neurons were recorded and stained intracellularly in male *Manduca sexta* moth. We impaled neurons randomly and used the same suite of neat odors as in previous multi-unit recordings. Standard histology allowed a classification as local or projection neuron. For the

latter, a species-specific glomerular map was used to identify the innervated glomerulus. Peri-stimulus rasters characterize activity patterns across the identified glomeruli with high temporal resolution and with the highest possible precision regarding neural firing patterns. Odor representations of the virtual ensemble are compared with multi-unit data. Additionally, glomerular space-time-activity maps are developed. They will be comparable to imaging-based maps, thus providing a unique opportunity to compare all 3 methods.

This work was supported by NIH-NCRR RR015574 to K.C.D. and DFG grant SCHA 678/3-3 to J.S.

Poster Session Sat AM

Dynamic Odor Perception and Neural Code in an Insect

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We studied how perception arises from sensory processing in the brain by correlating stimulus-evoked neural activity with behavioral assays of perception in the hawkmoth (*Manduca sexta*). A wide range of odor stimulus conditions (odorant types, concentrations, pulse durations) reliably evoked local field potential oscillations in the mushroom body (MB). Intracellular recordings from projection neurons (PNs, $n = 15$) and local neurons (LNs, $n = 31$) in the antennal lobe showed that their spikes were tightly phase-locked to the MB oscillations, revealing their widespread oscillatory coordination. Injection of GABA_A blocker into the antennal lobe abolished the oscillations. Seventeen intra- and 78 extracellular recordings from PN's followers, Kenyon cells (KCs), revealed very sparse spikes that were phase-locked to the MB oscillations, indicating that oscillatory synchrony is important for odor coding by PNs and decoding by KCs. Long odor pulses (4 s), commonly used in conditioning paradigms, activated different KC ensembles at stimulus onset and offset, indicating that odor representations in the MB evolved over the course of the odor response. Moths were classically conditioned in a proboscis extension reflex paradigm so that either the onset response (evoked by short pulses, 500 ms, $n = 49$) or the offset response (evoked by long pulses, 4 s, $n = 59$) overlapped with sucrose reward. Our behavioral results showed moths can readily distinguish odor onset from offset, consistent with the time-varying odor representations in the MB.

Poster Session Sat AM

Enantiomers and Their Neuronal Activation Patterns in the Olfactory Bulb

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University of Tuebingen

Chirality plays an important role in many interactions with biological sites such as drug response, insect communication and odor perception. A variety of optical isomers (pairs of mirror-symmetric, non-superimposable molecules that differ only in optical activity and their interaction with other chiral molecules) have been described as having different odor qualities and/or different odor intensities for humans. There are also reports of enantiomeric odor pairs in which one form has a distinct odor quality whereas the other form is odorless. It is

known that each odorant molecule activates at least one, possibly several receptor cell types and the axons of each receptor cell type converge onto a specific glomerulus in the olfactory bulb. As a result of this neuronal connections an odorant dependent neuronal activation patterns arises. Such activation patterns can be analyzed by applying, for example, the immunohistochemical c-fos method. We compared the neuronal activation patterns elicited by the 2 enantiomeric pairs *S*(+)- and *R*(-)-carvone ($n = 10$) as well as *S*(+)- and *R*(-)-sclarymol ($n = 5$). *S*-carvone has a caraway note, while *R*-carvone has a more spearmint note; *R*-sclarymole smells herbaceous and green while *S*-sclarymol smells sulfury and onion-like. We found that each odor of an enantiomeric pair elicit neuronal activity in different however also overlapping areas. We, therefore, conclude that the detection of the enantiomers carvone and sclarymol is mediated by different populations of receptors with opposite chiral selectivity.

Poster Session Sat AM

Temporal Dynamics of Receptor Neuron Input to the Olfactory Bulb of Behaving Rats

Ryan M. Carey, Justus V. Verhagen, Daniel W. Wesson and Matt Wachowiak

Boston University

We have previously demonstrated that the dynamics of olfactory receptor neuron (ORN) input to the olfactory bulb can encode odorant information and are shaped by sniffing in anesthetized mice. To test whether this occurs during natural sniffing, we trained head-fixed rats to discriminate between odorants, then imaged presynaptic calcium signals (indicative of ORN firing) during odor discriminations. Slow sniffing (<2.5 Hz) evoked brief bursts of ORN input that were phase-locked to each sniff. We measured parameters of the sniff-evoked calcium response in each glomerulus, such as latency (sniff peak to 50% of the peak response: 117 ± 45 ms; reflects the delay from odor sampling to bulbar input), rise time (10–90% of the calcium response: 119 ± 71 ms; reflects the temporal spread of ORN firing), and decay time. As in anesthetized mice, calcium response dynamics differed among glomeruli and for different odorants and concentrations, but remained uniform within a glomerulus for a given odorant and concentration. These dynamics occurred mostly within the time from the earliest calcium response to the behavioral response (~440 ms). Phase-locking between calcium signals and sniffing did not occur during fast sniffing (>4 Hz); as frequency increased, responses became desynchronized from sniffing. These data suggest that odorant information can be encoded by the temporal properties of receptor input to the olfactory bulb, and that this encoding can be modulated by sniffing frequency.

Funded by NIH DC06441 (M.W.) and DC008197 (J.V.V.).

Poster Session Sat AM

Toward an Estimate of the Number of Receptor Neuron Spikes Needed for Odorant Identification

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In the rodent olfactory system each glomerulus receives input from approximately 10 000 olfactory sensory neurons. The input from re-

ceptor neurons to glomeruli has been imaged using calcium indicator dyes or with synapto-pHluorin. It is not known how well these signals correlate with the animal's ability to identify odorants across a range of concentrations. We have compared the ability of 6 rats to identify odorants with these same animals' input calcium signals using an awake/restrained preparation. Behavioral measurements showed that the rats achieved greater than 80% correct odorant identification at all odorant concentrations we tested; from 0.0007% to 35% of saturated vapor. For most animals and odorants the calcium signals were smaller than the noise at odorant concentrations of 0.1% of saturated vapor. Clearly, the calcium signals and the behavior were poorly correlated. The concentration dependence of the calcium signals was well fit by a Michaelis–Menten equation. The amplitude of the fitted curve for the most sensitive glomeruli was only 0.05% of the maximum signal at an odorant concentration of 0.0007%. A calcium signal of 0.05% is equivalent to the high concentration odorant response of only 5 olfactory sensory neurons.

Supported by DC05259 and by AA014871.

Poster Session Sat AM

Directional Asymmetry in Responses of Crayfish Brain Interneurons to Hydrodynamic Stimulation of the Lateral Antennular Flagellum

DeForest Mellon and Joseph A.C. Humphrey

University of Virginia

We have recorded spiking responses from single, bimodally-sensitive local interneurons (type I) in the crayfish deutocerebrum to hydrodynamic and odorant stimuli flowing in 2 directions along the major axis of the lateral antennular flagellum. Using a reversible flow olfactometer we changed the direction of seamless introductions of odorant flow past the flagellum, from proximal-to-distal to distal-to-proximal, finding that flow direction did not consistently affect the dose-dependent spiking responses of type I neurons to odors. By contrast, changing the direction of an abruptly initiated flow of water (or odorant) past the flagellum resulted in consistently larger short-latency spiking responses to hydrodynamic stimulation when the flow was proximal-to-distal. Morphological findings, kinematic analysis and computer simulations predict that this direction of water flow mimics that of a downward flick for medial and lateral putative mechanoreceptive sensilla in the basal 33% of the upwardly-curved flagellum, whereas along the distal 67% of the flagellum, the favored direction of water flow will be experienced by all sensilla during a downward flick. Mathematical models of drag forces and torques acting on one class of putative mechanoreceptive sensillum—beaked sensilla—during favored hydrodynamic flow within the olfactometer and during downward flicking suggest that they are similar in both direction and magnitude.

Poster Session Sat AM

Imaging Juxtglomerular Responses to Odorants in Mice Using 2-Photon Microscopy

Ryota Homma¹, Lawrence Cohen¹, Olga Garaschuk² and Arthur Konnerth²

¹*Yale University* and ²*TUM*

There are few published recordings of odorant responses in juxtglomerular cells. We used the multi-cell bolus loading method of Stosiek et al. to load neurons in the glomerular layer of the mouse olfactory bulb with calcium sensitive dyes (OregonGreen 488 BAPTA-1 AM or Fura PE3 AM). Mice were anesthetized and the bone over the olfactory bulb was removed for imaging using 2-photon microscopy with an Olympus Fluoview 300 scanner. Frame rates of between 5 and 10 Hz were used. Three kinds of responses to 2 s odorant pulses were found. The most common was an increase in calcium during odorant application (ON cell). The second response type was an increase in calcium that occurred at the offset of the odor pulse (OFF cell). The third response type was a decrease in calcium concentration during odorant application (INHIBITED). When 3 odorant pulses were presented with 5–10 s intervals some ON cells showed little desensitization while others only responded to the first pulse. Cells of different response type were often clustered and the clustering appeared to be related to the kind of response measured in a nearby glomerulus. In one preparation we measured odorant responses in 8 Z-planes that encompassed one glomerulus. We detected activity in 200 ON cells, 40 OFF cells and 1 INHIBITED cell.

Supported by grants DC05259, SFB 391, SFB 596, and NGFN-2.

Poster Session Sat AM

Constructing Odor Representations: Learning, Genetics, and Pharmacology

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Secondary odorant representations across the population of mitral cells are substantially transformed in both form and content from the primary representation in olfactory sensory neurons. Feed-forward and feedback inhibition mediated by local interneurons and neuromodulation by descending inputs contribute to a secondary representation that reflects not only stimulus features, but learning and contextual factors. These processes are likely to be critical for the identification (segmentation) of odors of interest embedded within natural scenes. Generalization acuity is the degree of variance in odor quality that is considered by an animal to share the learned contingency of a conditioned odor stimulus, hence serving as a measure of the scope of a constructed odor. The breadth and form of olfactory generalization curves are shaped by learning-theoretic variables, olfactory bulb pharmacology, and certain genetic mutations in characteristic ways reflecting the bulbar circuitry that mediates them. We use computational modeling to integrate results from behavioral, genetics, electrophysiological, and anatomical studies and outline a general model of olfactory bulb stimulus processing.

Supported by NIH grants DC007725 (T.A.C.) and DC008702 (C.L.) and a Marie Curie Fellowship (N.M.).

Poster Session Sat AM

Neural Basis of Latent Inhibition to Odors in Honeybees

Fernando Locatelli¹, Giovanni Galizia² and Brian Smith¹

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Honeybee foragers learn about rewarded floral odors. They also must learn to ignore odors that have no predictive value. This form of learning, called latent inhibition (LI) is induced by unrewarded exposure to an odor. LI is revealed by reduced learning performance when the preexposed odor is subsequently paired with reinforcement. We used calcium imaging to evaluate if exposure to an odor in a way that induces behavioral LI affects the neural representation of that odor in the primary olfactory neuropil, the antennal lobe (AL). AL projection neurons were labeled with the calcium sensor dye Fura2. Odor-evoked activity was measured for 2 pure odors and the binary mixture before and after preexposure to one of the pure odors. Analysis based on intensity and spatial distribution of the evoked activity shows odor specific activity patterns characterized by partially overlapping groups of glomeruli. Binary mixtures evoked activity in the same glomeruli as the single odors. Induction of LI produced an odor-specific reduction in activity. This effect was not evident in all animals, which is consistent with genetic variation in expression of LI. The reduction in response was specific to the preexposed odor, so it was not due to a generalized reduction of activity in the AL. After LI induction the activity evoked by the mixture is less similar to the preexposed odor and shifts toward the pattern for the other odor. We are currently investigating modulatory pathways that mediate this form of plasticity in the AL.

Supported by DC007997 from NIH-NIDCD.

Poster Session Sat AM

Interhemispheric Connections of the Rat Anterior Olfactory Nucleus

Jennifer Eudy and Kurt Illig

University of Virginia

The anterior olfactory nucleus (AON) is a central olfactory cortical structure with heavy reciprocal connections with both the olfactory bulb (OB) and piriform cortex. The main portion of the AON (*pars principalis*) is comprised of 2 layers: an outer plexiform zone and a deeper cellular layer, and is often divided simply by “compass” points (dorsal, lateral, etc.). It has been firmly established that the AON is a primary source of bilateral projections in the olfactory system through extensive connections with both the ipsilateral and contralateral OB, AON and piriform cortex. However, few studies have examined this circuitry in detail. In the present study, we used small injections of the anterograde tracer *Phaseolus vulgaris* Leucoagglutinin (PHA-L) and the retrograde tracer Fluorogold in specific subregions of the AON to explore the topography of the interconnections between the left and right AONs. Labeled fibers were found in the contralateral AON following injections in all areas. Nevertheless, detailed results demonstrated that different regions of the AON have distinct patterns of interhemispheric projections; contralateral fibers were most heavily targeted to dorsal and lateral AON, while the medial and ventral areas received relatively light projections. Further, projections differentially targeted superficial or deep zones within the cellular layer. These results corroborate other recent work indicating that subregions of the AON are anatomically distinct, and thus may play separate roles in olfactory information processing.

Funded by NIH grant DC05557 (K.I.) and a Harrison Undergraduate Research Award (J.E.).

Poster Session Sat AM**Spatial Organization of Activity in the Anterior Olfactory Nucleus**

Elizabeth Meyer, Rachel Kay, Kurt Illig and Peter Brunjes

University of Virginia

The anterior olfactory nucleus (AON) plays a central role in odor information processing through reciprocal connections with the olfactory bulb (OB) and piriform cortex (PC). Information about odor identity appears to be organized spatially in the OB, but is distributed in the PC. No detailed studies have examined the organization of odor-evoked activity in the AON. Fos protein expression was used as a marker for activity following exposure to 2 odorants with different molecular structure, perceived odor, and spatial representations in the bulb: caproic acid, a 6-carbon aliphatic acid that activates the dorso-medial OB, and limonene, a hydrocarbon that activates large regions in the lateral and medial parts of the OB. Additionally, we examined Fos expression following focal electrical stimulation of spatially disparate areas of the OB glomerular layer in anesthetized and tracheotomized animals with bilateral naris occlusion. 3-D reconstructions of the AON revealed no discrete clusters of Fos positive neurons for either odor-exposed or electrically stimulated animals. Further, there were no differences in the spatial distribution of labeled cells for different odors, or for electrical stimulation of different locations in the OB. Rather, neurons expressing Fos were widely distributed, suggesting that mitral/tufted cells from widely separated glomeruli in the OB have functional connections with neurons in overlapping areas throughout the AON.

Funded by NIH grants DC05557 (K.I.) and DC00338 (P.B.).

Poster Session Sat AM**The Medial Amygdala Receives a Direct Input from Ventrally Located Mitral Cells in the Main Olfactory Bulb of Mice**

Ningdong Kang, Alice Wey, James Cherry and Michael Baum

Boston University

Information about social odorants detected by the main and accessory olfactory pathways converges in the rodent medial amygdala (MeA) before being conveyed to the hypothalamus. Whereas the inputs from the accessory (vomeronasal) system to the MeA are direct, previous studies suggested that inputs from the main olfactory system reach the MeA via an indirect polysynaptic pathway that includes the anterior cortical amygdaloid nucleus. Recently we demonstrated using anterograde tracing that the molecular layer of the MeA receives direct projections from both the main and accessory olfactory bulbs (Society for Neuroscience 2006 annual meeting). However, the precise location of MeA-projecting mitral cells in the main olfactory bulb is unknown. We addressed this question by injecting the retrograde tracer Cholera toxin b (CTb) iontophoretically into the MeA to label cells that send terminals to this site. As expected, retrogradely labeled cells were found throughout the accessory olfactory bulb, but a small population of labeled cells was also seen in a ventromedial location of the main olfactory bulb mitral cell layer. By contrast, retrogradely labeled cells following either piriform cortex or lateral amygdala CTb injections were found throughout the main

bulb mitral cell layer. This study provides strong evidence that a monosynaptic pathway exists from the main olfactory bulb to the MeA in mice. The function of these ventral-medial located MOB mitral cells is subject to ongoing study in our group.

Supported by HD 44897.

Poster Session Sat AM**Medial Amygdala Response to Territorial, Reproductive and Predator Stimuli**

Chad Samuelsen, C. Blake and M. Meredith

Florida State University

The medial amygdala (ME) of both male hamsters and mice is activated by chemosensory stimuli from females and males of their own species (conspecific) and of other species (heterospecific). Studies examining hypothalamic connections of the amygdala suggest the anterior medial nucleus (MeA) and ventral posterior medial nucleus (MePv) are related to defensive responses and the dorsal posterior MeP (MePd) to reproductive responses. In experiments with hamsters, conspecific stimuli activated both MeA and MeP, regardless of gender or reproductive/competitive relevance. Heterospecific stimuli, including predator (cat collar CC), activated only MeA, but also activated intercalated nucleus (ICN) which may suppress MeP response. In experiments with mice, all stimuli cause increased expression of FRAs (immediate early gene proteins) in the MeA. Female mouse urine activates both MePv and MePd, whereas male mouse urine only activates MePv. The heterospecific hamster vaginal fluid had no effect in either MePd or MePv. Interestingly, the amount of time a CC was worn affected both behavior and ME response. "Weak" CC (12-h) increased FRAs expression in MeA, but not in either division of MeP. "Strong" CC (2 weeks) activated MeA and the putative defensive MePv. Mice exposed to strong cat collar spend significantly less time investigating the collar compared to control. We are conducting experiments to characterize activated cell phenotypes. We are also using oxytocin-knock-out mice to investigate the role oxytocin plays in the ME response to conspecific and heterospecific odors.

Supported by NIDCD: DC05813, T32 DC0044 and F32 DC.

Poster Session Sat AM**Initial Survey of Integration of Sensory Information in the Olfactory Cortex in Awake Mice**

Jonah J. Scott-McKean, Wilder Doucette and Diego Restrepo

University of Colorado at Denver and Health Sciences Center

Most natural olfactory stimuli are made up of a mixture of odorants but an organism perceives the mixture as a singular odor. Due to convergence of a heterogeneous group of mitral cells onto pyramidal cells in the olfactory cortex we hypothesize that some pyramidal cells display an odor response to a binary mixture but not to either individual odorant. To test this an 8 channel electrode array was implanted bilaterally into the anterior piriform cortex where pyramidal cell responses were recorded in C57/B6J mice. Recordings were made at least 1 week after recovery from surgery in awake mice. The odorants were presented for 2 s either individually or as a binary mixture with 60 s in between trials. A small fraction of cells recorded from did

respond only to the mixture. Our findings show that individual pyramidal neurons in the olfactory cortex can respond to a mixture of odorants but not to the individual odorants alone.

Supported by grants: DC00566, DC04657, DC006070, DC008066 and HD41697.

Poster Session Sat AM

Pattern Completion and Separation in Anterior Piriform Cortex

Donald Wilson

University of Oklahoma

Synthetic sensory processing involves 2 components. First, there must be an anatomical basis for convergence of diverse feature input onto individual target neurons. Second, a mechanism often exists for remembering previously experienced patterns of input. This latter component makes synthetic processing both robust in the face of degraded or noisy inputs (i.e., allows pattern completion), while at the same time it allows enhanced sensory discrimination of familiar stimuli (i.e., pattern separation). Evidence for both convergence and experience-dependent processing exists in piriform cortex (PCX). In olfaction, pattern completion could allow stable recognition of complex mixtures even with disturbance in mixture components. However, the need for perceptual stability must be balanced by the need to discriminate between overlapping but distinct inputs. Here, we looked for evidence of pattern completion and separation in anterior PCX using complex 10-component mixtures. Single-units were recorded in the anterior PCX of urethane-anesthetized rats. Responses to: 1) 10-component equal volume mixture of molecules with similar vapor pressures, 2) the same mixture with 1 component missing, 3) the same mixture with 2 components missing, and 4) the same mixture with 1 substituted component. Mixtures were tested at 2 concentrations, 350 and 10 ppm. Population responses were compared with correlations to determine similarity of responses. Preliminary results suggest pattern completion occurs at high intensities although addition of a single novel component allows clear separation. At low intensities pattern separation occurs even with loss of a single component.

Funded by NIDCD.

Poster Session Sat AM

Piriform to Orbitofrontal Transthalamic Pathway Involved in Olfactory Attentional Processing

Jane Plailly, James Howard and Jay Gottfried

Northwestern University

Odor is thought to access higher-order cortical regions by 2 different pathways. In the direct pathway, piriform cortex (PC) projects directly to orbitofrontal cortex (OFC); in the indirect (transthalamic) pathway, PC projects to OFC via mediodorsal thalamus (MD). We hypothesized that in humans the indirect pathway is involved in attentional processing of odor. We used fMRI to measure brain activity from 12 participants during a selective attention task, in a mixed-event factorial design where attention to smells or auditory tones was independently manipulated, and used dynamic

causal modelling (DCM) techniques to examine attentional influences on the neuronal connections linking PC, MD, and OFC. Behaviorally, detection performance was similar in both tasks suggesting that task difficulty was matched. Random effects analysis comparing “odor attention” and “tone attention” blocks revealed that selective attention to odor involved neural activation in left anterior PC, and in left and right OFC. We observed that selective attention to odor (versus selective attention to tone) increased the connection strength from MD to OFC. Interestingly, this effect was seen in the absence of attention-dependent signal change in MD. Our results confirm that the transthalamic pathway is functionally active in humans, and may represent a modulatory target of olfactory attentional processing. We suggest that olfaction, like all other sensory modalities, requires a thalamic relay, if only to consciously analyze a smell.

Supported by NIDCD grant 1 K08 DC07653-01A1 (to J.G.) and Fyssen Foundation (to J.P.).

Poster Session Sat AM

A Nanotube-Based Electronic Nose

Reza Naima¹, Rehan Khan¹, Brad Johnson², Jean-Christophe Gabriel², Ying-Lan Chang², Qian Wang² and Noam Sobel^{1,3}

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We describe a nanotube field-effect transistor (FET) based electronic nose (e-nose). The e-nose was constructed from an array of FETs where the doped channel was replaced by a carbon nanotube random network, and the entire structure was coated with varying materials to alter their specificity. A programmable system controlled the flow rate and duration of “sniffs” over the sensor as the gate voltage was swept (0.2 Hz) from –10 to +10 V to compute an I-V curve which was then fit with a function so that the response to any stimulus was represented in a common parameter space. We tested the ability of the e-nose to identify the odors of 4 household items (coffee, tea, banana, vodka) after only minimal training. Each sniff consisted of 3 microsniffs: two 500 ms and one 3000 ms sniff, with 500 ms ISIs. During a sniff, ambient air at 8 l/min was pulled over the household items, and passed over 3 sensor integrated circuits (IC) arranged serially. Each sensor IC contained 10 FETs of various structures. Two of the sensor ICs were coated with varying materials, and one was uncoated. These initial measurements were used to build a discriminability function in the response parameter space. A further set of samples for each of the 4 odors was acquired using the same procedure and each sample was then classified using the derived discriminability function. The discriminability function correctly classified single “sniff” acquisitions more than 80% of the time for each of the 4 odors.

Poster Session Sat AM

The Dog Can Detect the Expiration-Odor of Cancer Patient

Yuji Satou¹ and Keiichi Tonosaki²

¹OJPC and ²Meikai University, School of Dentistry

Several researchers have reported that dogs are able to detect mole or tumours on the basis of odour. Recently, it is reported that dogs scent detection in lung and breast cancers. Since it is well known that the patient is scented unique odour, for example, diabetic patients are scented sweet body and sweet expiration odour. The ability of dogs to recognize and cross-match many kinds of odours are studied by electrophysiological and behavioral experiments. Here we show that dogs can sniff out the expiration odour of cancer patients. We trained 3 female black Labradors. The training objective was to enable the dogs to discriminate a cancer patient's expiration odour from the other 3 healthy men's. We used sampling gas bag which is made by Teflon with a Teflon one way tight bulb connector. We observed the dog's behavior and the dog's EEG from behind a partition, concealed from the dog and handler. The handler usually took an air from the prostate cancer's breath air bag with a 20 ml syringe. Then the air was puffed from the syringe to the nostrils of the dog about 10 s. The dog smelled it and stayed and waited until utter a command "look for." Then the dog searched and she was sitting beside the bag when she found out the same odor-bag among 4 bags. She has always rightly chosen it.

Poster Session Sat AM

Diagnosis of an Odor-Producing Genetic Disorder: Trimethylaminuria Using Salivary Analysis

Chris L. Whittle, Jason Eades and George Preti

Monell Chemical Senses Center

Trimethylaminuria (TMAU) is a metabolic disorder characterized by the inability of individuals to oxidize and convert trimethylamine (TMA) to trimethylamine N-oxide (TMAO) in the liver. This disorder results from a spectrum of changes in the gene that codes for the flavin-containing monooxygenase enzyme 3 (FMO3). Malodorous TMA is formed in the gut by bacterial metabolism. In normal individuals, TMA is converted/oxidized to TMAO at >95% efficiency. TMAO is non-odorous and readily excreted in the urine. Individuals suffering from TMAU have a reduced capacity to oxidize TMA to TMAO. The inability to efficiently oxidize TMA results in the sporadic production of a body odor that is perceived as foul, unpleasant, and in its most extreme cases fish-like. This odor is caused by excess, unoxidized TMA present in the systematic system that is excreted in urine, sweat, breath, and saliva. We hypothesized that individuals positive for TMAU would have elevated levels of salivary trimethylamine in contrast with individuals who did not have the disorder. Stimulated saliva samples were collected and acidified from individuals during a 10-min period. Samples were analyzed by solid-phase microextraction-gas chromatography/mass spectrometry. Individuals that tested positive for TMAU were found to have greater concentrations of TMA present in the saliva. However, the levels appear to vary depending upon expression of the disorder.

This research was supported, in part, by NIH institutional training grant 2T32DC00014.

Poster Session Sat AM

Folate Chemoreceptor and Lipid Rafts in *Paramecium*

Y. Pan, S.D. Weeraratne, J. Yano and J.L. Van Houten

University of Vermont

Paramecium tetraurelia is attracted to folate, but the folate receptor has been difficult to solubilize, probably because of its association with surface membrane microdomains. We identified candidate sequences for folate chemoreceptors and a transporter using the annotated *Paramecium* genome. We used RNAi to down regulate the candidate mRNAs and found one that qualified as a folate receptor, that is, RNAi eliminated response to folate while responses to other stimuli were normal. RNAi for the transporter had no effect on chemoresponse to folate. The folate receptor is a GPI anchored protein. Antisera against a GST fusion peptide recognizes a 30 kDa band among the GPI anchored protein, and shows surface expression of the protein on the cell surface, but not cilia. The GPI anchor of the folate receptor should allow it to associate with lipid rafts and other signaling molecules in the plasma membrane. Solubility studies and sucrose gradients of membrane fractions show that the folate chemoreceptor is in membrane rafts. Methyl-beta-cyclodextrin (MBCD) reduces sterols in the *Paramecium* membrane and dramatically disrupts the distribution of 2 surface proteins on the cell surface and where we find them in lipid rafts fractions, the plasma membrane calcium pump 2 and GPI anchored surface antigens. However, the folate receptor appears not to be affected by MBCD in its association with the Triton X-100 insoluble pellet or in its distribution on the cell surface. The folate receptor appears to differ from other membrane proteins in its raft association.

Supported by R01 DC 00721, R01 GM 59988.

Poster Session Sat AM

NMDA-Receptor-Like protein in *Paramecium* Chemoresponse to Glutamate

C. Jacobs, S.D. Weeraratne, J. Yano and J.L. Van Houten

University of Vermont

Paramecium tetraurelia is attracted to glutamate. Through analysis of the annotated *Paramecium* genome, we identified several candidate glutamate receptors, all of which show homology to NMDA-receptor-like proteins. Using RNAi, we have identified one that fits criteria for a receptor because RNAi reduces chemotaxis to glutamate only among other stimuli. We have confidence in these results since RNAi for other sequences has no effect on glutamate chemoresponse, implying that there are several glutamate binding proteins in *P. tetraurelia*, only one of which functions as a surface chemoreceptor. We are using a GFP-fusion protein to localize the receptor on the cell using deconvolution microscopy. The GFP-fusion protein traffics to the cell surface where it is found on cilia and along ridges on the cell body surface. The sequence of the *P. tetraurelia* glutamate receptor (pGluR1) shows the potential for 7 transmembrane spans, as do the other species' NMDA-receptor-like proteins, which are thought to function as ion channels. Previously we established that a kinase (probably PKA) and cAMP function in the glutamate chemosensory signal transduction pathway. Consequently, we explored whether RNAi for a G protein beta subunit would affect glutamate chemoresponse, and found that it inhibits response to glutamate. We are determining the specificity of the G-beta RNAi effect on chemoresponse.

Supported by NIH R01 DC 00721 and NIH R01 GM 59988.

Poster Session Sat AM

The Effect of Periodic Input on Antennal and Antennal Lobe Responses in the Moth *Manduca sexta*

Shreejoy Tripathy¹, Oakland Peters² and Kevin Daly²

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Biomechanical studies of the insect wing beat suggest that each down stroke periodically increases axial airflow over the antennae; this could be sufficient to drive penetration of odorants through the sensillar array providing a temporal pattern to input. However, there is no physiological data establishing that the antenna or antennal lobe (AL) can respond to odor stimulation on this timescale. To address this, we first measured the responsiveness of the antenna to odor stimulation at frequencies around the wing beat in the moth *Manduca sexta*, using electroantennograms (EAG). We varied pulse frequency, duty cycle and airflow velocity. Using power spectral analysis we show that the antenna responds to periodic stimulation up to but not beyond the wing beat (20–25 Hz); this was true of both odor stimuli and a blank control. EAGs were always stronger in response to odor suggesting both mechano and olfactory receptor aspects to the response. Furthermore, EAGs tracked pulses with odor:air duty cycles up to 10:40 ms. Responses faded when the overall flow velocity was lowered to a range of 5–30 cm/s. Using multi-unit and local field recordings, we then varied stimulus frequency in both odor and blank control conditions. Power spectral analysis established that AL neurons and the local field will track odor pulsed at the wing beat frequency only in the presence of odor. These results suggest that periodic changes in airflow velocity caused by the wing beat could drive a periodic olfactory input in the AL.

Support: NIH-NCRR RR015574 to K.D.

Poster Session Sat AM

OMP Mechanism of Action: A Model

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The function of OMP has been an enigma since its discovery. Although OMP was reported to enhance mitosis of OSN precursors, it was the generation and characterization of the OMP-KO mouse that provided insights to OMP function. The OMP-KO mouse has a slowed EOG, deficits in odor detection and quality perception, altered innervation of the olfactory bulb, delayed efflux of stimulus-induced Ca²⁺ entry, and slowed odor-induced onset and offset kinetics. These observations implicate OMP in olfactory sensory transduction. However, its mechanism(s) of action has remained elusive. It is known that a common and critical participant at most steps of olfactory signal transduction is the calcium sensor calmodulin (CaM). We hypothesize that OMP, in concert with its partner protein Bex, regulates the participation and targeting of CaCaM to multiple effectors in the olfactory sensory neuron signal transduction cascade. We propose an OMP/Bex/CaM model of protein-protein interaction to explain the diverse phenotypes of the OMP-KO mouse. This novel model is supported by several exper-

imental observations and will have broad impact on understanding CaM regulation of olfactory signal transduction mechanisms.

Supported by NIH DC 03112 and DC03904.

Poster Session Sat AM

IP3 Receptors Play a Critical Role in the Secretion of Olfactory Mucosal Proteins

Nanaho Fukuda and Katsuhiko Mikoshiba

RIKEN Brain Science Institute

IP3 receptors (IP3Rs) are Ca²⁺ channels that release calcium from endoplasmic reticulum to the cytoplasm in response to the second messenger, IP3. Although calcium signalings via IP3Rs have been shown to play important roles in various tissues, their physiological role in the mammalian olfactory system remained unclear. In this study, we found that IP3Rs play a critical role in the mouse olfactory tissue as key components of the olfactory mucus secretion. In situ hybridization and immunohistochemical studies showed that 2 subtypes of IP3Rs are expressed in the anterior gland of nasal septum (AGNS) and lateral nasal gland (LNG). Examination of the LNG acinar cells isolated from wild type and IP3R knockout (KO) mice revealed that cholinergic stimulation cause calcium increase in LNG acinar cells via IP3Rs, leading to secretion of the olfactory mucosal proteins. Acetylcholine-induced calcium increase and secretion were severely diminished in the 2 subtypes of IP3Rs-double KO mice. MAS-spectrometry analysis suggested that various members of lipocalin family including odorant binding proteins (OBPs) are secreted from LNG by cholinergic stimulation. Finally, we found impaired odorant perception and tissue degeneration in the IP3Rs-double KO mice. Taken together, these results suggest that the IP3Rs play physiological roles in the odorant perception and the olfactory tissue maintenance by mediating the secretion of mucosal proteins from the nasal glands.

Poster Session Sat AM

A Specific Heat Shock Protein Enhances the Expression of Mammalian Olfactory Receptor Proteins

Lian Gelis, Anastasia Mashukova, Weiyi Zhang, Jon Barbour, Hanns Hatt and Eva M. Neuhaus

Ruhr-University Bochum

Multiple trials failed to express significant amounts of olfactory receptors in heterologous cells, as they are typically retained in the endoplasmic reticulum. Evidence is accumulating that cell-type-specific accessory proteins regulate the folding of olfactory receptors, their exit from the endoplasmic reticulum, and the trafficking to the plasma membrane of the olfactory cilia where the receptors gain access to odorants. We found Hsc70t, a testis-enriched variant of the Hsp70 family of heat shock proteins which is specifically expressed in post-meiotic germ cells, in the olfactory epithelium of mouse and human. Co-transfected HEK293 cells with Hsc70t and different GFP-tagged odorant receptors from mouse and man showed a significantly enhanced odorant receptor expression. Hsc70t expression also changed the amount of cells functionally expressing olfactory receptors at the cell surface, as the number of cells responding to odorants in Ca²⁺ imaging experiments significantly increased. Our results show that Hsc70t helps

expression of odorant receptors in heterologous cell systems and helped the characterization of an “orphan” human olfactory receptor.

Workshop: Genomics Approaches to Study Chemosensory Receptors

Evolution of Vertebrate T1R and T2R Taste Receptor Genes

Jianzhi Zhang

Department of Ecology and Evolutionary Biology, University of Michigan, USA

Taste reception is fundamental to diet selection in many animals. I report comparative genomic analysis of T1R and T2R taste receptor genes from 9 vertebrates, including 3 fishes, 1 amphibian, 1 bird, and 4 mammals. The results show that orthologous T1R sequences are relatively conserved in evolution and that the T1R gene repertoire remains virtually constant in size across most vertebrates, except for the loss of the T1R2 sweet receptor gene in the sweet-insensitive chicken and the absence of all T1R genes in the tongueless western clawed frog. In contrast, orthologous T2R sequences are more variable, and the T2R repertoire diverges tremendously among species, from only 3 functional genes in the chicken to 49 in the frog. Although the rate of gene duplication is much lower in T1Rs than in T2Rs, signals of positive selection are detected during the functional divergences of paralogous T1Rs, as was previously found among paralogous T2Rs. Thus, functional divergence and specialization of taste receptors generally occurred via adaptive evolution.

Workshop: Genomics Approaches to Study Chemosensory Receptors

Olfactory Receptor Genomics: Ancient Roots and Recent Demise

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Olfactory receptors (ORs) that mediate smell perception constitute the largest gene family in the vertebrate genome. This remarkable gene family has evolved towards expanding and diversifying the OR repertoire of different species to meet their individual evolutionary needs. Interestingly, in human and other apes this process has been reversed in the last 10–20 million years resulting in massive accumulation of pseudogenes. We employ various genomics analyses to explore the ancient root and recent demise of this extraordinary gene family in multiple organisms. Elucidation of the complete olfactory sub-genome in human, opossum and platypus (<http://bioportal.weizmann.ac.il/HORDE>) revealed species-specific expansions of OR gene families, implying different importance of receptor subtypes in these species. Whole genome comparison of the ORs genomic organization in 5 mammals revealed that 90% of these genes are disposed in multi-species conserved clusters (CLICs). This indicates that most mammalian OR gene clusters have a common ancestral origin. To study the extent of the OR genes diminution in human we developed a probabilistic Classifier for Olfactory Receptor Pseudogenes (CORP). Using this tool, we predict that ~70% of human OR genes encode non-functional pseudogenes, a much higher num-

ber than hitherto suspected. Our studies shed new light on the range of evolutionary pathways of OR genes in multiple species.

Workshop: Genomics Approaches to Study Chemosensory Receptors

Inter-species Differences in Olfactory and Vomeronasal Receptor Gene Families

Janet Young¹, Hillary Massa¹, Leo Goodstadt², Chris Ponting² and Barbara Trask¹

¹Fred Hutchinson Cancer Research Center and ²University of Oxford

We and others have used bioinformatic tools to identify members of the olfactory receptor (OR) and vomeronasal receptor (V1R and V2R) gene families from various sequenced mammalian genomes. We find only ~10–20 V2R pseudogenes in each of the dog, cow, human, chimpanzee, and macaque genomes and no intact V2Rs. This contrasts with the 87–123 intact V2Rs present in the most recent rat, mouse and opossum genome assemblies. Numerous post-speciation gene duplications led to “private” V2R repertoires in each of these 3 species. We previously found that only ~9 intact V1Rs remain in dog. We hypothesized that loss of V1Rs from the dog genome might have occurred during domestication, because docile, obedient animals could have been strongly favored during breeding. However, by sequencing wolf DNA we found that for 5 of 5 V1R pseudogenes examined (those with fewest inactivating mutations), inactivation preceded wolf-dog divergence. We find that about 1/3 of ORs inferred in the mouse-rat ancestor experienced subsequent duplication in one or both genomes, about 1/3 remain as one-to-one orthologs, and the remainder have been deleted in one of the 2 genomes. ORs that are the product of recent duplication evolve at a faster rate than those that remain as one-to-one orthologs, as expected if duplication events are followed by acquisition of novel function. Some OR subfamilies evolve faster than others, experiencing more recent duplication events and tolerating more sequence changes.

Workshop: Genomics Approaches to Study Chemosensory Receptors

Characterizing the Expression of Human Olfactory Receptor Genes Using a Novel DNA Microarray

Yoav Gilad

University of Chicago

Olfactory receptor (OR) genes were discovered more than a decade ago, when Buck and Axel observed that, in rats, certain G-protein coupled receptors are expressed exclusively in the olfactory epithelium. Subsequently, protein sequence similarity was used to identify entire OR gene repertoires of a number of mammalian species, but only in mouse were these predictions followed up by expression studies in olfactory epithelium. To rectify this, we have developed a DNA microarray that contains probes for most predicted human OR loci and used that array to examine OR gene expression profiles in olfactory epithelium tissues from 3 individuals. We detected expression of 437 (76%) human OR genes in these olfactory epithelia, thereby corroborating their functional annotation as odorant receptors. Interestingly, we detected widespread expression of OR pseudogenes, an observation that may shed light on the mechanism of OR gene choice in the olfactory sensory neurons. To address the hypothesis that OR genes may carry out additional

functions, we also characterized the expression of OR genes in a number of non-olfactory tissues. We find that a large number of putative human OR genes are expressed in non-olfactory tissues, sometimes exclusively so, casting doubt on their current functional annotation.

Presidential Symposium

Watching Neurons in Fluorescent Mice

Joshua Sanes, Jean Livet and Jeff Lichtman

Department of Molecular and Cell Biology, Center for Brain Science, Harvard University, USA

The green fluorescent protein (GFP) has revolutionized biology in general and neurobiology in particular. Transgenic mice in which neurons express GFP enable high-resolution time-lapse imaging of axonal and dendritic arbors in live mice over time scales that range from seconds to months. By expressing spectral variants of GFP (XFPs) in distinct subsets of neurons, it becomes possible to image multiple neuronal types, and the connections among them, in a single animal. Over 100 different colors can be distinguished in newly generated fluorescent mice called “Brainbow,” allowing reconstruction of 100 s of neurons in a small region of the brain.

Presidential Symposium

Multimodal Fast Optical Interrogation of Neural Circuits

Karl Deisseroth

Department of Bioengineering, Stanford University, USA

Our understanding of the genetic and cellular underpinnings of systems-level neural processes like action, thought, and emotion is limited by the availability of tools to interrogate specific classes of cells within neural tissue. To enable precise perturbation of living circuits, we recently developed a technology for genetically-targeted, millisecond-timescale optical excitation of neurons employing an algal molecule channelrhodopsin-2 (ChR2). ChR2 as a method of controlling neurons works well together both with GFP as a method for visualizing neurons, and with fura-2 calcium imaging as a method of listening to neurons. Together these 3 probes form a complete and complementary optogenetic system for multimodal, high-speed, genetically-targeted, all-optical interrogation of living neural circuits.

Presidential Symposium

Seeing What the Nose Tells the Brain: Using Optical Probes in Olfaction

Matt Wachowiak

Department of Biology, Boston University, USA

Genetically-encoded optical probes hold great promise as tools for investigating brain function. The olfactory system—and the olfactory bulb in particular—is among the most amenable of all neural systems to the use of these tools. We have used one such probe—synaptopHluorin (SpH)—to investigate odor coding and processing in the mouse olfactory bulb. Mice expressing SpH in olfactory receptor neurons (generated by T. Bozza and P. Mombaerts) are

now commercially available, and are powerful tools for studying olfaction. SpH allows receptor input to the olfactory bulb to be visualized/in vivo/with a spatial resolution and sensitivity equivalent to that of synthetic calcium indicators. Odor representations can be imaged repeatedly in the same animal for months. SpH can also serve as a rapid and linear reporter of transmitter release from receptor neurons, allowing us to ask how olfactory input to the brain is modulated. Finally, used in combination with synthetic or genetically-encoded calcium indicators targeted to specific postsynaptic cell classes, it should be possible to directly visualize the transformation of odor codes at successive processing stages.

Poster Session Sat PM

Effects of Mitochondrial Ca²⁺ Transport on Ca²⁺ Responses in Taste Cells

Kyle Hacker and Kathryn Medler

University at Buffalo

Ca²⁺ is an important second messenger in the transduction of taste stimuli since a primary response to taste stimuli is an increase in cytosolic Ca²⁺ levels. Taste cells use 2 different mechanisms to increase cytosolic Ca²⁺ levels. One of these mechanisms is Ca²⁺ influx through voltage gated Ca²⁺ channels (VGCCs) while the other is Ca²⁺ release from internal stores followed by Ca²⁺ influx through store operated channels. Since an increase in [Ca²⁺]_i represents the final step in taste transduction, the amplitude and duration of this response has an impact on the signal output of the taste cell. Ca²⁺ signals are determined by 2 things: the source of the Ca²⁺ increase and the signal shut off mechanisms. As a result, Ca²⁺ buffering or “shutting off” impacts the signal used by taste cells to communicate with the brain. Mitochondrial Ca²⁺ transport is an important Ca²⁺ buffering mechanism in many cell types. Using Ca²⁺ imaging, we have determined that mitochondria are also important regulators of Ca²⁺ signals in taste cells. Disrupting the mitochondria’s ability to buffer Ca²⁺ in taste cells has a significant impact on the amplitude and duration of the evoked Ca²⁺ response. Mitochondria affected Ca²⁺ responses to both cell depolarization by 55 mM KCl and to a bitter mixture of denatonium (10 mM) and cycloheximide (0.5 mM). These data demonstrate that Ca²⁺ buffers have a significant contribution to the formation of taste signals.

Work supported by DC006358.

Poster Session Sat PM

Are Type III Taste Cells Normal in P2X2/P2X3 Double Knockout Mice?

Leslie Stone-Roy, Tod Clapp and Sue Kinnamon

Colorado State University

Taste buds use ATP to communicate with gustatory fibers and this communication is dependent upon the presence of P2X2 and P2X3 ionotropic ATP receptors. Mice lacking these receptors (P2X2/X3 DKO) are unable to detect certain tastants although the exact signaling mechanism of ATP is unclear. Taste buds comprise a heterogeneous population of cells. Type II cells, which contain the transduction machinery for bitter, sweet and umami transduction lack classical chemical synapses which instead are found associated

with type III taste cells. This suggests that upon stimulation, type II cells may communicate with type III cells, which then relay the signal to the nervous system. The inability of P2X2/X3 DKO animals to detect bitter, sweet and umami tastes thus may reflect an inability of type II cells to communicate effectively with type III cells. In fact, type III cells may be abnormal or absent in P2X2/X3 DKO animals. To test this hypothesis, we used immunocytochemistry and calcium imaging to identify and evaluate type III taste cells in P2X2/X3 DKO mice. We found that type III cells are present in the double knockout animals, and that these cells express immunoreactivity to SNAP-25 and NCAM. Further, these cells respond to K^+ -mediated depolarization with increases in intracellular Ca^{2+} , suggesting that they possess voltage-gated Ca^{2+} channels. We conclude that the lack of responses to bitter, sweet and umami taste stimuli in the DKO mice is not due to a defect in the type III taste cells.

Supported by NIH grant DC007495-02.

Poster Session Sat PM

The Sour Taste Receptor, PKD2L1, Is Expressed by Type III Taste Cells in the Mouse

Shinji Kataoka¹, Anne Hansen¹, Yoshiro Ishimaru², Hiroaki Matsunami² and Thomas Finger¹

¹University of Colorado Medical School and ²Duke University Medical Center

Mammalian taste buds comprise 3 distinct morphological types of cells (type I, type II and type III) which can be identified by distinct ultrastructural and immunohistochemical features. Different type II cells express the T1R and T2R families of G-protein-coupled taste receptors for sweet, umami and bitter tastants. Recently, polycystic-kidney disease-like ion channel, PKD2L1, has been reported as a likely candidate for a mammalian sour taste receptor based on molecular biological and functional methods. These investigators report that PKD2L1 is expressed in a subset of taste cells different from those expressing either T1R or T2R receptors. To examine which taste cell type expresses this sour receptor, double immunolabeling was performed using NTPDase2 as a marker for type I cells and PLC β 2 as a marker for type II cells. PKD2L1 was not co-expressed with either type I or type II markers. Serotonin, PGP 9.5 and NCAM all have been described as being present in morphologically defined type III taste cells. PKD2L1 was co-expressed with each of these type III markers. Further investigation by immunoelectron microscopy is underway to determine in detail the morphological distribution of this sour receptor.

Support: NIH grants to T.F. and H.M.

Poster Session Sat PM

Responses of Mouse Fungiform Taste Cells with Action Potentials to Glutamate

Yoshihiro Murata, Ryusuke Yoshida, Toshiaki Yasuo, Keiko Yasumatsu, Noriatsu Shigemura and Yuzo Ninomiya

Kyushu University

Glutamate is believed to elicit a unique taste perception known as umami, which is distinct from sweet, bitter, salty and sour. Glutamate is also one of the transmitter candidates for cell communica-

tion in taste buds. To examine the dual functions of glutamate in the response of taste cells, we recorded action potentials from single taste cells when glutamate is apically or basolaterally applied to the taste buds isolated from mouse fungiform papillae. The apical application of 100–300 mM monosodium glutamate (MSG) induced an increase in firing frequency in a subset of the taste cells. The response characteristics of these cells were classified into 4 categories by best stimulus and synergistic response with 5'-inosine monophosphate: MSG-best with (M1) or without (M2) synergism, sweetener-best with (S1) or without (S2) synergism. The basolateral application of <1 mM glutamate induced an increase or a decrease in spontaneous spike frequency in some taste cells responding to apical MSG, suggesting that basolateral glutamate modulates the basal activity of MSG-sensitive taste cells. Our results support the hypothesis that glutamate functions both as a umami compound and as a modulator of taste cell activity.

Supported by JSPS Grants-in-Aid 18077004, 18109013 (Y.N.) and 17791325 (R.Y.).

Poster Session Sat PM

Responses of Taste Receptor Cells and Presynaptic Taste Cells to Taste Stimuli

Seth M. Tomchik, Craig D. Roberts, Elizabeth Pereira, Robert Stimac and Stephen D. Roper

University of Miami Miller School of Medicine

A major controversy in taste research has been whether individual taste cells respond to a single taste quality (bitter, sweet, umami, sour, or salty) or are broadly tuned to multiple qualities. Taste receptor cells (TRCs; type II) express receptors for only one taste quality, yet physiological studies indicate that some taste cells are broadly tuned to multiple stimulus qualities. Taste cells communicate with each other via paracrine signaling within the taste bud and recent data show that TRCs activate presynaptic cells (type III) through purinergic signaling. Presynaptic cells may receive converging input from multiple TRCs, and could therefore be more broadly tuned than individual TRCs. We tested this hypothesis using calcium imaging in a lingual slice preparation. Responses of individual taste cells were recorded using bitter, sweet, umami, sour, and salty tastants and cells were identified as either TRCs (PLC β 2-expressing) or presynaptic cells (SNAP-25 expressing) following functional imaging. We found that TRCs were narrowly tuned; 80% were sensitive to only one taste quality: bitter, sweet, or umami. However, presynaptic cells were broadly tuned; 83% responded to 2 or more stimulus qualities. Presynaptic cells responded to bitter, sweet, and umami tastants, as well as sour and salty tastants. Thus, cell-cell signaling within the taste bud may shape the signal output in ways not predicted by receptor expression patterns.

Support: R01DC000374/5R01DC007630.

Poster Session Sat PM

Response Properties of Mouse Taste Receptor Cells within a Single Taste Bud of Fungiform Papillae

Ryusuke Yoshida, Yoshihiro Murata, Keiko Yasumatsu, Noriatsu Shigemura and Yuzo Ninomiya

Kyushu University

Each taste bud comprises a group of 50–150 heterogeneous cells, such as receptor cells, supporting cells and precursor cells. Among these cells, we have recently shown that taste receptor cells generating action potentials are the cells that transmit major taste information to gustatory nerve fibers. The plural numbers of taste receptor cells with action potentials may exist in a single taste bud. However, it is unclear whether these cells in a bud would possess different taste selectivity or they would share the same taste selectivity. In this study, therefore, by using a loose patch recording technique, we recorded action potentials from 2 different taste cells within a single taste bud separately or simultaneously, and compared their taste selectivity to 4 conventional taste stimuli. In some cases, 2 taste cells in a bud responded to the same class of taste stimuli, whereas in other cases they did to different classes of taste stimuli, such as saccharin versus quinine, saccharin versus NaCl, etc. These results suggest that a single taste bud may contain the plural numbers of taste receptor cells having similar and different taste responsiveness.

Supported by JSPS Grants-in-Aid 18077004, 18109013 (Y.N.) and 17791325 (R.Y.).

Poster Session Sat PM

Voltage-Dependent Potassium Channels Expressed in Taste Buds

Makoto Ohmoto, Ichiro Matsumoto, Takumi Misaka and Keiko Abe

The University of Tokyo

The molecular mechanisms regulating membrane potentials of taste receptor cells (TRCs) are not well-defined. In this study, we searched for molecules playing a role in regulating membrane potentials of TRCs by comparing DNA microarray data on 3 different tissues with one another; the tissues were taste buds isolated from circumvallate papillae, papillal epithelia after removal of the taste buds, and non-papillal epithelia. Subsequent *in situ* hybridization analysis revealed that 2 voltage-dependent potassium channels, KCNQ1 and KCNH2, were expressed exclusively in the taste buds. By double staining experiments, we analyzed the types of cells expressing the channels in taste buds. The signals of KCNQ1 included those of TRPM5 expressed in the sweet, umami and bitter TRCs. Recently identified candidate sour taste receptor as well was expressed in a subset of KCNQ1-expressing cells. On the other hand, KCNH2 was expressed in a subset of TRPM5-expressing cells and the signals of KCNH2 were partially overlapped with those of TIR3 and Ggust. The BrdU-labeling analysis revealed that KCNH2 was expressed only in young TRCs. These results suggest that KCNQ1 plays a common role in TRCs, and that KCNH2 functions in a cell age-dependent manner in TRCs expressing GPCR-type taste receptors.

This work was supported in part by Grants-in-Aid for Scientific Research (16688006 to I.M., 16108004 to K.A.) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

Poster Session Sat PM

Arachidonic Acid Influences Electrical Excitability of Taste Receptor Cells

Fang-li Zhao and Scott Herness

The Ohio State University

Recently the lipid signaling molecule arachidonic acid (AA) has been suggested as a potential regulatory molecule in taste transduction. For example, phospholipase A₂ expression has been reported in a subset of taste receptor cells (TRCs) and AA has been demonstrated to influence potassium currents and, in heterologous systems, TRPM5 currents. In the present study we investigate a role of AA in rat posterior TRCs using pharmacology with patch clamp recordings. Application of AA (10 μM, 50 μM) significantly inhibited outward potassium currents in almost all tested cells. Additionally, some enhancement of a heterogeneous inward current was also noted. Recovery, as expected for a lipid signaling molecule, was slow but significantly enhanced with addition of bovine serum albumin (BSA), which can act as an AA scavenger. To test if AA could modulate taste stimuli, caffeine, SC45627, and cycloheximide were tested. R59022, an inhibitor of DAG kinase that increases DAG levels (a source of AA), acted to enhance the responses of all 3 stimuli as well as responses to neuropeptides NPY, CCK, and SOM. On the other hand, application of OAG, a DAG analogue that does not produce AA, was ineffective in modifying these currents. RHC80267, an inhibitor of DAG lipase, which produces AA from DAG, reduced caffeine responses but had not only mild effects on SC45627 or CHX. Collectively, these data suggest that liberation of AA can influence electrical excitability of taste receptor cells via its actions on potassium currents.

Supported by NIDCD DC00401.

Poster Session Sat PM

Norepinephrine Uptake But Not Synthesis in Mouse Taste Buds

Gennady Dvoryanchikov, Seth M. Tomchik and Nirupa Chaudhari

University of Miami Miller School of Medicine

Norepinephrine (NE) is proposed to play a modulatory role in taste, based on its inhibition of K currents in taste cells and the ability of NE reuptake inhibitors to affect taste thresholds. We looked for tyrosine hydroxylase (TH) and dopamine β-hydroxylase (DBH), enzymes necessary for NE synthesis. By RT-PCR, we detected expression of TH but not DBH in taste buds. Neither TH nor DBH was found in taste cells by immunofluorescence. In contrast, using RT-PCR and qRT-PCR, we detected the plasma membrane NE transporter (NET). NET immunoreactivity was evident in nerve fibers penetrating taste and non-taste epithelium. Within the taste bud, NET is expressed in some presynaptic (type III) and type I cells but not in receptor (type II) cells. Using enzyme immunoassay, we found that taste buds specifically uptake NE when incubated in 10 μM NE. This uptake was blocked by the specific NET inhibitors, desipramine and nisoxetine. Further, proteins involved in inactivating (catechol-o-methyltransferase, COMT; monoamine oxidase, MAO-A) and packaging (vesicular monoamine transporter, VMAT1,2; and chromogranin, ChrgA) NE are also expressed in taste buds. Within the taste bud, ChrgA, associated with NE-containing vesicles, is found only in type III cells. Hence, we postulate that in taste epithelium, only nerve fibers synthesize NE; both types I and III cells may uptake and degrade NE released from nerves; type III cells may also repackage and secrete this NE.

Supported by NIH/NIDCD R01DC6308 to N.C. and T32NS7044 (S.M.T.).

Poster Session Sat PM

Inflammation-Stimulated Signal Transduction Pathways in Taste Bud Cells

Hong Wang, Minliang Zhou, Joseph Brand and Liquan Huang

Monell Chemical Senses Center

Recent studies in the molecular biology of taste have identified a number of taste signaling molecules. However, little is known about the molecular mechanisms underlying taste disorders. Infection-induced inflammation is believed to be one of the possible causes, and oral intake of one of the inflammation-induced cytokines, interferon (IFN- α), can distort taste sensations. In our endeavor to isolate taste bud cell-specific genes, we discovered the expression of cDNAs that encode proteins of the interferon-mediated signaling pathways, which prompted us to more thoroughly investigate the possible effect of inflammation on taste tissue. RT-PCR, in situ hybridization and immunocytochemistry studies showed that IFN-mediated signaling pathways are present in taste buds, and particularly that the IFN- γ receptor subunit IFNGR1 is coexpressed with the taste bud cell type markers: α -gustducin and NCAM, respectively. Incubation of gustatory lingual epithelia with the recombinant interferons activated these signaling pathways, resulting in the phosphorylation of the downstream transduction components. Intraperitoneal injection of lipopolysaccharide and poly [rI-rC], mimicking bacterial and viral infections, activated the IFN signaling pathways in mouse taste papillae and subsequently altered gene expression patterns in taste bud cells. Taken together, these findings suggest that bacterial and viral infection-induced IFNs can directly affect taste bud cells' physiology, leading to certain types of taste disorders.

Supported by NIH grants.

Poster Session Sat PM

Taste Cells Secrete ATP via Pannexin 1 Hemichannels

Yutaka Maruyama, Yi-Jen Huang, Elizabeth Pereira, Nirupa Chaudhari and Stephen D. Roper

University of Miami

Taste receptor cells (Type II cells) express taste receptors (T1Rs, T2Rs) and downstream effectors (e.g., PLC β 2) but do not possess ultrastructural features of conventional synapses. ATP has been shown to be a neurotransmitter in taste buds and we have shown that taste receptor cells are specifically responsible for its release. However, it is not yet known how receptor cells secrete ATP, especially given that they do not form synapses. Recently, it has been reported that the gap junction protein pannexin-1 (Px1) forms ATP-permeable hemichannels in the plasma membrane of erythrocytes. Furthermore, Px1 channels are gated open by increase of [Ca²⁺]_i. We hypothesized that gap junction proteins, and specifically Px1, may also mediate ATP release in taste cells. We tested this by investigating the ability of the fluorescent dye, carboxyfluorescein, to pass through pannexin hemichannels in CHO cells expressing Px1 and in isolated mouse taste cells. Dye uptake was significantly

increased by inducing intracellular Ca²⁺ release in Px1-expressing CHO cells and in taste cells. Carbenoxolone, a Px1 junction blocker, significantly diminished stimulus-evoked dye-uptake without affecting baseline uptake. These results are consistent with tastant-induced ATP via Px1 hemichannels.

Supported by NIH/NIDCD R01DC006308 (N.C.) and R01DC007630 (S.D.R.).

Poster Session Sat PM

The Role of Pannexin 1 Hemichannels in ATP Release from Mouse Taste Receptor Cells

Y. Anthony Huang, Yutaka Maruyama, Guennadi Dvorientchikov, Elizabeth Pereira, Nirupa Chaudhari and Stephen Roper

Miller School of Medicine, University of Miami

Taste stimulation causes taste buds to release serotonin (5HT) and ATP. We previously have shown that taste receptor (type II) cells secrete ATP, and presynaptic (type III) cells release 5HT. However, transmitter release mechanisms were not explored. Understanding how transmitter is released is particularly important for taste receptor cells (type II cells) because those cells lack morphological features of synapses such as presynaptic vesicle clusters and membrane densities. We used CHO cells expressing P_{2X} receptors as biosensors to monitor ATP secretion from individual taste buds and isolated taste receptor cells. A mixture of tastants (saccharin, 2 mM; SC45647, 100 μ M; cycloheximide, 10 μ M; and denatonium, 1 mM) elicited ATP secretion from individual taste buds and isolated taste receptor cells. Carbenoxolone (5 μ M), a selective blocker of pannexin 1 (Px1) hemichannels at this low concentration, reversibly blocked taste-evoked ATP secretion. Single cell RT-PCR indicated that taste receptor cells express Px1 mRNA. Double immunostaining showed that taste receptor cells that express PLC β 2 also express Px1. Our data indicate that gustatory stimulation causes taste receptor cells to secrete ATP via Px1 hemichannels.

Supported by NIH/NIDCD R01DC006308 (N.C.) and R01DC007630 (S.R.).

Poster Session Sat PM

Co-expression Patterns of SNAP-25 with Neuropeptides, GAD, and NCAM Suggest Its Expression in Multiple Cell Types

Scott Herness, Paul El Dahdah, Tamara Kolli and Yu Cao

The Ohio State University

It is now thought that many taste receptor cells (TRCs), both with and without neural synapses, participate in cell to cell communication using neurotransmitters and neuropeptides. However, some studies suggest that SNAP-25, a protein important in transmitter vesicular release, is expressed only TRCs with neuronal synapses. Here we explore co-expression patterns of SNAP-25 with signaling molecules using immunocytochemical methods in rat taste buds. TRCs expressing cholecystokinin and neuropeptide Y, which co-express gustducin and are thought to lack neuronal synapses, demonstrate significant co-expression with SNAP-25. Additionally, TRCs expressing GAD, a marker for GABAergic TRCs which also

show large gustducin overlap, strongly co-express SNAP-25. Forty percent of SNAP-25 cells double label with GAD whereas about 2/3s of GAD cells overlap with SNAP-25. We next examined expression patterns of SNAP-25 with NCAM since NCAM is thought to be expressed only in synapse forming TRCs. Double labeled cells for SNAP-25 and NCAM were observed as expected. However, many TRCs expressed SNAP-25 but not NCAM or NCAM without SNAP-25. These results place SNAP-25 expression in both TRCs with and without neuronal synapses. They also suggest TRCs expressing signaling agents such as neurotransmitters and neuropeptides may express molecular machinery involved vesicular release as well as reinforce the notion that TRCs may utilize non-traditional mechanisms of signaling molecule release.

Supported by DC00401.

Poster Session Sat PM

Concentration-Dependent Effects of Shh and Agonist on Taste Papilla Formation

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Sonic hedgehog (Shh), a potent morphogen, is progressively restricted to taste papilla placodes and papillae in embryonic rodent tongue and has demonstrated roles in regulating fungiform papilla pattern and number. When Shh signaling is disrupted with the alkaloid, cyclopamine (CYCL) or with the Shh blocking antibody, 5E1, fungiform papillae form in doubled numbers and atypical posterior locations in tongue cultures. Therefore some investigators regard Shh as inhibiting papilla formation. Others have proposed models for Shh roles in both papilla induction and suppression. We used embryonic rat tongue cultures from gestational day 14 to assess potential concentration-dependent effects of Shh (R&D Systems, Minneapolis, MN) or a Hh agonist, Ag 1.10 (Curis Inc., Cambridge, MA), added to culture medium. At high concentration (10 µg/ml Shh; 100 nM Ag) fungiform papilla numbers were reduced by >50% relative to controls; in contrast, at low concentration (0.1 µg/ml; 0.1 nM) papillae were increased by >25%. Implanted beads soaked with Shh (5 mg/ml) in the anterior tongue induced disruption of the tongue epithelium and novel papilla formations. A potent analog of CYCL, KAAD-cyclopamine, induced supernumerary fungiform papillae on anterior and posterior tongue and altered circumvallate papilla morphology, with concentration dependence. Interpretation of Shh signaling in papilla formation and pattern must incorporate peptide concentration as a key variable. Shh effects in papilla formation not only are stage and tissue specific but also reflect Shh activity gradients.

Supported by NIH NIDCD Grant DC00456 to C.M.

Poster Session Sat PM

Bitterness of Iso-alpha-acids Is Localized to Posterior Oral Cavity and Is Enhanced by the Addition of NaGluconate

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Monell Chemical Senses Center

The iso-alpha acids (IAAs) derived from hop cone flower components have a strong bitter taste that is commonly known for its characteristic bitterness in beer. These acids vary structurally in their *cis/trans*- configuration, their side chains and can be converted to their reduced analogues making a family of 36 compounds. Their historical use in brewing was due to their preservative properties, but are used today largely for their bitterness. We focused on these compounds because the bitterness of tetralone is enhanced by sodium salts. This is surprising, since most bitter compounds are suppressed by sodium salts to some degree. We believe that sodium's inhibitory action is at the level of the receptor cell (and perhaps the receptor); thus this observation suggests that IAA receptors are unusual among the TAS2Rs. Further evidence of the distinctness of IAAs derives from the spatial localization of their bitterness. While most compounds are bitter on the anterior tongue and elsewhere, IAAs stimulate very little bitterness in anterior taste fields yet are very bitter in posterior fields. Presently, we demonstrate the stimulating potency, spatial localization, and interactions with NaGluconate, adenosine monophosphate (AMP) and monosodium gluconate (MSG) on 6 synthesized IAAs. Our data suggest that not every IAA shares these unique properties and, thus, they may stimulate different bitter receptors.

This work was funded in part by NIH DC02995 to P.B.

Poster Session Sat PM

Spatial Summation of Taste Revisited

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It is generally accepted that spatial summation occurs in taste, yet the amount of summation reported has ranged from marginal to abundant. A potential confound in prior studies was that touch stimulation always co-varied spatially with taste stimulation. In the present study taste stimulation was varied while touch was held constant. A plastic holder was used to space 5 cotton swabs 5 mm apart in an arc that conformed to the tongue tip. On each trial taste solution was applied to 1, 3 or 5 swabs while the remaining swabs contained water, and all 5 swabs were stroked simultaneously against the front edge of the tongue. Summation was measured for 5 concentrations of sucrose (0.1–1.0 M) and QSO₄ (0.01–0.1 mM), and Ss rated sweetness, sourness, saltiness, bitterness and burning/stinging on the gLMS. The same stimuli were also rated in a sip-and-spit procedure. The swab procedure revealed large amounts of spatial summation for both stimuli (up to a 6-fold difference in intensity between 1 and 5 taste swabs), although the amount varied directly with concentration for QSO₄. The latter relationship meant that the slope of the bitterness function increased with the number of taste swabs, whereas the slope of the sweetness function stayed constant. Consistent with this difference, the sip-and-spit procedure yielded intensity functions that converged at higher concentrations; the slope of the bitterness function was much steeper (0.96) than the slope of the sweetness function (0.55). Studies are continuing to further elucidate how normal taste perception depends on interactions among tactile stimulation, stimulus intensity, and spatial summation.

Supported by NIH grant DC005002.

Poster Session Sat PM

Adaptation to Sucrose and NaCl Tracked Discretely or Continuously

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A series of studies using precise automated delivery of taste stimuli as mists demonstrates substantial gustatory adaptation and recovery from adaptation each occurring within 15 s. Like filter-paper delivery, atomized stimuli of little liquid volume remain restricted to the region of the anterior surface of the tongue to which they are applied. Adaptation and recovery were evaluated with automated delivery of greater liquid volume stimulus streams to see if, with precise temporal control, adaptation would be rapid. Two methods of rating were used: discrete, with the cursor set to zero afterwards, and continuous LMS intensity tracking. Triplicate ratings for 0.5 M sucrose and 0.3 M NaCl, applied and rinsed off by water during consecutive 60-s periods, were made at 9 discrete time points at 15-s intervals in one session, and continuously in another session ($n = 6$). In discrete trials, stimulus ratings were reduced to 35% within 45 s ($P < 0.001$), and recovered to 86% by 30 s after water rinsing began ($P < 0.01$). The continuous trials were less successful in demonstrating adaptation/recovery, with only ratings after 60-s adaptation lowered ($P = 0.05$). Thus, mists and discrete ratings, not the dynamic stimulus spread of solution streams or the response hysteresis in continuous tracking, reveal rapid taste adaptation.

Supported by NIH grant DC004849.

Poster Session Sat PM

Chlorhexidine Induced Salt-Taste Distortions and Stimulus Cation Valency

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Chlorhexidine (CHX) mouthrinses alter perceptions of bitter and salty tastes. To further evaluate effects of CHX on salt-taste perception, 8 salts with cationic and anionic valences of 1 or 2 were selected for study (NaBr, KBr, CaBr₂, MgBr₂, Na₂SO₄, K₂SO₄, CaCl₂, MgSO₄) and matched for intensity. Two non-salts, sucrose and water, were also used. Ten subjects participated in a taste confusion matrix experiment, in which salt stimuli were identified by cation names after 2 training trials. Data analyzed were percent correct and an information theory measure of discriminability, T_2 . Compared to water rinses, three 1-min rinses with 1.34 mM CHX disrupted taste qualities of all the salts with monovalent cations ($P = 0.0001$), but did not affect 3 of the 4 salts with divalent cations. Correct identification of water also deteriorated ($P = 0.0001$), but sucrose identification remained perfect. Monovalent-cation salt versus water discriminability ($T_2 = 0$ for identical responses to each; $T_2 = 1$ for no responses in common), was 0.91 after water, but resembled random responding, 0.36, after CHX. Comparable values for divalent-cation salts were 0.98 for water rinse, and 0.88 for CHX

rinse. Salts with divalent cations may displace dicationic CHX from binding sites more readily than salts with monovalent cations.

University of Connecticut Biomedical Sciences PhD Thesis (A.J.), funded by NIH DC004849.

Poster Session Sat PM

Detection and Recognition Thresholds for Sucrose and Quinine HCl for Moderate Dry Mouth Sufferers (MDMS) and Sjogren's Syndrome Sufferers (SS)

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GlaxoSmithKline

Recent sensory studies regarding the assessment of oral formulations for dry mouth sufferers raised the question of the validity to extrapolate sensory data obtained from normal subjects to dry mouth sufferers. Detection (DT) and recognition (RT) thresholds for sucrose and quinine HCl were determined for a panel of MDMS and a panel of SS. Values were compared with the thresholds obtained from non-sufferers. *Quinine HCl*: For normal subjects, DT and RT were consistent with the literature (0.14×10^{-3} g/l and 1.2×10^{-3} g/l, respectively). For MDMS and SS, DT and RT were above the limit of the concentrations assessed ($>2.0 \times 10^{-3}$ g/l and $>11.4 \times 10^{-3}$ g/l, respectively). *Sucrose*: For normal subjects, DT and RT were consistent with the literature (1 and 5 g/l, respectively). For MDMS, DT and RT were higher than the values obtained for normal subjects or SS. DT was 6 g/l and 2 RT subtypes were observed (11.5 and 20.4 g/l). For SS, there were 2 DT subtypes (2 and 11.5 g/l) and 2 RT subtypes (6.4 and 11.5 g/l). The greater loss in taste sensitivity was observed for the bitterness both for MDMS and SS. Sweetness was more affected for MDMS than for SS. Using sensory data generated by normal subjects to predict the sensory responses of MDMS may be misleading. However, since MDMS were more sensitive to bitterness than SS, a product deemed not bitter by a panel of MDMS is unlikely to be perceived as bitter by SS.

Poster Session Sat PM

Examination of Taste Recognition Thresholds with Edible Taste Strips

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Edible taste strips composed of pullulan and hydroxypropyl methylcellulose (HPMC) polymers were used as vehicles for administering precise amounts of taste stimuli to the oral cavity. Test subjects rated virtually all control strips as non-detectable for taste intensity on the generalized Labeled Magnitude Scale. In this study, a 3-strip psychophysical protocol was used to determine taste recognition thresholds for sweet, sour, salty, and bitter tastants; namely, sucrose, L-ascorbic acid, sodium chloride, and quinine HCl. Each sample population contained 25–30 subjects, and average ages of all 4 sample populations were between 26 and 30 years. Mean taste recognition thresholds for sour tastant was 3.6 μ mol, for salty tastant was 3.8 μ mol, and for sweet tastant was 2.6 μ mol. In contrast, the mean taste recognition threshold for bitter tastant

was near 35 nmol. Also, salty tastant yielded the narrowest range of subject response for detection thresholds. These mean recognition thresholds are up to a magnitude of order lower than those reported with water-based testing protocols for comparable age groups, conceivably reflecting localized stimulus presentation with minimal dilution from saliva. These and other data suggest that pullulan-HPMC edible taste strips may prove to be excellent substrates for delivery of tastants to the oral cavity.

Supported by NIDCD R43 DC007291, and a Return of Overhead Research Incentive Grant from Temple University.

Poster Session Sat PM

A Test for Gustatory Function

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Edible taste strips composed of pullulan-hydroxypropyl methylcellulose (HPMC) polymers allow the administration of precise amounts of tastants to the tongue. These strips rapidly dissolve in the oral cavity, have a long shelf-life, are easily transported outside the lab or clinic, and exhibit virtually no background taste. All 5 major classes of taste stimuli are readily incorporated into taste strips at amounts well above suprathreshold levels. As shown by quinine HCl fluorescence, taste strips contained uniform amounts of tastant in each strip. In this study, 25 college students rated the whole-mouth intensity of NaCl incorporated in these strips using the generalized Labeled Magnitude Scale (gLMS). Additionally, magnitude matching was performed for the same NaCl stimuli by matching their intensity values to that of an incandescent light source illuminated at varying intensities. Monotonic increases in perceived whole-mouth intensity were found in both cases. Similar results were observed for the sour tastant L-ascorbic acid with a 10-item category scale. These studies suggest that edible taste strips composed of pullulan and HPMC can be reliably employed in whole-mouth taste tests.

Supported by NIDCD R43 DC007291, and a Return of Overhead Research Incentive Grant from Temple University.

Poster Session Sat PM

Modifying Bitterness Depends on Vegetable Type and PROP Tasting

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University of Connecticut

Chemopreventive agents in vegetables promote health yet can diminish pleasantness via bitterness. Here, we measured taste and hedonic ratings of brussels sprouts, asparagus and kale after adding tastants known to suppress bitter—aspartame (ASP), sodium acetate (NaOAc), and NaCl (10.32 mM)—in 29 Ss phenotyped for propylthiouracil (PROP) bitterness. Pilot testing showed zinc sulfate as highly unpalatable. In RM-ANOVA, ASP improved vegetable liking most effectively by increasing sweetness and decreasing bitterness. For brussels sprouts, all tastants increased liking with concurrent increased saltiness and decreased bitterness for NaOAc, decreased bitterness for ASP, and no qualitative shift for NaCl. For

asparagus, only ASP worked to shift disliking to liking with concurrent bitter reductions. NaOAc increased saltiness and decreased bitterness without hedonic shift (presumably due to sour/astringent sensations for some Ss). NaCl did not modify asparagus taste or hedonic ratings. For kale, tastant effects were PROP dependent. NaOAc increased saltiness or reduced bitterness for PROP medium or supertasters without changing disliking. ASP increased sweetness for all PROP groups and decreased bitterness in tasters. Liking increased for medium but not supertasters. 32 mM NaCl increased kale liking for tasters, with some bitter reduction. In summary, the unique profile of bitters in vegetables and consumer palates presents a need to translate basic science on taste suppression to support healthy food choices by improving palatability.

Funded by American Diabetes Association.

Poster Session Sat PM

The Relationship between Caffeine, Taste and Anxiety

Lucy Donaldson, Tom Heath, Emma Richardson, Charlotte Kenyon, Victoria Smith, David Nutt and Jan Melichar

University of Bristol

This study was designed to determine the effect of caffeine on taste sensitivity in humans and its relationship with anxiety. All subjects completed Speiberger's State and Trait Anxiety questionnaires prior to testing. A series of quinine, sucrose, sodium chloride, hydrochloric acid and monosodium glutamate (MSG) solutions were presented to the dorsum of the tongue in 12–20 healthy human subjects. The subjects indicated whether or not they could recognise the stimulus at each concentration. Each volunteer was then given either caffeine (50 mg) or placebo (lactose), in a double blind cross-over design. Taste recognition tests were performed again 2 h after drug administration. Psychophysical taste function curves were constructed for the group, for each taste modality before and after drug intervention. Comparison of responses showed that caffeine significantly decreased quinine (threshold 47 μ M [39–57 95% CI] before, 31 μ M [25–38] after; $P < 0.01$) and sucrose (threshold 25 mM [25–32 CI] before, 15 mM [13–15 CI] after; $P < 0.001$), and increased sour (7.2 mM [6.3–7.9 CI] before, 8.7 [7.9–9.7 CI] after) recognition thresholds. Salt and MSG thresholds were unchanged by caffeine, and placebo had no significant effects on any taste modality. There was a significant relationship between the change in sweet taste threshold and Trait anxiety levels. Caffeine increases sensitivity of bitter and sweet taste recognition. Anxiety enhances the action of caffeine on sweet taste.

Poster Session Sat PM

Sensory Perception and Characterization of Novel Sensory Evoking Flavor Ingredients

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Cooling substances are an emerging class of ingredients that intensify or extend the flavor impact of foods. Several substances derived from menthol impart cooling to chewing gum and confectionary products, and are therefore economically important to the flavor industry. This study examined the sensations of cooling, heat/burning, bitterness, and tingling from 3 novel compounds structurally

similar to *l*-menthol, herein referred to as Coolact® 5, Coolact® 10, and Coolact® 38D. Healthy adults ($n = 118$) rated 3 concentrations (75, 150 and 300 ppm) of each Coolact® type using 15-cm line scales at 4 time points over 10-min (0, 2.5, 5 and 10 min after tasting). They also indicated the locations of each sensation in the mouth and throat. The intensity of all attributes was maximal directly after tasting ($P < 0.0001$) and decreased with time ($P < 0.0001$). Coolact® 5 produced the strongest cooling and tingling, followed by Coolact® 10, then Coolact® 38D ($P < 0.0001$). There was a clear dose response for cooling and tingling for each Coolact® material. Heat/burning and bitterness were barely detectable at any time point or concentration. Cooling was predominantly perceived on the tongue ($P < 0.0001$) and roof of the mouth ($P < 0.05$) but not elsewhere. Power functions for cooling at time zero were 0.86, 0.36, and 0.08 for Coolact 5, 10, and 38D, respectively. These data demonstrate that Coolact® flavor ingredients produce cooling and tingling in the mouth with minimal bitterness or burning.

Supported by Takasago International Corp.

Poster Session Sat PM

Transsynaptic Effects and Topographic Re-innervation of Olfactory Bulb after Binge Alcohol

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Neuronal degeneration and death occurs in many regions of the CNS in response to ethanol (EtOH) administration in both humans and animals. In humans, deficits in the sense of smell associated with alcoholism are partially reversible on abstinence. Most studies have considered that the olfactory deficit derives from EtOH-mediated damage to CNS pathways involved in processing olfactory information. However, the possibility of transsynaptic effects seems not to have been considered. Previously we demonstrated that EtOH induces a cycle of mature olfactory sensory neuron (OSN) degeneration and replacement from immature precursor cells in the olfactory neuroepithelium (OE). We now show that the loss of mature OSNs following EtOH is accompanied by a reduction in synaptic innervation of the olfactory bulb (OB) as manifested by a decrease in OB tyrosine hydroxylase immunoreactivity (TH-ir) in the target neurons of the OSNs. On abstinence, OSN neurogenesis proceeds and is accompanied by synaptic reinnervation of the OB and recovery of TH-ir. This is perhaps the first demonstration of transsynaptic effects after EtOH administration. To determine if the reinnervation is topographically correct we are using 2 mutant mouse lines, M71-GFP and P2-tau-LacZ, that have different projection profiles in the OB. Our data indicate that abstinence following EtOH administration permits newly formed OSNs to connect to their OB targets in a topographically correct pattern.

Supported by ABMRF, NIH-CINTGT32NS07375, NIH-DC03112.

Poster Session Sat PM

Impact of ApoE Deficiency on Regeneration of Olfactory Receptor Neurons Post Injury in Mice

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Previous studies from our laboratory show that apolipoprotein (apoE), a lipid transporting protein, is extensively expressed in the primary olfactory pathway. We hypothesized that apoE may play a role in the rate of regeneration of olfactory receptor neurons (ORN). We examined the rate of ORN recovery following olfactory epithelium (OE) lesion in wild-type (WT) and apoE-deficient/knockout (KO) mice to directly test this hypothesis. OE was lesioned in 2–3-month-old mice by intranasal irrigation with Triton X-100 (TX). OE was collected at 0, 3, 7, 21, 42, and 56 days post-lesion, and apoE concentration and distribution in the OE of WT mice were evaluated. ORN recovery in the OE and synaptic recovery in the OB were monitored by immunoblotting and immunohistochemistry of olfactory marker protein (OMP) and synaptophysin (Syn). The results revealed that 1) apoE levels declined until 3 days post injury in WT mice, followed by a gradual increase to about 1.5-fold normal level by 42 days post injury; 2) recovery rate of OMP in the OE was significantly delayed in the KO mice compared to WT mice, although the endpoints were similar; and 3) recovery of Syn in the glomerular zone was similarly slower in KO mice. These results show that apoE facilitates the rate of ORN recovery and synaptogenesis post OE lesioning.

Supported in part by NIDCD grant (DC003889) and Illinois Department of Public Health Alzheimer's Fund.

Poster Session Sat PM

Regeneration of the Olfactory Nerves following Mild and Severe Injury and Efficacy of Dexamethazone Treatment

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To investigate factors that influence the degree of neural recovery in the olfactory system, we studied 2 injury models using OMP-tau-lacZ mice. Models of mild and severe injury were obtained by performing olfactory nerve transections. Histological assessment of recovery in the olfactory bulb was made at 5, 14 and 42 days after injury using X-gal staining to label olfactory marker protein (OMP) in degenerating and regenerating olfactory nerve fibers, and immunohistochemical staining of glial fibrillary acidic protein (GFAP) and CD68 to measure injury-associated changes in astrocytes and histiocytes. Dexamethazone sodium phosphate (DXM) was injected for 5 days after nerve transection in the severe injury model. With mild injury, there was less injury-associated tissue present between the olfactory bulb and the cribriform plate and regenerated olfactory nerves were observed in the glomerular layer of the olfactory bulb. At 42 days recovery there were more astrocytes and histiocytes with severe injury than for mild injury. DXM-injected mice showed less injury-associated tissue, better olfactory nerve recovery and fewer astrocytes and histiocytes. These results show that regeneration and recovery in the olfactory system occurs with mild injury, and that DXM treatment may have therapeutic value by reducing injury associated tissue reactions in severe injury, and potentially improving recovery outcome.

Supported by a grant from the Jeffress Memorial Foundation.

Poster Session Sat PM

Neurogenesis in the Adult Rat Olfactory Epithelium and Subventricular Zone: Differential Effects of Atypical Antipsychotics

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Neuroprotection and neuroplasticity, including neurogenesis, have been associated with neuroleptic medication treatments in both animals and humans. We proposed that atypical antipsychotics would increase cell division in 2 neurogenic tissues, the subventricular zone (SVZ) of the brain and the basal region of the peripheral olfactory epithelium (OE). Young male rats were given risperidone (1 mg/kg/day), an atypical antipsychotic commonly prescribed to schizophrenic patients, paliperidone (1 mg/kg/day), a recently introduced atypical containing the active metabolite of risperidone, or diluent (1 mM acetic acid) in drinking water, with IACUC approval. After 28 days, animals were injected with bromodeoxyuridine (BrdU), perfused with 4% formaldehyde and nasal and brain tissues were cryostat sectioned and stained with anti-BrdU. In the OE, BrdU-positive cell counts for risperidone and paliperidone groups were significantly higher ($P < 0.05$) than controls, but did not differ between drugs. In the SVZ, drug treatment did not alter BrdU counts in posterior SVZ areas, but more anteriorly, risperidone but not paliperidone decreased counts. This suggests a differential effect at these doses: stimulating potential neurogenesis in the OE, but not changing or decreasing cell division in the SVZ. Comparisons with previous studies in the SVZ will be discussed. Further work will examine other drugs, doses and drug delivery methods.

Supported by Jansen Pharmaceutica, L.L.C.

Poster Session Sat PM

Integration of Adult-Generated Granule Cells into Synaptic Circuits

Mary C. Whitman and Charles A. Greer

Yale University

Throughout life neuroblasts from the subventricular zone (SVZ) migrate into the olfactory bulb (OB) and differentiate into granule (GC) and periglomerular (PG) cells that are presumed to integrate into the synaptic circuits of the OB. We have used retroviral infection of a GFP construct into the SVZ of mice to label adult born cells and follow their differentiation and integration into OB circuitry. Ten days post-infection (DPI), cells reach the granule cell layer (GCL) and extend an apical dendrite toward, but not fully into, the external plexiform layer (EPL). GCs first exhibit elaborate spiny dendritic arbors in the EPL at 14 DPI. The density of spines is initially low, but increases from 14 to 28 DPI, with a later decrease in density by 56 DPI. Despite the first appearance of spines at 14 DPI in the EPL, they do not express pre- or postsynaptic markers until 21 DPI. Ultrastructural evidence of symmetrical and asymmetrical dendrodendritic synapses between GCs and mitral cell lateral dendrites is shown at 42 DPI. In contrast to the dendrites/spines in the EPL, postsynaptic markers are expressed on granule cell somata within 10 DPI, prior to dendritic elaboration in the EPL. Thus, it appears that synaptic input to newly differentiating GCs

occurs first in the granule cell layer where inputs from centrifugal and collateral axons terminate. From this evidence it is tempting to speculate that mitral cell axon collaterals as well as central inputs to the OB may play an important role in regulating the survival or differentiation of new neurons.

Supported in part by NIH DC006972, DC00210, DC006291 to C.A.G. and the Yale MSTP GM07205 to M.C.W.

Poster Session Sat PM

GABA Modulates Ventral Migration of Subventricular Zone Progenitors in Neonatal Mice

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University of Maryland School of Medicine

The subventricular zone (SVZ) is a residual germinal layer of the lateral ganglionic eminence (LGE) surrounding the ependymal layer of the lateral ventricle. The postnatal/adult SVZ is best known as the source of neurons migrating along the rostral migratory stream toward the olfactory bulb (OB). Our lab has previously shown neurons also migrate ventral from the postnatal SVZ and form the islands of Calleja. Since neuronal migration from the LGE to cortex, and from the SVZ to the OB, can be modulated by neurotransmitters we hypothesized that SVZ derived ventral migration may also be modulated by neurotransmitters. SVZ derived migratory cells were labeled by cell tracker green injection into SVZ, and migrating cells monitored by time-lapse confocal microscopy. CTG-labeled progenitors migrate at an increased velocity (+40%) in the presence of the GABA_A receptor agonist muscimol, and decrease (-20%) in the presence of the antagonist gabazine. Interestingly, GABA has opposite effects on SVZ derived progenitors migrating to the OB. In addition to altering migration rate, CTG-labeled cells migrate toward a point source of GABA suggesting GABA can act as a guidance factor. RT-PCR identified expression of the α -3 GABA_A receptor subunit in migratory progenitors, consistent with a direct GABA action. These data show that GABA can promote and direct SVZ progenitor ventral cell migration and may be an important cue in the organization of this region.

Supported by NIDCD DC005739.

Poster Session Sat PM

BDNF Immunoreactive Periglomerular Cells May Modulate Survival and Plasticity of Neurons in the Olfactory Bulb

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Florida State University

The olfactory bulb (OB) retains the ability to acquire new neurons throughout its life and shows remarkable regeneration properties. The mechanism and neuroanatomical substrate involved in regeneration, however, is not fully understood. Here we show that following 20 days of unilateral-naris occlusion in C57/B6 mice, 80% of the brain-derived neurotrophic factor (BDNF)-immunoreactive (ir) periglomerular cells disappear from the olfactory bulb that is ipsilateral to the naris occlusion. The number of BDNF-ir mitral cells, however, did not vary significantly between the occluded and non-occluded sides. These changes were accompanied by an

increase in the expression of the GTP-binding protein, G_{olf} , in the main olfactory epithelium of the occluded side. Periglomerular cells make dendrodendritic reciprocal synapses with both mitral and tufted cells and also receive sensory input from primary sensory neurons. Thus, periglomerular cells are positioned in the OB to supply both input and output cells with BDNF. Given the trophic role of BDNF in the development and survival of neurons and in the modification and plasticity of synapses, BDNF-ir periglomerular cells may modulate activity-dependent OB neuron survival. Our future experiments are aimed at studying the BDNF-ir profile of the periglomerular cells after reopening of the nostril and how intrinsic properties of the projection neurons might affect regenerative properties of the OB.

Supported by NIH DC03387 (NIDCD) and a Florida Neuroscience Fellowship.

Poster Session Sat PM

Canonical Wnt Signaling Defines a Novel Cell Population in the Mouse Olfactory Bulb

Tiara Booker-Dwyer, Sarah Hirsh and Haiqing Zhao

Johns Hopkins University

Olfactory sensory neurons (OSNs) in the nose form precise connections with neurons in the brain. However, mechanisms that account for the formation of such precise neuronal connections are incompletely understood. Recent evidence implicated the function of Wnt growth factors in the formation of neuronal connections. To assess the role of Wnt signaling in the olfactory system, we examined the expression of β -galactosidase (β -gal) in the TOPGAL mouse, a transgenic strain where β -gal expression reports the activation of the canonical Wnt signaling pathway. In the olfactory epithelium (OE), no β -gal expression was observed at any developmental stages, suggesting that canonical Wnt signaling does not participate in OE development. In the olfactory bulb (OB), β -gal expression was observed in a population of cells located at the interface of the olfactory nerve layer and the glomerular layer. The β -gal expression in the OB was developmentally regulated with the peak number of β -gal positive cells occurring at late embryonic and early postnatal stages. The number of β -gal positive cells was drastically reduced in adulthood, but could be recovered by forcing regeneration of OSNs. The temporal coincidence between the peak of β -gal expression and periods of formation of OSN connections, together with the spatial location of these cells, suggest a potential role for these cells and canonical Wnt signaling in the formation of OSN connections in the OB. The identity of this population of cells remains elusive, as they do not express any molecular markers for a broad panel of cell types.

Poster Session Sat PM

Development of the Glial Investment of Glomeruli in the *Drosophila* Olfactory Lobe

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University of Arizona

A robust, multi-layered glial envelope surrounds olfactory glomeruli in the adult *Manduca sexta* olfactory lobe (OL) and a complex glial envelope surrounds mammalian glomeruli; glomerulus formation in both involves critical neuron-glia interactions. The glial investment of *Drosophila melanogaster* glomeruli and possible roles for those glia are poorly understood, even as the *Drosophila* OL is increasingly used as a model for development and for various disorders, including disorders thought to be glia-based. We are using a combination of electron microscopy and confocal microscopy of OLs from a Nervana 2 line (gift of P. Salvaterra), in which glial cells express GFP, to examine in detail the development of glia during development of the adult OL. Glial cells and their processes are confined to a shell around the neuropil at 30 h APF. As glomeruli begin to coalesce around 40 h, a fringe of glial processes extends into the incipient glomerular layer but only few glial processes extend fully between new glomeruli even at 60 h. By 72 h, the mature pattern is essentially established: glial processes surround ORN axon fascicles in the nerve fiber layer, and they form thin, incomplete glomerular borders. They also lightly invest the synaptic interior of the glomeruli. Because glomerular architecture is established by \sim 60 h, *Drosophila* glial cells cannot play the critical role in glomerulus formation that they do in moth and mammal.

Funded by NIH-NS28495 and HHMI 52003749.

Poster Session Sat PM

Glial Identity of Neuronal Stem Cell Niches in the Olfactory Midbrain of Adult Spiny Lobsters, *Panulirus argus*

Manfred Schmidt and Charles Derby

Georgia State University

In the spiny lobster, *Panulirus argus*, adult neurogenesis in the olfactory midbrain is localized to a small proliferation zone (PZ) in each of the clusters of neuronal somata (MC, LC). One neuroblast is associated with each PZ and is itself surrounded by a clump of small cells apparently constituting a stem cell niche. The cells of the clumps share characteristics with perivascular cells and with a newly identified type of glial cells in the MC and LC. To further elucidate the identity of the cells of the clumps, we surveyed the MC and LC of *P. argus* by labeling with \sim 50 antibodies and \sim 20 lectins. We found an antibody (anti-phosphorylated histone H3: anti-pH3) that labeled the glial cells in the MC and LC (in addition to a few nuclei in M-phase in the PZs) and a lectin (*Amaranthus caudatus* lectin: ACL) that labeled the vasculature. The cells of the clumps were labeled by anti-pH3 but were not labeled by ACL. Among the tested antibodies were 3 against glutamine synthetase (anti-GS) since glial cells in the neuropils of the olfactory midbrain are labeled by anti-GS. One anti-GS antibody labeled these neuropilar glial cells but failed to label the glial cells in the soma clusters or the cells of the clumps. From these findings, we conclude that glial cells of a distinct type (GS-negative) reside in the soma clusters of the olfactory midbrain of *P. argus* and that the clumps of cells surrounding the putative adult neuroblasts are constituted by these glial cells.

Supported by DFG grant Schm738/8-1 and NIH grant DC00312.

Poster Session Sat PM

Does Acetylcholine Play a Role in Olfactory Bulb Synaptogenesis and Morphogenesis?

Ambarish Ghatpande and Alan Gelperin

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The rodent olfactory bulb (MOB) undergoes significant morphological development postnatally. This increase in size is a result of a large influx of new interneurons that integrate into the bulbar circuit postnatally. Recent evidence suggests that acetylcholine may play an important role in olfactory bulb morphogenesis by influencing survival of newborn neurons. The mechanistic basis of such a role for acetylcholine is poorly understood. In whole-cell patch clamp recordings from mitral and granule cells in 350 μm thick rat MOB slices from 6–15 day rats, we found evidence for a functional role of carbachol (an acetylcholine analog) in modulating glutamate and GABA release onto these neurons. Furthermore, our experiments indicate this modulation is age-dependent and changes in the first 2 postnatal weeks during which the MOB undergoes dramatic morphological development. We will present these experimental results and a plausible model for a morphogenetic role of acetylcholine in the MOB suggested by the findings.

Support provided by Army Research Office, grants from the National Alliance for Autism Research and the Whitehall Foundation to A.Gelperin.

Poster Session Sat PM

Visualization and Manipulation of Mitral Cell Dendritic Maturation In Vivo by Lentivirus

Dennis Hawisher, Ting-Wen Cheng and Qizhi Gong

University of California–Davis

The emergence of appropriate functioning circuits in the brain requires synaptic reorganization from the immature connections developed during embryonic stages. Although sensory experience is believed to be the driving force in the remodeling and refinement of the neuronal circuitry, intrinsic genetic programs could also play critical roles. The first relay of the olfactory connections between olfactory sensory neurons and mitral cells are remodeled during early postnatal stages. Mitral cells in the olfactory bulb undergo a morphological change from bearing an elaborate dendritic tree to having only one apical dendrite targeting one glomerulus. Lentiviral mediated manipulation allows visualization of single cell morphology and investigation of genetically modified mitral cells in an otherwise wild-type background in vivo. Lentiviral mediated EGFP expression labels mitral cells and allows detailed colocalization studies. Mitral cell dendritic maturation, examined between P3-P15, is unaltered by lentiviral infection when compared with Golgi and DiI labeled cells. We found that microtubule binding protein, MAP1A, is transiently expressed in mitral cell dendrites. MAP1A is preferentially localized in the emerging apical dendrite and is absent in the degenerating dendrites. In addition, knockdown of a candidate gene, *opg*, by lentiviral delivery of shRNA results in dendritic degeneration and lacking MAP1A ex-

pression in mitral cells. Cre mediated conditional knockout is also studied by lentiviral mediated technique.

Supported by: NIH DC006015, NSF0324769.

Poster Session Sat PM

Expectancies about Harmfulness Influence Early Odor Sensations

Patricia Bulsing¹, Monique Smeets¹, Thomas Hummel² and Marcel Van den Hout¹

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Health symptoms attributed to odor exposures are not well understood. Cognitive factors seem to be a candidate for explaining odor-related illness: In previous research it was shown that beliefs about the harmfulness of an odor mediated the number of symptoms reported. We investigated if such influences are mainly *cognitive* (“interpretations” of odors), or also *perceptual* (affect perceptions of the characteristics of the odor). ERPs were recorded in response to an odor believed to be painful versus not painful. The experiment consisted of 2 conditions: one where subjects expected to smell an odor (“non-painful”); one where they also expected to feel irritation in the nose (“painful”). Subjects received painless H₂S stimuli during all conditions. To reinforce pain expectancy, a CO₂ pulse was given occasionally during the painful condition. Comparisons were made between reactions to H₂S, under the 2 expectancy conditions. Detection sensitivity (reflected by amplitudes and latencies of the early N1 peak) and stimulus salience (reflected by amplitudes and latencies of the late cognitive positivity) were examined. Peak amplitudes were unaffected by expectancy condition. A main effect of expectancy on N1 latency was found: expecting a painful stimulus reduced the time to detect a harmless odor. These results shed new light on the way individuals concerned about exposure effects perceive their olfactory world. Expectancies seem to alter very basic aspects of odor perception.

Funded by NWO 452-03-334.

Poster Session Sat PM

Thresholds and Chemosensory Event-Related Potentials to Malodors: Differences Related to Sex and Age

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Background: Sex differences in olfactory sensitivity to androstadienone (4,16-androstadien-3-one) have been reported to occur during puberty. The study reported here extends this work to investigate whether sex and age differences exist during pre- and post puberty for 2-methyl 3-mercaptopropanol (2M3M) and hydrogen sulphide (H₂S). **Method:** A total of 121 participants took part in the study (58 females, 63 males; age range 9–20 years). Participants were divided into 3 groups 1) pre-puberty, 2) puberty and 3) post-puberty. Threshold measurements for 1) androstadienone, 2) 2M3M and 3) H₂S were recorded. Chemosensory event-related potentials (CSERPs) were recorded using air-dilution olfactometry. **Results: Thresholds**—Female thresholds for the 3 odorants were stable between the 3 age groups. Pubescent males had a higher threshold

to androstadienone and 2M3M, for 2M3M this continued into post puberty. No significant differences were observed for H₂S thresholds. *Evoked Potentials*—Significant sex differences were present for CSERP P2 latency; pubescent males had a prolonged P2 latency for 2M3M and androstadienone compared to female pubescents. An age related increase was observed in the P2 amplitude in the pre-puberty group for 2M3M and androstadienone. **Conclusions:** The P2 latency increase in male pubescent may be due to increased endogenous levels of sweat and androstadienone.

Poster Session Sat PM

More Precise Measurements of Olfactory Event Related Potentials and Magnetic Fields

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There are many studies reporting chemosensory event related potentials (CSERPs). Almost all these studies reported, however, using average data obtained with dozens of stimulus repetition. And in the previous established olfactory stimulator, neither temporal delay from signal onset nor dispersion of changing time from air into odor has considered, because of lacking techniques high-speed gas monitoring. Additionally, the number of works of chemosensory event related magnetic fields, which have potential to investigate both temporal and spatial cortical information, is still a few. In this study, therefore, we tried to obtain average EEG and MEG data about 200 trials, by repeating several sessions, including 30 trials (ISI was 40 s), for one participant. Simultaneously, we monitored odor change from control air, using newly developed ultrasonic gas sensor. This stimulus monitoring revealed the rising time included more dispersion than measurement without participant. The averaged EEG data about 200 trials demonstrated that positive peak in frontal area (Fpz) about 200 ms after stimulus onset which was measured by gas sensor. And we now analyze MEG data with adjustment of sensor positions for each session.

Poster Session Sat PM

Olfactory Sensitivity in Euthymic Bipolar Patients

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This study aimed to investigate the olfactory function in relation to bipolar disorder (BD). There is evidence of olfactory dysfunction in BD patients correlated with increased emotional vulnerability. This might be due to the close anatomical and functional connection between orbitofrontal cortex (OFC) and olfactory processing, as the OFC is involved in the regulation of emotional and behavioural responses to external events. Therefore, a first pilot study compared the olfactory function in BD patients with heightened emotional reactivity and BD patients who do not exhibit this sensitivity. Seven patients with event-triggered mood episodes and 9 without such episodes were investigated using olfactory event-related potentials (ERP), and psychophysical tests for odor threshold, identification and quality discrimination. Lower odor thresholds and shorter N1

peak latencies of the olfactory ERP were found in BD patients with event-triggered episodes. The findings indicate disinhibition of orbitofrontal areas involved in the processing of emotional events in a subset of BD patients. In addition to these findings, psychophysical, electrophysiological and imaging data regarding the olfactory function in euthymic BD patients compared to controls will be presented.

Poster Session Sat PM

Orthonasal and Retronasal Perception of Binary Odor Mixtures

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Several studies investigated perceptual interactions in binary odor mixtures. However most were interested in orthonasal perception and very few is known on the differences between orthonasal and retronasal perception of binary mixtures. In a previous study, Atanasova et al. reported perceptual interactions in woody-fruity mixtures presented orthonasally. Here we designed an experiment (30 subjects), relying on psychophysical and olfactory event-related potentials recordings (ERPs), to compare perceptual interactions in woody-fruity mixtures presented ortho- and retronasally. A medium intensity of fruity odor (isoamyl acetate) was mixed with a low and a medium level of woody odor (whisky lactone). Single odorants or mixtures were delivered either orthonasally or retronasally using a computer-controlled olfactometer. Psychophysical results revealed a synergy of the fruity odor induced by the woody component only in the retronasal condition ($P = 0.03$). On the contrary, the fruity odor was found to be masked by the woody component in the orthonasal condition ($P = 0.02$). Synergy was associated to an increase in N1 amplitude for mixtures with low woody odor intensity levels ($P = 0.002$) whereas masking was related to an increase in P2 amplitude for mixtures with high woody odor intensity ($P < 0.0001$). These results suggested that perception through the ortho- or retronasal way induced differentiated olfactory processing that directly impact on perceptual interactions in binary odor mixtures.

Poster Session Sat PM

Olfactory Evaluation with Olfactory Event-Related Potentials and MRI in Patients with Olfactory Dysfunction

Daofeng Ni and Jianfeng Liu

Olfactory Research Lab

Objectives: To study primary application of OERPs in clinics. **Methods:** Subjects included 37 cases (male/female 17/20) with chief complains of olfactory dysfunction. A detailed medical history inquiry, a thorough ENT physical examination and a subjective olfactometry with T&T olfactometer were performed in all patients. OERPs with isoamyl acetate, and or intranasal trigeminal nerve chemosensory event-related potentials with stimulus ammonia, were obtained in patients. Nasal endoscopic evaluation, a computed tomography scan and MRI of brain and olfactory pathway were available in 17 cases. **Results:** In 37 cases, OERPs were elicited in 4 cases

with normosmia and 6 cases with hyposmia. Compared with age and gender matched control subjects, patients with hyposmia showed longer latency for P2. In patients with anosmia, no OERPs to maximum stimulus were obtained, but event-related potentials to ammonia were elicited. Five patients. Results of MR imaging: The absence of olfactory bulbs and tracts was noted 3 cases with Kallmann's syndrome. Unilateral or bilateral olfactory bulbs volumes were smaller in 6 cases than in age and gender matched controls. Normal olfactory bulbs and tracts exhibited in 2 cases; subfrontal regions were impaired with loss of olfactory bulbs and tracts in 3 cases with head trauma. Abnormal maxillary sinus were noted in 2 cases with phantosmia. Temporal lobe was damaged in 2 cases. **Conclusions:** OERPs can objectively evaluate olfaction, and have important clinical significance. MRI of olfactory pathway facilitates location diagnosis of olfactory impairment.

Poster Session Sat PM

Adaptation/Compensation in Trigeminal Processing in Subjects with Acquired Anosmia

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The olfactory and trigeminal systems have been shown to be closely related. Research indicates that anosmic subjects exhibit decreased trigeminal sensitivity of unclear underlying mechanisms. Aim of the present study was to investigate the adaptive and compensatory changes in the trigeminal pathway of subjects with acquired anosmia (AA) to improve our understanding of the interactions between the 2 sensory systems. To assess trigeminal activation at different levels of processing 123 subjects (50 AA subjects) were investigated using electrophysiological (event-related potentials [tERP] and negative mucosa potential [NMP]) as well as psychophysical methods (lateralization task, intensity rating). At the peripheral level AA subjects showed larger responses (NMP) to CO₂ than healthy controls. However, at the central level they were found to have smaller tERP as well as smaller psychophysical responses to irritants. After 9 months trigeminal sensitivity on a peripheral level had increased significantly in AA subjects with recovering olfactory function. These data suggest shifting mechanisms of mixed sensory adaptation/compensation in the interaction between the olfactory and trigeminal systems, where trigeminal activation is increased on the peripheral level in AA subjects and amplified on central levels in subjects with a functioning olfactory system.

This research was supported by Philip Morris USA Inc. and Philip Morris International.

Poster Session Sat PM

Comparison of Visual versus Olfactory Distractions on Pain Threshold and Tolerance

Robert Bayley, Peter D'Amore, Lindsay Coyne, Kathryn Repicky, Daniel Felbaum and Bryan Raudenbush

Wheeling Jesuit University

A variety of distraction techniques (visual, physical, olfactory, etc.) have been effective in mediating pain perception and tolerance. The present study compared the efficacy of visual versus olfactory pain distraction methods. In a within-subjects design, participants

completed 4 conditions: peppermint scent, high arousal images, low arousal images, and a control condition. Images were acquired from the International Affective Picture System (IAPS). After an 8-min exposure, participants completed a cold pressor test and questionnaires assessing mood (POMS), task load (NASA-TLX), and anxiety (STAI). Physiological measurements were monitored. High arousal images produced lower pain intensity ratings than both the low arousal images and the control condition. Peppermint scent produced lower pain ratings than the control and low arousal conditions. Both peppermint scent and high arousal images promoted increased pain tolerance. High arousal images led to higher ratings of anxiety. Physiologically, visual stimuli led to lower systolic blood pressure recordings than the peppermint scent and control conditions, and there was an interaction indicating higher post systolic ratings between the peppermint and control condition. Finally, mean arterial pressure increased following the cold pressor task. Thus, peppermint scent and high arousal visual images are equally effective in managing pain and altering physiological measurements during a cold pressor pain task.

Poster Session Sat PM

Differential Effects of Chocolate and Coffee Scents on Enhancing Cognitive Ability and Clerical Office Work Performance

Daniel Felbaum, Justin Schmitt, Kristen Koval and Bryan Raudenbush
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Past research has shown that the consumption of chocolate and coffee, due to their stimulating qualities, have been effective in enhancing cognitive alertness and ability, thus increasing clerical office work performance. The present study assessed the effects of chocolate and coffee scent administration on cognitive ability and tasks associated with clerical office work. In a within-subjects design, participants completed 3 scent conditions: chocolate scent, coffee scent, and a non-scented control condition. Scents were delivered via a nasal cannula. After a 10-min scent exposure (or no scent exposure in the control condition), participants completed computer-based neuropsychological tests assessing word discrimination, verbal memory, design memory, attention span, reaction time, problem solving, and response variability. Participants also completed clerical office work tasks, such as a typing test. Following each condition, participants completed surveys related to mood (Profile of Mood States) and workload demands (NASA-TLX). Chocolate scent was found to increase visual motor speed and impulse control, whereas coffee scent was found to improve typing accuracy and speed. Thus, these 2 scents differentially impact and enhance cognitive ability and clerical office work performance.

Poster Session Sat PM

Dream and Recent Memory Narratives Reveal Differential Effects of Floral Odors

Patricia Wilson¹, Caroline Coffield², Estelle Mayhew² and Jeannette Haviland-Jones²

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Recent research has demonstrated that floral odors, as a group, elicit greater use of positive emotion words in recall of recent memories compared to other kinds of pleasant odors. This corresponds with our other research showing that a variety of living fragrant flowers increase positive emotion and social displays more than other positive stimuli. Here we compared 5 floral fragrances to each other and to etoh and a non-odor control to determine how the floral odors might individually differ in mood change. 129 women were in 1 of 7 odor conditions: muguet, hyacinth, gardenia, jasmine, jasmine pale white night, etoh, or no-odor. The women were exposed to 130 μ l of stimulus. They wrote 2 narratives for 10 min each: a recalled dream and a recent memory. Both were submitted to content analyses for frequency of use of different word categories. For the recent memory, all floral odors significantly reduced the use of anxious words in the narratives. For the dream narrative, the no-odor condition was associated with the highest use of anxiety words; only jasmine, gardenia, and hyacinth significantly reduced this use in dream narrative. In the dream narrative, only jasmine and hyacinth elicit significantly higher use of positive emotion words compared to the other conditions. This research lends further support to the notion that pleasant odors cannot be collapsed into simple categories; even within a category such as florals, some odors may be more “emotionally active” than others.

Poster Session Sat PM

Investigation of Breathing Parameters during Odor Perception and during Olfactory Imagery

Anna Maria Kleemann, Jessica Albrecht, Veronika Schöpf, Rainer Kopietz, Maria Demmel, Andrea Anzinger, Tatjana Schreder, Johanna May, Jennifer Linn and Martin Wiesmann

Ludwig-Maximilians-University

Objectives: Only little is known about olfactory imagery in comparison to visual and auditory imagery. There is evidence that respiration may be altered by both olfactory perception and olfactory imagery. In order to investigate this relationship, we measured breathing parameters (respiratory minute volume, respiratory amplitude and breathing rate) in human subjects during olfactory perception and olfactory imagery. **Methods:** We tested 56 subjects (35 females, 21 males) with a normal olfactory function. Nasal respiration was measured using a respiratory pressure sensor (OL014, Burghart Instruments, Wedel, Germany). Using an experimental block design we alternatively presented odors or asked the subjects to imagine a given smell. Four different pleasant odors were used: banana, rose, coffee, and lemon. **Results and Conclusion:** We detected a significant increase in respiratory minute volume between olfactory perception and the baseline condition, as well as between olfactory imagery and the baseline condition. Differences in respiratory amplitude and breathing rate between olfactory perception, olfactory imagery and baseline were not statistically significant. We conclude from our results that olfactory perception and olfactory imagination both have effects on the human respiration profile, and that these effects are based on a common underlying mechanism.

Poster Session Sat PM

Androstenol/Androsterone May Condition a Human Hormonal Effect/Behavioral Affect

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Chemical signals communicate affective and motivational states by eliciting hormonal effects that translate to unconscious behavioral affects in non-human animals. In human females, androstenol elicits hormonal effects and, in a pilot study, androsterone appeared to elicit unconscious behavioral affects. Mammalian conditioning paradigms suggest that androstenol may condition hormonal effects that are unconsciously associated with the possible behavioral affects of androsterone. In this present study, we evaluated the interaction of ovulatory phase human female subjects with a male confederate who either applied or did not apply a standardized androstenol/androsterone mixture diluted in propylene glycol. We are determining whether study participants, and non-participants who rated the interactions, provide data that discriminate between the confederate's 2 conditions in a cooperative task. After task completion, an odor assessment questionnaire was completed by the female participants with assessment of the subject's mood by POMS. In addition, males and non-task females completed a questionnaire to determine whether odor assessment might be sexually dimorphic. Preliminary results suggest that combining the known hormonal effects of a putative human pheromone (e.g., androstenol) with the possible behavioral affects of androsterone may help to extend non-human animal models of sexually dimorphic chemical communication to humans. Final results will be presented at the conference.

Poster Session Sat PM

Increase in Anhedonia Level in Menopausal Women is Accompanied by a Shift of Olfactory Function

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Universite Lyon 1

Menopausal women between 55 and 65 years of age exhibit psychological and hormonal changes that are reflected in modification in experiencing sensory pleasure. In the present study we set out to characterize whether that modulation of anhedonia level was accompanied by a modification of olfactory function during that period of life. Thirty-two menopausal women (55–65 years of age) without neurological diseases and not under menopausal substitutive treatment were tested. Anhedonia level of each subject was assessed with the Physical Anhedonia Scale, a 61-item true/false inventory that attempts to access a wide variety of positive physical experiences. Subjects' olfactory performances were estimated using the European Test of Olfactory Capabilities (ETOC). After completing the localisation and identification tasks included in the ETOC, subjects sniffed each odorized vial that they properly localized and rated compound intensity and pleasantness on a

1–9-point scale. Results showed that anhedonia level was negatively correlated with olfactory function [$F(1,31) = 5.982$, $P = 0.0205$] and nearly negatively correlated with the percentage of odors perceived as pleasant [$F(1,31) = 4.023$, $P = 0.0540$]. Control analyses revealed no significant relationships between physiological age [$F(1,31) = 0.322$, NS] or menopausal age [$F(1,31) = 0.371$, NS] and olfactory function. This result suggests therefore that affective perturbations occurring in menopausal women are accompanied by both a decreased olfactory function and a shift in olfactory hedonism affecting mainly its pleasant side.

Poster Session Sat PM

Individual Differences in Processing Olfactory Information: Comparing Behavioral Measures to Self-report

Monique Smeets¹, Hendrik Schifferstein² and Sarai Boelema¹

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People's reactions to odors may be determined by *perceptual style*: a preferential style for processing sensory information. To assess individual differences in processing olfactory information we constructed the Odor Awareness Scale (OAS: study 1) and determined whether differences in OAS scores were correlated with behavioral endpoints of processing of olfactory information (study 2). Study 1: 332 respondents completed the OAS, asking about experiences with odors in everyday situations. Principal component analyses on 34 items revealed a 4-component solution explaining 39% of total variance. The components were labelled as General Odor Awareness, Body Odor, Alarm and Product Preference. Study 2: Respondents in the High Odor Aware (HOA) group (upper 25% of the scale), indicating increased awareness of odors in general, were compared to respondents in the Low Odor Aware (LOA) group (lower 25% of the scale), on nasal chemosensory performance using the Sniffin' Sticks, and processing of odor words using a lexical decision task (LDT). Preliminary results ($n = 24$) showed that the HOA group performed better on the Sniffin' Sticks task ($P = 0.05$), with expected group differences on the LDT still falling short of statistical significance. Thus, differences in self-reported awareness of odors may be correlated with differences in the processing of olfactory information. Results on a larger sample will be presented.

Supported by NWO VIDI grants 452-02-028 and 452-03-334.

Poster Session Sat PM

Effect of Contextual Information on Short-Term Olfactory Memory

Naomi Streeter and Theresa White

Le Moyne College

Odors are typically named for their sources. But does information about the source improve memory for an odor? Dual encoding Theory suggests that memory is enhanced when encoding occurs in more than one representational system, indicating that

congruent source information should improve odor memory. We investigated the influence of complex scenes and words on short-term memory for odors via congruent and incongruent exemplars that accompanied target odors. Participants were divided into 4 groups. All were presented with 6 odors accompanied by a visual stimulus that varied by group: congruent verbal label, incongruent verbal label, congruent complex scene, or incongruent complex scene. Participants knew a memory task would follow, but not that it would test odor recognition. The memory test contained the 6 target odors, as well as 6 distracter odors (half congruent and half incongruent to the visual stimuli). Participants judged each test odorant as old or new and rated their confidence. Preliminary results from 20 normosmic participants suggest that when odors are presented with information that is effectively redundant, olfactory information is discarded while other information is retained. Thus, people in the congruent groups did not remember target odors as well and were more easily deceived by congruent distracter odors than people in incongruent groups. These findings illustrate reliance on non-olfactory cues at encoding and suggest that source information might be retained to the exclusion of olfactory information.

Funded by the Le Moyne Student Research Fund.

Poster Session Sat PM

Role of Innate Information in Learning in the Moth *Manduca sexta*

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Animals can use innate information to locate their first sources of food; their subsequent feeding experiences later guide them, as well. Do animals learn about innately preferred odors more readily than others? Some animals have been shown to have more receptors for important odors; further, odors have vastly different volatilities. And, it has been shown that higher concentrations of odors are more effective for associative conditioning. To analyse the contributions of odor identity and intensity to learning, we prepared a set of host-plant (methyl salicylate, benzyl acetone and benzaldehyde), non-host plant ((+)- β -citronellene), and non-plant (cyclohexanone) odorants, and using an electroantennogram (EAG)-based method, mutually diluted the odorants to elicit responses of equivalent intensity. Before normalization, EAGs elicited by either undiluted odors or odors normalized by vapor pressure differed significantly in amplitude (ANOVA, $P < 0.001$ and $P < 0.005$, respectively). Using the EAG-normalized odorant set, we trained 75 moths in a classical conditioning, proboscis extension paradigm to associatively learn that an odor (conditioned stimulus, CS) predicts food reward (sucrose; unconditioned stimulus, US). Responses to the CS significantly increased during training (repeated measures logistic regression [RMLR], $P < 0.0001$); an unpaired, control group (1 min CS–US interval) showed no learning. We found no significant differences in learning among our odor set (RMLR, $P = 0.83$), suggesting any preferential responses to odors would be due to intensity differences, either from odorant volatility, or numbers of odorant receptors.

Poster Session Sat PM

Behavioral and Pharmacological Evidence for 2 Different Mechanisms of Habituation Learning in the Olfactory System

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Male mice readily investigate the odors of conspecifics, which is useful for identifying strangers versus familiars and dominants versus subordinates; however, males will habituate to the odors with repeated presentations. We examined the effect of manipulating intertrial interval length on habituation to a conspecific male's bedding odors. We habituated male mice using either 20 s or 5 m intertrial intervals (ITI). First, we found a significant difference in memory duration as a function of ITI length: mice habituated with 5 min ITIs remembered the odor for 30 min whereas mice habituated with 20 s ITIs remembered the odor for only 1 min. Second, we found that in agreement with recent electrophysiological data, habituation with very short ITIs was significantly impaired when class II/III metabotropic glutamate receptors were blocked (LY341495, 2.5 mg/kg IP), but not at the longer ITIs. Third, we found that blockade of NMDA receptors with the specific antagonist MK-801 (0.2 mg/kg IP) did not affect habituation at the shorter ITIs, but significantly impaired habituation at longer ITIs. Together, these results show that 2 pharmacologically distinct mechanisms underlying olfactory habituation exist in mice. All animal use was performed in accordance with NIH guidelines.

Funding: NBB Animal Behavior Grant and AAUW American Fellowship (A.M.M) and IOB0338981 (NSF) to C.L. and D.A.W.

Poster Session Sat PM

Is There a Simple Relationship between Odour Discrimination and Odour Memory

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Objective: To investigate the relationship between odour discrimination and memory. **Method:** Ten different well-known odours were presented in a 4AFC identification task to 279 subjects (78 males) younger and 229 subjects (73 males) older than 50 years of age. Half of the subjects in each age group was cued to remember 3 of the 10 odours, whereas the other half was not. Memory in both groups was tested with a 3AFC procedure. Two other groups of 20 young and 20 elderly Ss provided data on odour discriminability among the odours used as targets and distractors in the memory experiment. **Results:** Overall we find no effect of cued versus non-cued learning condition, but young Ss remember odours better than the elderly (Wilcoxon, $Z = -5.549$; $P < 0.000$). Young Ss outperform elderly in the discrimination task (86.1% vs 62.5% overall correct). However, for some targets, the young do not have a better memory than the elderly despite superior discrimination between these targets and their distractors. Furthermore, young Ss discriminate equally well between some targets and their distractors, while at the same time they remember one target better than the other. **Conclusions:** Despite having used well-known odours and explicitly

cued half of the Ss about the memory task, they did not perform better than the non-cued group, suggesting only a minor influence of semantic coding. Since we can only smell one odour at a time, any odour discrimination relies on some sort of memory. The double dissociation between odour discrimination and odour memory (over ~10 min) is consistent with the existence of a short term odour memory system.

Olfaction and Taste: Human and Animal Behavior

BDNF Modulation of Adult Neurogenesis: Impact on Neuroblast Migration and Survival as well as Spontaneous Olfactory Discrimination

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Neurotrophins are a class of molecules known to influence the development and survival of the nervous system. BDNF signaling is important for postnatal modification of the nervous system such as synaptic remodeling. In adult animals, application or overexpression of exogenous BDNF impacts neurogenesis, however whether BDNF is endogenously involved in regulating adult neurogenesis has not been clarified. Through the use of several genetically engineered lines of mice in combination with a new reagent, an antibody against phosphorylated TrkB receptor, we show that BDNF signaling through TrkB and not p75 is critical for adult OB neurogenesis. Activated TrkB receptors were most highly expressed in migrating neuroblasts (type A cells) implicating BDNF-TrkB signaling in the migration of neuroblasts. Using a newly developed transgenic line of mice, the BDNF Val66Met mouse, we show that secretion of BDNF and subsequent TrkB receptor activation is critical for OB neurogenesis. Finally, we show that the loss of these new cells leads to specific impairments in spontaneous olfactory discrimination. These observations identify BDNF-TrkB signaling as a critical mechanism for adult OB neurogenesis and proper functioning of the olfactory system.

Olfaction and Taste: Human and Animal Behavior

Wild Type Zebrafish That Were Selected Due To Their Ability to Discriminate Structurally Related Odorants Pass This Ability on to Their Offspring

Nika Fon Leben and Tine Valentincic

Univeristy of Ljubljana

The discrimination of olfactory stimuli is based upon convergent inputs from olfactory receptor neurons (ORNs) to the olfactory bulb where chemotopically organized mitral cells are activated. It is known that odorants having differential bulbar chemotopic organizations can be discriminated behaviorally. However, for fish, a degree of variability exists in olfactory discrimination capabilities for specific amino acid stimuli. We used the differential swimming of fish to conditioned and non-conditioned amino acid (AA) stimuli to investigate the discrimination capabilities of the zebrafish olfactory system. Wild (aquarium) zebrafish (*Danio rerio*) discriminated

nearly every AA from all other AAs except for 2 pairs of compounds: L-Ile/L-Val and L-Phe/L-Tyr. Only 3 of 10 zebrafish tested were capable of discriminating L-Ile from L-Val. First generation zebrafish ($n = 7$), raised by inbreeding those capable of discriminating L-Val from L-Ile, were, after reaching adulthood, able to successfully discriminate between these 2 AAs. Similarly, a transgenic zebrafish line (donated by R. Friedrich) that exhibits more distinct spatio-temporal activity patterns in the olfactory bulb for L-Phe and L-Tyr produced offspring that were also capable of behaviorally discriminating L-Phe from L-Tyr. This is the first direct evidence that fish selected either for their ability to behaviorally discriminate specific amino acids or selected due to their differential bulbar activity patterns to amino acids that are closely related structurally pass this ability on to their offspring.

Olfaction and Taste: Human and Animal Behavior

Entrainment of the Circadian System of the Newborn Rabbit by Pheromonal Cues

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In the rabbit the typical once-daily nursing is an effective non-photic synchronizer of the neonatal circadian system; pups display circadian rhythms at behavioral, physiological and molecular levels entrained by this vital event. However, information is lacking on the sensory stimuli mediating this. Here we ask if a pheromonal cue (2MB2) present in rabbit milk can entrain the pups' circadian system as measured by their rhythm in body temperature. On postnatal day 1 we implanted pups IP with telemetric sensors for continuous recording of core body temperature. Litters were randomly assigned to one of 3 groups: 1) fed normally by the mother at the same time each day, 2) hand fed on the same schedule as group 1, or 3) hand fed as for group 2 but simultaneously exposed to 2MB2. We used Fourier analysis to test for rhythmicity in pups' body temperature, including period and acrophase. The normally raised pups of group 1 showed clear rhythmicity as reported previously, with an anticipatory rise in temperature immediately before nursing; the hand-fed pups of group 2 showed weak, non-synchronized rhythmicity; and the hand-fed, pheromone-exposed pups of group 3 showed clear rhythmicity similar to the pups of group 1. Thus, we show for the first time that pheromonal cues can entrain the circadian system in this newborn mammal.

CONACYT 48504; PAPIIT IN226107; TWAS 04-155 RG/BIO/LA.

Olfaction and Taste: Human and Animal Behavior

Pharmacologic Antagonism of the Oral Aversive Taste-Directed Response to Capsaicin in a Mouse Brief Access Taste Aversion (BATA) Assay

Kyle Palmer, Daniel Long, Heather Devantier, Raymond Salemm and Robert Bryant

Redpoint Bio

Oral aversiveness of tastants is operationally defined as the suppression of taste-directed behavior. We used a mouse BATA assay with a Davis Rig to rapidly quantify the oral aversiveness of capsaicin and other ligands known to activate the transient receptor potential V1 (TRPV1) cation channel. Lick-rate dose-response functions for capsaicin, obtained in C57BL/6J mice, yielded an EC₅₀ of 1 μ M (0.5–1.5 μ M, 95% CI). Relative to other agonists, capsaicin was maximally effective at suppressing lick-rate. The order of potency for the oral aversiveness of TRPV1-related agonists was resiniferatoxin > capsaicin > piperine > olvanil. Further, we evaluated the ability of TRPV1 antagonists BCTC, AMG9810, and SB366971 to block the oral aversiveness of capsaicin. Inclusion of 1 μ M BCTC in the capsaicin solutions resulted in 2- to 3-fold rightward shifts of the capsaicin dose-response function. Lick-rate suppression by capsaicin at 100 μ M (the highest concentration tested) was completely blocked by 30 μ M BCTC. Similar antagonistic effects of BCTC were obtained against the oral aversiveness of piperine. Higher concentrations of AMG9810 were required to antagonize capsaicin, with 10 μ M being the lowest concentration that caused measurable shifts of the capsaicin dose-response function. Unexpectedly, SB366971 (up to 100 μ M) was ineffective at antagonizing capsaicin-mediated lick suppression. Our results represent the first demonstration of pharmacologic antagonism of a receptor-mediated oral aversiveness in taste-directed behavior.

Olfaction and Taste: Human and Animal Behavior

Construction of a Quantitative Taste-Preference Assay System and Investigation on Abnormal Feeding Behaviors of Transgenic Taste-Blind Medaka Fish

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Whether or not a vertebrate ingests food is critically dependent on taste stimuli received by taste bud cells. Small fish species with simple nervous systems would offer a good model for studying vertebrates' taste systems. We previously identified fish homologues of T1rs and T2r expressed in discreet subsets of taste bud cells, finding that both were co-expressed with Plc- β 2 as in the case of mammals. However, fish taste preference research needs some behavioral assay. Here we report a new device to quantify a taste-preference relation in medaka fish, *Oryzias latipes*, and its taste-blind mutant which has reduced taste receptor-mediated signals. First, we developed tastant-containing artificial foods in particle which were designed to float so that fishes could bite ad libitum for ingestion or ejection. Each food also contained fluorescent dye to quantify the intake by fluorometric observation with a microscope. To suppress the signaling for taste reception, we generated transgenic medaka fish which express a dominant-negative G α_{i2} mutant in taste receptor cells. The use of the devised assay system revealed that the transgenic fish showed abnormal behavioral reactions for amino acids and against denatonium. Our taste-preference assay system as well as the taste-blind fish would be useful also for investigation of higher-order taste information processing.

Olfaction and Taste: Human and Animal Behavior

Odor Detection of Ozone and *d*-Limonene: Reactants in Indoor Spaces

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Adult subjects who occupied a well-ventilated space with low background level of ozone achieved via carbon-filtration could detect ozone odor at 7 ppb, lower than expected from archival compilations. The outcome was not inconsistent, however, with some observations of recognition, beyond mere detection, at about 15–20 ppb. Individual differences in sensitivity lay at or just below an order of magnitude, rare in olfactory testing and indicative of precision. In a study of *d*-limonene, a common reactant with ozone indoors, subjects again showed high sensitivity and small individual differences. The subjects could detect the odor at 8 and 15 ppb, depending upon whether they occupied a space with or without carbon filtration, respectively. The protocol used here, with 1) collection of hundreds of judgments in a day per subject, yet with little net exposure of the subject to odorant, 2) verifiably stable delivery, and 3) analytical confirmation of level, should reduce tolerance for outcomes of large differences among subjects and among studies. Published databases, with errors of $\pm 1000\%$, often badly underestimate sensitivity and can thereby encourage use of higher concentrations of compounds than relevant in studies of reactive indoor chemistry.

Funding was provided by Phillip Morris International.

Olfaction and Taste: Human and Animal Behavior

Older Adults with the APOE4 Risk Factor for Alzheimer's Disease Show Altered Topographical Brain Response in an Odor Recognition Memory Task

Claire Murphy^{1,2} and Andrew Bender¹

¹San Diego State University and ²University of California

Olfactory function, and specifically olfactory recognition memory, is impaired in old age, dramatically more so in Alzheimer's disease. Individuals with the apolipoprotein E4 allele are at significant risk for developing Alzheimer's disease. The current study investigated the topographical ERP response in older adults with and without the APOE4 allele, while they performed a cross-modal odor recognition memory task. Participants were presented with a series of common odors for encoding before EEG was recorded from a multi-electrode array. During recording, subjects 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. ERP activity corresponding to memory performance was recorded as participants correctly identified targets, missed targets, misidentified foils as targets, or correctly rejected foils. Older adults with the E4 risk factor showed patterns of component brain activity during hits and correct rejections that were markedly different from those generated by the older adults without the E4 allele. The altered patterns of topographical ERP activity suggest altered brain response that may reflect the cortical substrate for differences in performance in those at genetic risk for Alzheimer's disease.

Supported by NIH grant DC02064 to C.M. We gratefully acknowledge contributions of John Polich, Paul Gilbert and the Life-Span Human Senses Laboratory.

Olfaction and Taste: Human and Animal Behavior

Pleasantness of Binary Mixtures

Hadas Lapid, Rehan Khan, David Harel and Noam Sobel

Weizmann Institute of Science

Although the rules underlying the perceived intensity of binary mixtures have been investigated, only minimal efforts have been directed at elucidating the rules underlying the perceived pleasantness of such mixtures. To address this, 11 subjects ranked the relative and absolute pleasantness of binary mixtures (18 pairs, ISI = 4 s, ITI = 30 s, flow = 5 l/min, pulse = 2.5 s) made up of different ratios (0:100%, 25:75%, 50:50%, 75:25% and 100:0%, olfactometer generated vapor phase) of 3 distinct pairs each consisting of one relatively pleasant and one unpleasant odorant (*L*-carvone–cyclohexanol, isoamylacetate–propylbutyrate, ethylbutyrate–isovaleric acid). Each trial was repeated twice with odorant order counter-balanced and trial order randomized. The perception of the mixtures varied in a near linear fashion from pleasant to unpleasant as a function of the relative ratios of the 2 components. The midpoint (50:50%) had decreased values in all mixtures, suggesting a sub-additive effect. Surprisingly, the increase in pleasantness following the addition of 25% pleasant odorant to an unpleasant odorant was significantly steeper than the decrease in pleasantness following the addition of 25% unpleasant odorant to a pleasant odorant [$F(1,10) = 9.8, P < 0.01$]. In other words, an unpleasant odor can “more easily” become pleasant than a pleasant odor can become unpleasant. We intend to continue our work, using these results in an effort to generate a predictive platform from mixture chemistry to mixture perception.

Parallel Processing by Multiple Olfactory Subsystems

Olfactory Neurons Expressing TRPM5 Are Involved in Sensing Semiochemicals

Diego Restrepo¹, Robert Margolskee², Gerald Donner³, Stefan W. Hell³, Anne Hansen¹ and Weihong Lin⁴

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Some olfactory sensory neurons (OSNs) in the main olfactory epithelium (MOE) respond to pheromones. Previously we determined that responses to pheromones in the main olfactory system were not eliminated in mice defective for the canonical cAMP transduction pathway and we suggested an involvement of phospholipase C (PLC). In the present study we obtain data consistent with expression of an effector of the PLC pathway—the transient receptor potential channel M5 (TRPM5)—in the MOE. In mice where the TRPM5 promoter drives expression of GFP we find abundant GFP expression in a subset of microvillar cells. In addition, a subset of OSNs expresses GFP. Using STED fluorescence microscopy—a novel technique allowing resolution below the diffraction limit—we find that TRPM5 is expressed in ciliary microdomains ~70 nm in

diameter. Importantly, OSNs expressing TRPM5 also co-express the cyclic nucleotide-gated channel (CNGA2) implying potential interactions between the pathways. These neurons project axons primarily to the ventral olfactory bulb where information from socially relevant signals is processed. We find that these chemosignals activate glomeruli targeted by TRPM5-expressing OSNs. Thus, TRPM5-expressing OSNs are involved in processing information from semiochemicals.

Funded by DC00566, DC004657 and DC006070 (D.R.), DC03055 and DC03155 (R.M.), DC07732 (A.H.) and DC006828 (W.L.) and Exzellenzfond grant from the Max Planck Society (S.W.H.).

Parallel Processing by Multiple Olfactory Subsystems

Detection of Carbon Dioxide at near Atmospheric Level by a Specialized Mammalian Olfactory Subsystem

Ji Hu¹, Chun Zhong¹, Cheng Ding¹, Qiuyi Chi², Hiroaki Matsunami² and Minmin Luo¹

¹National Institute of Biological Science and ²Duke University Medical Center

Carbon dioxide (CO₂) is an important environmental cue, but it remains unclear whether it can be sensitively detected by the mammalian olfactory system. Here we show rich expression of carbonic anhydrase II (CAII) in a subset of olfactory receptor neurons containing elements of a c-GMP-mediated signaling cascade and projecting to the necklace glomeruli in the olfactory bulb. Wild-type mice detected CO₂ with thresholds near atmospheric level (0.066%) but CAII mutants had profound deficits. Physiological recordings and c-Fos labeling revealed activation of the necklace glomeruli by CO₂. Recordings identified bulbar neurons with CO₂ response sensitivity comparable to behavioral threshold. These neurons also responded to pheromones, suggesting integration of CO₂ and pheromonal signals. T-maze assays demonstrated negative valence of CO₂ for mice. We conclude that mammals detect CO₂ at near atmospheric level through a specialized olfactory pathway.

Parallel Processing by Multiple Olfactory Subsystems

The Grueneberg Ganglion—A Novel Chemosensory Organ in the Nose?

Joerg Fleischer, Karin Schwarzenbacher, Nicole Hass, Stefanie Besser and Heinz Breer

University of Hohenheim

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.

Parallel Processing by Multiple Olfactory Subsystems

What Can We Learn from the Septal Organ?

Minghong Ma

University of Pennsylvania

Sensory neurons in most systems are primarily specialized to detect one modality. We recently discovered that the olfactory sensory neurons in the mouse septal organ can detect 2 distinct modalities transmitted by chemical and mechanical stimuli. As revealed by patch-clamp recordings, most septal organ neurons (70%) respond not only to odorants, but also to mechanical stimuli delivered by pressure ejections of odor-free Ringer's solution. Similar mechanosensitivity also exists in 50% of the neurons in the main olfactory epithelium. The mechanical responses directly correlate with the pressure intensity and exhibit several properties similar to those induced by odorants, including onset latency, reversal potential and adaptation to repeated stimulation. Blocking adenylyl cyclase or knocking out the cyclic nucleotide gated channel (CNGA2) eliminates the odorant and mechanical responses, suggesting both are mediated by a shared cAMP cascade. We further demonstrate that the mechanosensitivity enhances the firing frequency of individual neurons when weakly stimulated by odorants and likely drives the rhythmic activity (theta oscillation) in the olfactory bulb to synchronize with respiration.

Supported by NIDCD/NIH.

Parallel Processing by Multiple Olfactory Subsystems

Deconstructing Smell

Linda Buck

Fred Hutchinson Cancer Research Center

We are interested in how mammals detect odorants and pheromones and how the brain translates those chemicals into diverse perceptions and instinctive behaviors. We found that odorants are detected in the nasal olfactory epithelium (OE) by ~1000 different odorant receptors (ORs). We and others also identified 2 smaller families of candidate pheromone receptors in the vomeronasal organ (VNO). Our studies showed that ORs are used combinatorially to encode odor identities. Exploring the patterning of OR inputs, we found that each sensory neuron in the OE expresses a single type of OR and that neurons with the same OR are scattered in one zone, but synapse in a stereotyped fashion in OR-specific glomeruli in the olfactory bulb. At the next level, the olfactory cortex, we discovered another stereotyped map of OR inputs, but here signals from different ORs are targeted to

partially overlapping clusters of neurons and single neurons receive combinatorial OR inputs. By comparing responses to binary odorant mixtures versus their components, we found evidence that the cortical neurons act as coincidence detectors whose activation requires combinatorial OR inputs. This may provide an initial step in the reconstruction of an odor image from its deconstructed features. To explore how pheromones alter reproductive physiology and behavior, we made mice expressing a transneuronal tracer in gonadotropin releasing hormone (GNRH) neurons. These studies revealed that GNRH neurons receive pheromone signals from the OE as well as the VNO. We recently discovered a second class of chemosensory receptors in the OE, called TAARs, that recognizes at least one pheromone and may also be involved in the detection of other social cues by the OE.

Poster Session Sun AM

The Mouse NST: A Cytoarchitectonic Atlas

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¹Tel-Aviv University, ²Hebrew University and ³University of California–San Diego

The murine model in molecular biology has provided insights into neural development, structure and function. Regarding the mouse taste system, a standard cytoarchitectonic reference for the nucleus of the solitary tract (NST) would facilitate comparisons, for example, between normal and transgenic mice, and between connectional and marker expression patterns. Nissl-stained, 40 μ m sections of the 2 mm rostro-caudal extent of the NST of C57BL/6J mice were parcellated into subnuclei based on the shape, size and distribution of cells, and location of subnuclei relative to the solitary tract. NST nomenclature follows that in hamster. Subnuclei were further characterized after Cholera-toxin B (Ctb) labeling of chorda tympani inputs, or of connections with the parabrachial nuclei (PBN). Subnuclei include a dense collection of small cells dorsally in the NTS (rostral-RC and caudal-CC central subdivisions) medial to the tract, subdivisions with sparser cellularity medial (M) and lateral (RL and CL) to these, and large cell regions ventrally (V and VL). RC received densest input from the chorda tympani; primary afferent endings extended from 1 mm rostral to the obex to the level of the rostral area postrema. RL and VL received sparse endings. The terminal field at the rostral pole of NST abuts the dorsomedial spinal trigeminal n. oralis. Retrograde Ctb labelling of PBN projection cells was heaviest in RC and CC.

Support: NIH ROI DC01091.

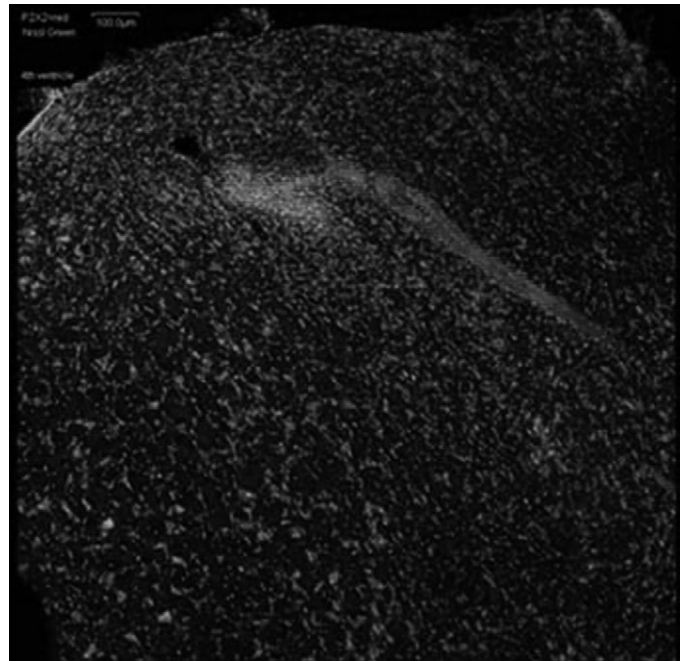
Poster Session Sun AM

Immunohistological Map of the Nucleus of the Solitary Tract (NTS) in Mice

Dianna L. Bartel¹, Mark C. Whitehead² and Thomas E. Finger¹

¹University of Colorado Health Sciences Center and ²University of California–San Diego

The nucleus of the solitary tract (NTS) is a complex structure which contains numerous subnuclei serving a variety of functions, only some of which are gustatory. To understand such a structure it is necessary to develop careful anatomical, histochemical and



functional maps of the different areas. Accordingly, we are using immunochemical probes for a variety of substances to map the various territories of the NTS. Since P2X receptors on primary gustatory afferents are essential for communication in this system, we used antisera directed against the C-terminus of the P2X receptors to delimit the primary afferent terminal areas in the NTS. The presence of these receptors was not restricted to gustatory regions as staining was also noted in the area postrema and other carotid body afferent projections, a chemosensory system also known to be purinergic. P2X2 results in more robust staining and was used to routinely label the gustatory NTS in the presence of various other immunohistochemical neuronal markers such as GAP-43, VGlut, Nissl and glial markers GFAP and Iba1. Our map is a baseline anatomical map of the gustatory NTS which can be compared to NTS after peripheral nerve damage.

Supported by NIH grants to T.E.F. and M.C.W.

Poster Session Sun AM

Juxtacellular Labeling as a Technique for Studying Structure–Function Relationships in the Nucleus of the Solitary Tract

Andre Roussin¹, Patricia Di Lorenzo¹, Andrew Rosen¹ and Laura Schweitzer²

¹Binghamton University and ²Bassett Healthcare

Previous anatomical studies of the nucleus of the solitary tract (NTS) in rats have characterized cells into 3 types depending on the shape and size of their soma and the number and orientation of their dendrites. More recent studies have revealed a more complex categorization scheme including spine density and breadth of tuning as important defining characteristics. Juxtacellular labeling techniques were used in the present study to mark NTS cells from which we had recorded electrophysiological responses to

representatives of the 4 basic taste qualities (NaCl, HCl, quinine and sucrose) in rats. Rats were anesthetized and prepared for electrophysiological recording in the NTS. Extracellular responses to taste stimuli were recorded from 12 single cells thus far. These cells were then labeled with biocytin or neurobiotin by passing positive current pulses (400 ms on/off) through the micropipette used for recording. Spike activity was entrained to the electrical stimulation in all cases. The locations of these cells, as well as the size and shape of the soma and the length and orientation of primary and secondary dendrites were determined. Results indicate that juxtacellular labeling can be an effective method for identifying taste-responsive cells in the NTS without cellular damage or the suppression of activity associated with barbiturate anesthesia.

Supported by NDCD grant DC005219 to P.D.L.

Poster Session Sun AM

Ultrastructural Analysis of Synaptic Organization of Chorda Tympani Nerve in Normal Developmental Rats

Siting Wang, Alev Erisir and David Hill

University of Virginia

We recently showed that the terminal field of the chorda tympani nerve (CT) in the nucleus of the solitary tract (NTS) decreases in size with postnatal age in rat. In particular, the dorsal zone of the chorda tympani field changes the most during development through an apparent "pruning" of exuberant processes. With the use of electron microscopy, we examined the synaptic targets of CT nerve in the NTS during normal development. Biotin dextran amine was used to reveal the CT terminals in NTS of rats aged postnatal day 15 (P15), P25, P35 and >P50 (adult, $n = 3$ for each age group). GABA postembedding reaction was used to reveal the GABA contents of the postsynaptic targets. Our results showed that CT primarily contacted non-GABAergic dendrites (more than 90%) in all age groups. Furthermore, there was an age-related decrease in the percentage of GABAergic contacts (9.1% for P15; 5.1% for P25; 4.5% for P35; 3.3% for adults). Since the same period is marked with proliferation of GABAergic cell dendrites, the rapid decrease in GABAergic targets between day 15 and day 25 cannot be explained by the loss of targets. It may rather indicate a selective pruning of CT synapses on GABAergic circuitry, and corresponds well with the CT terminal area decrease.

Supported by NIH grant R01 DC00407.

Poster Session Sun AM

Persistent Injury Induced Decrease of the Chorda Tympani Terminal Field in the NTS of Adult Rats

Rebecca Reddaway and David Hill

University of Virginia

Unilateral chorda tympani nerve section (CTX) produces persistent morphological changes in the peripheral and central taste systems in adult animals. CTX leads to a 20% reduction of CT axons in the regenerated nerve and a decrease in ipsilateral fungiform taste bud volume at more than 50+ day post injury. In addition, preliminary data from our lab indicates a dramatic reduction of the CT terminal field volume in the central target, the nucleus of the solitary tract (NTS).

In order to confirm and to extend the preliminary data of the central effects of CTX, we labeled the CT with an anterograde tracer at multiple periods following injury in order to detect changes in the morphology of CT terminal field. Degeneration of CT terminal field is statistically significant at 14 days following CTX. Despite peripheral reinnervation of taste buds by 40 days postsection, the volume of nerve terminal field 60+ days following CTX remains shrunken to about half of that seen in control animals, and is similar to that in animals 14 days following CTX. These findings indicate that nerve transection in adult animals leads to dramatic and permanent degeneration of primary sensory nerve terminal field. It is our aim to build on these results to further characterize the mechanisms underlying injury-induced changes in sensory nerve terminal fields in the brainstem.

Supported by NIH grant DC006938.

Poster Session Sun AM

TRPV2 Expression in Geniculate and Petrosal Ganglia and the Rostral Nucleus of the Solitary Tract

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TRPV2 immunoreactive neurons have been demonstrated in the dorsal root and trigeminal ganglia and in the dorsal horn of the spinal cord. We have now demonstrated expression of TRPV2 in the geniculate and petrosal ganglia as well as in the rostral nucleus of the solitary tract (rNST), using fluorescent immunolabeling combined with retrograde tracing in rats. Alexa Fluor 488 was applied to the chorda tympani and lingual-tonsillar branch of the glossopharyngeal nerve. After fixation with 4% paraformaldehyde brainstem and ganglia were removed and sectioned. Sections were immunoreacted with antibodies to TRPV2 using tyramide amplification. In both the geniculate and petrosal ganglia subpopulations of neurons stained intensely with the TRPV2 antibody. The afferent glossopharyngeal fibers and terminal fields in the rNST also reacted strongly to the antibody. A few large rNST neurons stained weakly with the antibody. Motoneurons of the nucleus ambiguus showed strong immunolabeling for the TRPV2 antibody. In addition to thermal reception, TRPV2 has been hypothesized to contribute to a variety of other functions. Since the TRPV2 positive fibers terminate in all rNST subdivisions it is possible that they may participate in the neuromodulation of synaptic transmission as demonstrated for TRPV1 receptor activation in the caudal NST.

Support by: NIH grant DC000288 to R.M.B.

Poster Session Sun AM

Taste Information from Both Sides of the Tongue Converge on the Neurons in the Nucleus of the Solitary Tract

Young K. Cho¹ and Cheng-Shu Li²

¹Kangnung National University and ²Southern Illinois University

The chorda tympani (CT) fibers carry taste information from the anterior tongue ipsilaterally to the nucleus of the solitary tract (NST). However, no subjective change occurs in taste experience after unilateral CT damage. The "release of inhibition" theory

explains this phenomenon (taste constancy) which emphasizes that within the NST there are inhibitory interactions between inputs from the anterior (CT nerve) and posterior (IXth nerve) tongue, and that the loss of taste information from the CT nerve is compensated by a release of inhibition on the IXth nerve input. However, the possibility of compensation by taste input from the contralateral side has never been investigated. We examined if taste cells in the NST receive taste information from the contralateral side of the tongue. After a taste neuron was isolated in the NST, the contralateral CT nerve was stimulated. This stimulation activated 46 of 95 taste cells; 42 were excitatory and 4 were inhibitory. Responses to the contralateral CT nerve stimulation were not affected by anaesthesia of the PbN, but were blocked by that of the NST in the CT nerve stimulating side. The taste responses of NST cells were reduced by blockage of the contralateral NST. These results demonstrate that gustatory neurons in the NST receive taste information from both sides of the tongue and that the taste input from the contralateral side of the tongue pass through the NST.

Supported by NIDCD006623 to C.-S.L.

Poster Session Sun AM

Characterization of Synaptic Potentials at the First Synapse in the Central Taste Pathway

M. Wang and R.M. Bradley

University of Michigan

Central taste processing first begins in synaptic circuits of the rostral solitary tract nucleus (rNST). Postsynaptic potentials (PSP) elicited from unidentified rNST neurons by stimulation of the solitary tract (ST) are composites of excitatory and inhibitory PSPs. To investigate the properties of the synapse between afferent taste input and identified second order rNST neurons, we used afferent fluorescent tracing and latency measures. An anterograde fluorescent tracer was used to label the chorda tympani synaptic field around unlabeled rNST neurons. Unlabeled neurons were imaged using DIC microscopy and patch clamped in brain slices. Current stimulation was applied to the ST to elicit PSPs. Two types of PSPs were recorded. One was characterized by a graded amplitude to increasing stimulus magnitude. The second had an all-or-none response to increasing current stimulation. Measures of the variability in the shock-to-shock PSP latency (jitter) to trains of ST stimulation (0.5–50 Hz) were used to confirm that the synapses were monosynaptic. Most rNST neurons surrounded by fluorescently labeled terminals had short latency PSPs of about 2.5–4.8 ms and small jitter <200 ms, characteristics of second order monosynaptically connected neurons. In addition all PSPs exhibited frequency dependent depression to stimulus frequencies that exceeded 50 Hz. These results suggest that second order rNST neurons respond to afferent input with 2 patterns of PSPs that would influence transmission of gustatory information.

Support by: NIH grant DC000288 to R.M.B.

Poster Session Sun AM

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

Andrew M. Rosen and Patricia M. Di Lorenzo

Binghamton University

Previous electrophysiological studies of the nucleus of the solitary tract (NTS) in the rat have demonstrated that stimulation of the chorda tympani (CT) nerve, both electrical and chemical, results in inhibition that can alter the responsiveness to the basic taste qualities. Inhibition resulting from CT stimulation may also modulate the temporal parameters of the underlying neural code for taste. In the current study we electrically stimulated the CT nerve using a paired-pulse (0.1 ms) paradigm while recording from single taste-responsive neurons in the NTS. Stimulating electrodes were implanted in the middle ear of urethane anesthetized rats for passage of current across the CT. Single taste-responsive cells were identified by significant responses to taste stimuli (0.1 M NaCl, 0.01 M HCl, 0.01 M quinine and 0.5 M sucrose). Paired-pulse stimulation of the CT (interpulse interval range = 10–2000 ms) was then applied in blocks of 100 trials. Consistent with the effects of paired CT pulses on NTS evoked field potentials, preliminary results show that paired CT pulses in single taste-responsive NTS cells also produced paired pulse suppression. In addition, paired pulsed facilitation was also observed in some cells. Individual cells show differential time courses for these effects, suggesting that they may play a role in the interpretation of incoming, temporally encoded information.

Supported by NIDCD grant DC005219 to P.M.D.L.

Poster Session Sun AM

The Role of Group III Metabotropic Glutamate Receptors in Transmission of Gustatory Inputs to the Brainstem

R.M. Hallock and T.E. Finger

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Primary taste afferents use glutamate as the transmitter at their central terminals in the nuc. solitary tract. We have investigated the role of metabotropic glutamate receptors (mGluR) in regulating transmission in the primary gustatory nucleus of goldfish. In an *in vitro* slice preparation, the primary gustatory afferents are electrically stimulated, while evoked dendritic field potentials (fEPSP) are recorded in the sensory layers wherein the afferents terminate. fEPSPs consist of N1, the initial spike from activation of fibers, as well as N2, the activation of second-order cells, and N3 due to activation of higher-order neurons. Application of broad agonists to group I (DHPG; 100 μ M) and group II (APDC; 10 μ M) metabotropic receptors had no effect on the fEPSP, although application of the broad group III mGluR agonist, L-AP4 (2.5 or 25 μ M) significantly attenuated N2 and N3 potentials. L-AP4 also significantly prevented the decrease in paired-pulse inhibition, suggesting that mGluRs are located presynaptically. More specific agonists suggested that only mGluR8 was mediating this effect: PPG (1 and 10 μ M), an mGluR8 agonist, attenuated N2 and N3, while agonists for mGluR6 (homoAMPA; 100 μ M) and mGluR7 (AMN-082; 1 μ M) did not. Lastly, PCR results confirmed the presence of mGluR8 mRNA in the vagal ganglion. Collectively, these results show that the group III mGluRs may be present presynaptically

on the primary gustatory nerve terminals, and that they function to modulate transmission of afferent information in this system.

Supported by NIH grant RO1 DC 00147.

Poster Session Sun AM

Contribution of the T1R3 Taste Receptor to the Response Properties of Central Gustatory Neurons in Mice

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We explored how an absence of the T1r3 taste receptor impacts responses to sweets in neurons of the nucleus of the solitary tract (NST). The consequences here bear on how taste networks in the brain are configured to handle input mediated by T1r3 and on the question of multiplicity of taste receptors for sweets. Taste responses (spikes/3 s) to effective concentrations of glycine, NaCl, HCl and quinine were recorded from single NST neurons in anesthetized T1r3 knockout (KO) and C57BL/6 (B6) control mice. Most cells were also tested with L-proline, sucrose, fructose, glucose, sorbitol, Na-saccharin, acesulfame-K, MSG, NaNO₃, Na-acetate, citric acid, KCl, denatonium and papaverine. Data have been acquired hitherto from 17 KO and 12 B6 neurons. Responses to sweets were suppressed in KO cells ($P < 0.05$). Yet the degree of response suppression varied among stimuli, ranging from 92% for 1 M sucrose to 53% for 1 M glycine. This suggests that T1r3-independent receptors contribute in part to sweet responses in NST cells. Input from T1r3 is necessary to establish NST cells that respond optimally to sweets, as such neurons were prominent (67% of cells) in B6 mice but seldom found (6%) in KOs. Yet sweet-optimal B6 cells varied widely in their sensitivities to non-sweet stimuli (entropy range = 0.36 [sweet specific] < 0.64 ± 0.22 [mean ± SD] < 0.99 [unspecific]), suggesting that a heterogeneous pool of central neurons processes taste input from T1r3.

Support: NIH DC008194, DC00353, DC003155.

Poster Session Sun AM

Gustatory Cortex Neurons Respond to the Reward Value of Sucrose Independently of Taste Signaling

Ivan de Araujo, Albino Oliveira-Maia, Tatiana Sotnikova, Raul Gainetdinov, Miguel Nicolelis and Sidney Simon

Duke University

Post-ingestive effects can influence food preferences independently of palatability, but the neurobiological bases of such mechanism are poorly understood. We show that mice lacking the machinery for sweet taste transduction (*trpm5*^{-/-}) can develop a preference for sucrose solutions based solely on caloric content, an effect that was related to increased brain dopamine levels. Furthermore, neuronal adaptations occurred in gustatory cortex, expressed as changes in both population activity levels and spike distribution, as these animals developed preferences for sucrose. We conclude that the primary taste cortex plays a more integrative role in feeding behavior that is not limited to chemosensory processing.

Supported by Phillip Morris USA, Phillip Morris International, NIH to S.S.

Poster Session Sun AM

Responses of the Chorda Tympani Nerve to NaCl following Brief Dietary Na⁺ Deprivation with NaCl Repletion

Joanne Vaughn and Robert Contreras

Florida State University

In rats, dietary sodium deprivation increases their consumption of normally avoided, highly concentrated NaCl solutions. Na⁺ deprivation also decreases the chorda tympani nerve (CT) responses to NaCl, suggesting that changes in CT responses may be necessary for increased NaCl intake. Data from our laboratory show that 2 days of Na⁺ deprivation leads to a pronounced licking response to 0.5 M NaCl. Interestingly, this increased licking occurred only during the first NaCl bout in which Na⁺-deprived rats consumed roughly 4 ml, while remaining NaCl bouts were similar to controls. This suggests that dietary Na⁺ deprivation-induced changes in NaCl taste may persist until repletion of body NaCl levels. The goal of this study was to determine if intragastric infusion of NaCl in Na⁺-deprived rats is sufficient to restore CT responsiveness to NaCl to control levels. We recorded whole nerve electrophysiological responses from the CT to lingual application of NaCl (75, 150, 300, 450, 600 mM) before and after a 4 ml intragastric infusion of 0.5 M NaCl. Previous results from our laboratory indicate that CT responses of Na⁺-deprived rats are reduced compared with controls. Preliminary data indicate that the NaCl infusion had no effect on CT responses of Na⁺-deprived or control rats. However, sample size needs to be increased before firm conclusions can be made. Currently, it appears that infusion of a NaCl load equivalent to the amount ingested following brief dietary Na⁺ deprivation is insufficient to restore CT responsiveness to NaCl.

Supported by NIH grant DC 04785.

Poster Session Sun AM

Potassium Deprivation Produces a Chloride Appetite in the Rat

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Potassium deprived (KDep) rats avidly consume KCl, NaCl, and CaCl₂ in long-term tests, but it is not known whether this is due to associative or unlearned factors, such as changes in taste sensitivity. In previous 20-s access tests, which preclude post-ingestive effects, KDep rats licked more for Cl⁻ salts but not for non-Cl⁻ salts than replete rats, suggesting an unlearned Cl⁻ appetite. However, both KDep and replete rats licked maximally (behavioral ceiling) for several tastants, potentially masking some group differences. We therefore evaluated licking for a variety of salts using stronger concentrations to reduce palatability and ceiling effects. Replete ($n = 8$) and KDep ($n = 8$) rats were fluid deprived, and licking for an array of tastants was recorded during 20-s access tests. Urine K⁺ levels and 24 h dH₂O/KCl 2-bottle tests were used to confirm K⁺ deficiency. KDep rats licked significantly more 0.5 M KCl, 0.5 M CsCl, and 0.5 M NaCl than did replete rats ($P < 0.05$), with no effect on licking for water, 0.5 M KHCO₃, and 1 mM

quinine-HCl. Addition of 200 μM amiloride, an epithelial Na^+ channel (ENaC) blocker, or 100 μM ruthenium red, a vanilloid receptor 1 (VR-1) antagonist, to NaCl, or 50 μM 4-aminopyridine, a K^+ channel blocker, to KCl, failed to block the K^+ -deprivation-induced increase in NaCl and KCl licking, respectively. These findings suggest that K^+ -deprivation produces a chloride appetite guided by unlearned factors; this response appears not to be mediated by ENaC, VR-1, or K^+ channels.

Supported by Amherst College and NIH DK-46791.

Poster Session Sun AM

Amiloride Blunts the Saltiness of NaCl after Adaptation to NaCl

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¹VCU and ²VAMC

Adapting the tongue to NaCl allows humans to distinguish sodium salts from other salts by eliminating the saltiness of non-sodium salts. We hypothesized that the epithelial sodium channel, ENaC, may play a role in the perception of saltiness after NaCl adaptation. To investigate this possibility we employed a protocol similar to that of Smith and van der Klaauw. On experimental days, subjects' tongues were adapted to 3 solutions: distilled water, 100 mM NaCl and 100 mM NaCl + 10 μM amiloride, an ENaC inhibitor. 10 μM amiloride is not tasted and is a partial inhibitor of lingual ENaC. After adaptation, tongues were exposed to 125 mM NaCl and 125 mM KCl, and subjects provided magnitude estimates of the component taste qualities (sweet, salty, sour and bitter). After water adaptation, NaCl was characterized as salty and slightly sour. KCl was also characterized as salty, but other qualities (sweet, sour and bitter) were present. After NaCl adaptation, NaCl remained salty, while KCl became bitter and far less salty. Amiloride addition to the adapting NaCl solution blunted the saltiness of NaCl by 40% ($P < 0.01$) and enhanced the bitterness of KCl by 11% ($P < 0.02$). These observations indicate that following NaCl adaptation an amiloride sensitive pathway is important for humans to perceive Na as salty. Perhaps earlier studies, which detected only a minimal effect of amiloride on salt taste, did so because tongues were adapted to water only.

This work is funded by the Department of Veterans Affairs.

Poster Session Sun AM

Oral Factors Mediating Equimolar NaCl and LiCl Taste Discrimination in Rats

Rebecca Dailey and John-Paul Baird

Amherst College

Sodium chloride (NaCl) and lithium chloride (LiCl) are inorganic salts with different biological properties but similar psychophysical taste qualities. For example, sodium deprived rats avidly consume LiCl even though it is potentially toxic. Further, oral LiCl and NaCl solutions produce similar electrophysiological neural responses that can be inhibited by the epithelial sodium channel blocker, amiloride (AMIL). Nevertheless, rats learn to discriminate equimolar concentrations of NaCl and LiCl in long-term taste tests independent of postgestive associations, suggesting an orosensory difference. We

used our recently developed conditioned taste aversion (CTA) rapid generalization test to isolate differences in LiCl and NaCl taste detection. Groups of water-deprived rats were offered 0.12 M LiCl solutions for 8 min (T1) followed by 8 min access (T2) to a variety of 0.12 M NaCl or LiCl taste solutions with or without 100 μM AMIL and/or 3.5 mM lanthanum, a tight junction blocker. Results show that licking suppression for T1 LiCl rapidly generalized to a T2 LiCl + AMIL solution but not to a T2 NaCl + AMIL solution, suggesting a differential LiCl-NaCl sensitivity across amiloride insensitive channels. Interestingly, the suppression induced by a T1 LiCl + AMIL solution did rapidly generalize both to T2 NaCl and T2 NaCl + AMIL solutions. Rapid generalization to NaCl was lost when a T1 LiCl + AMIL + lanthanum solution was offered. Overall, results suggest that LiCl solutions do not activate epithelial amiloride-insensitive channels and that NaCl and LiCl may differentially stimulate basolateral channels of taste receptor cells.

Poster Session Sun AM

After Chorda Tympani Nerve Transection, Rats Relearn a Presurgically Trained NaCl versus KCl Taste Discrimination Using Remaining Gustatory Input

Ginger Blonde, Mircea Garcea, Enshe Jiang and Alan Spector

University of Florida

Discrimination of NaCl versus KCl is strongly impaired during the first week of testing after chorda tympani (CT) transection in rats, but remains above chance. Glossopharyngeal nerve (GL) transection is inconsequential. Here, rats that were presurgically trained to discriminate NaCl versus KCl in an operant taste task were tested for ~30 sessions in 6 weeks after either CT transection (CTx, $n = 7$), CT and greater superficial petrosal nerve (GSP) transection (7x, $n = 8$), CT and GL transection (CTx + GLx, $n = 4$), or sham surgery (SHAM, $n = 7$; SHAM-INT, $n = 8$). The stimulus concentrations were postsurgically lowered for SHAM-INT rats. Performance by CTx, CTx + GLx, and 7x rats was significantly impaired during the first week after surgery. Over time, however, the performance of the rats with CTx or CTx + GLx improved significantly to near presurgical levels and was just as affected by amiloride as was the SHAM groups'. Performance by 7x rats remained at chance throughout postsurgical testing. SHAM-INT rats performed similarly to SHAM rats during the first week of postsurgical testing and recovered to presurgical levels by the second week. Thus, the effect of the nerve transections on performance is not easily explained by a postsurgical decrease in stimulus intensity. Apparently, rats with CTx can relearn the task using remaining gustatory input. The results implicate the GSP, but not the GL, as providing sufficient input for this process. Histological confirmation of the surgeries is in progress.

Supported by NIDCD R01-DC01628.

Poster Session Sun AM

Differential Effects of Fructose and Glucose on Intake Behavior in Rats

Keiichi Tonosaki

Meikai University, School of Dentistry

Fructose, glucose and sucrose are quite commonly found in soft drinks and baked goods and we can easily discriminate their quality of the sweetness each other. Receptor genes for sweet taste stimuli including sugars were identified as T1Rs. Then, the question arises. Are all sweet receptor pathway of taste cells identical or do multiple pathways exist? We have addressed this question in this report. Since it is believed that no significance differences were observed between either fructose or glucose diet. Recently, it has been reported that fructose consumption may induce some adverse side effects on the diabetes mellitus and normal aging process. Here we show some typical characteristics of fructose. 1) Behavioral experiment of rats with 2-bottle preference tests (fructose vs glucose) showed relative preference of glucose solution increasing day by day, while fructose solution exclusively with in a week. 2) The plasma insulin release (PIR) and plasma glucose level (PGL) parameters were significantly increased rapidly to glucose drinking rat, but not to fructose. Consequently, fructose feeding may decrease glucose tolerance and increase insulin resistance. 3) The magnitude of chorda tympani (CT) nerve responses to "fructose plus sucrose" and "fructose plus glucose" were prominently greater than that of "glucose plus sucrose." From our results, sugars such as fructose, glucose and sucrose have different characteristics each other.

Poster Session Sun AM

The Effects of Greater Superficial Petrosal Nerve Transection in Rats on Licking Responses to Sucrose and Putative Sweet-Tasting Amino Acids in Brief-Access Taste Tests

Enshe Jiang, Ginger Blonde, Mircea Garcea and Alan Spector

University of Florida

The rat greater superficial petrosal nerve (GSP) is exceptionally responsive to sucrose. To examine the role of the GSP relative to the chorda tympani nerve (CT), we conducted series of brief-access licking tests (5-s trials) in nondeprived rats using a range of concentrations of sucrose, L-alanine, glycine, and L-serine, with and without 1.0 mM inosine monophosphate (IMP) added, before and after SHAM surgery ($n = 6$), or transection of the CT (CTX, $n = 5$) or GSP (GSPX, $n = 5$). The group sizes reflect those rats that had histologically confirmed transections and initiated at least 2 trials for each concentration. Postsurgically, all groups showed significant concentration-dependent increases in licking to all stimuli. The CTX group had lower licking responses to some of the higher concentrations of sucrose (but only in the first block of postsurgical sucrose sessions), alanine, and alanine + IMP. Other than this, there were no major differences between the surgical groups. Overall, after GSPX, the input from the remaining nerves is apparently sufficient at maintaining normal concentration-dependent licking for all stimuli in this task. Based on prior work, it appears that both the CT and GSP must be removed for major changes in the sucrose concentration-response function in brief-access tests to be observed. The disparity between our results and those from the literature possibly relates to the degree of risk of inadvertent damage to the CT during GSPX surgery.

Supported by NIH R01-DC01628.

Poster Session Sun AM

Effect of IMP on Behavioral Response to D-Alanine in Mice

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Recent in vitro heterologous expression studies showed that L-amino acids activate the mouse T1R1 + T1R3 receptor, while D-amino acids activate the mouse T1R2 + T1R3 receptor. D-Alanine (D-Ala) has unusual properties. Like other D-amino acids, it activates T1R2 + T1R3 but not T1R1 + T1R3 receptor. However, D-Ala mixed with IMP activates T1R1 + T1R3 receptor. This suggests that addition of IMP changes taste quality of D-Ala. We tested this hypothesis using a conditioned taste aversion (CTA) technique. Separate groups of C57BL/6J mice were exposed to 50 mM D-Ala with or without 2.5 mM IMP or to water (control) and injected with LiCl to form CTA. Conditioned mice were presented with 5 basic taste solutions, L-methionine (Met) and D-Ala, and their lick responses were recorded. An aversion to D-Ala generalized to sucrose, while an aversion to D-Ala mixed with IMP generalize not only to sucrose but also to a mixture of 50 mM MSG, 2.5 mM IMP and 30 μ M amiloride (added to block sodium taste). This suggests that as predicted by the in vitro study, addition of IMP changes taste quality perception of D-Ala in vivo.

Supported by Fisheries Research Agency (Yokohama, Japan) Research Overseas Program (Y.M.), Ajinomoto Amino Acid Research Program grant (A.B.) and NIH grant DC 00882 (G.B.).

Poster Session Sun AM

The Nature of Fragrance Preferences in Young Women

Marie-Paule Bensoussan and Robin Freyberg

Yeshiva University

The current study explored the basis of fragrance preferences in adolescent girls. In a preliminary study, adolescent girls rated the pleasantness of their favorite perfume and an alternative perfume that they were given. Participants perceived the alternative fragrance to be significantly less pleasant than their favorite perfume, $t(26) = 5.94$, $P < 0.001$. The authors investigated this difference in pleasantness by analyzing participants' qualitative responses describing why they chose their current favorite perfume. Results revealed that participants with self references in their explanations reported greater differences in perceived pleasantness than participants who incorporated other types of references, $F(1,25) = 4.66$, $P = 0.041$. Such findings suggest that the difference in perceived pleasantness could be attributed to the personal meaning that the adolescents associated with their current favorite fragrance. To confirm this, in a follow-up study, undergraduates rated the pleasantness of the alternative fragrance and the 3 most popular fragrances from the preliminary study. There were no significant differences in perceptions of pleasantness between the alternative fragrance and the other fragrances, $F(3,204) = 2.48$, $P > 0.05$. Such findings demonstrate that the alternative fragrance was not perceived by the undergraduate raters as intrinsically unpleasant. Consequently, it is suggested that the adolescent female participants in the preliminary study perceived the alternative fragrance as more

unpleasant because it lacked the personal meaning ascribed to their favorite perfume.

Poster Session Sun AM

The Influence of Fragrance on Facial Attractiveness and Attraction

David Reynolds and Paraskevi Antonopoulou

University of Chester

While we may use fragrance to lift mood and enhance physical attractiveness there is little research into the influence of fragrance on facial attractiveness. Thirty subjects (15 female; age 18–35) selected preferred and dispreferred fragrance from 10 fragrances. Later Ss viewed opposite gender faces standardised for attractiveness. The procedure required Ss to sniff a cotton swatch dosed with their preferred, dispreferred or a neutral (blank) swatch whilst viewing a face. In expt. 1a, Ss imagined the face pictured wearing the presented fragrance and rated attractiveness on a 7 point scale. There was a main effect of fragrance. Tukey comparisons revealed faces were rated as more attractive paired with the preferred fragrance versus neutral ($P < 0.01$) or dispreferred ($P < 0.001$); neutral versus dispreferred did not differ. In expt. 1b, Ss imagined wearing the fragrance inhaled and reported the likelihood that they would approach and flirt with the individual shown. The main effect of fragrance was qualified by an interaction of fragrance by gender. Tukey comparisons revealed for males a higher likelihood to approach when imagining wearing a preferred fragrance than neutral ($P < 0.01$) and dispreferred ($P < 0.01$) but no difference between neutral versus dispreferred; females showed no difference in likelihood to approach preferred versus neutral, however they were less likely to approach when imagining wearing a dispreferred fragrance versus neutral ($P < 0.05$) and dispreferred versus preferred ($P < 0.01$). Results show fragrance quality increases perceived attractiveness and may increase likelihood to flirt for males but not for females.

Poster Session Sun AM

Concentration-Detection Functions for Odor from Homologous *n*-Alcohols

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We aim to measure and model concentration-detection (i.e., psychometric) odor functions from volatile organic compounds (VOCs), particularly within homologous series. Functions are measured via a dynamic vapor delivery device designed to meet stimulus demands under natural sniffing conditions and whose output is quantified by gas chromatography. Stimuli comprised ethanol, 1-butanol, 1-hexanol, and 1-octanol. Subjects ($14 < n < 16$) were tested using a 3-alternative forced-choice procedure against carbon-filtered air blanks. The outcome showed that functions for increasing homologs shift towards lower vapor concentrations, confirming that odor potency increases with carbon chain length. Functions were accurately modeled by a simplified sigmoid of the form: $y = 1/(1 + e^{-(x-C)/D})$ where y = detection probability, x = vapor concentration, and C and D are constants. C is the concentration pro-

ducing a detectability halfway between chance and perfect detection and D reflects the function steepness. Our ultimate goal is to apply a quantitative structure-activity relationship (QSAR) approach to calculate the values of C and D , and thus the complete odor detection function, for untested odorants. In the past, a comparable QSAR served well to model odor potency based on threshold values (instead of functions) measured via a static squeeze-bottle system.

Supported by grant R01 DC 002741 from the NIDCD, NIH.

Poster Session Sun AM

Time-Intensity Tracking of Retronasal Smelling

Jennifer Lee and Bruce Halpern

Cornell University

Four vapor-phase odorants (anise, coffee, peppermint, and strawberry) were 1st matched for intensity retronasal-only (retro) and orthonasal-only (ortho) in a non-tracking task to a common standard (orange odorant) by 19 subjects (median age = 20; 10 female). In subsequent sessions, smelled intensities (measured every 100 ms) of the matched 5 vapor-phase odorants were tracked in random order over 55 s, 5 times each. Tracking retro 1st or ortho 1st was balanced across subjects. Real-time visual feedback of judged intensity was provided on a computer display. Subjects controlled the vertical position of the display (intensity) while the horizontal position (time) advanced at a constant rate under program control, creating an image of change in intensity with respect to time. **Results:** Approximately 1/3 of subjects apparently tracked smelled-intensity on breath-by-breath bases. Times to initial and maximum intensity did not differ between ortho and retro ($|P| > 0.1106$, 2 tailed t -test), but times to final intensity were less for retro trials ($|P| < 0.0001$). For both ortho and retro, initial and final intensities were $<$ maximum intensities ($|P| < 0.0001$). Some gender differences occurred, for example, initial and maximum intensities were $<$ for females. **Conclusions:** Both retro and ortho smelled intensities increase to maxima and then decrease over 55 s. Decreased intensity occurs earlier for retro smelling.

Support from USDA Hatch NYC-191403, The Cornell Presidential Scholars Program, and a Susan Linn Sage Professorship.

Poster Session Sun AM

Quantification of Stimuli and Perceived Changes in Odor Stimulus Intensity

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Studies of olfactory function which are reliant on liquid-phase dilutions are sometimes hindered by the discrepancy between the calculated and actual values of such dilutions and perception of odors. However, few studies make the effort to deal with this issue by empirically quantifying the vapor concentration of compounds used in olfactory research, mainly due to the technical difficulties associated with the analytical process (usually gas chromatography). In recent years, a new generation of photoionization detectors (PID) has been developed with the necessary sensitivity (ppb range) and ease of use to make them practical instruments for olfactory

research. This study investigated the utility of using PID in validating the relationship between changes in real-time concentration and perceived intensity. Nine odorants commonly employed in studies of human olfactory functioning were utilized that varied in concentration and perceived intensity as judged by the human nose. Changes in PID measurements were consistent with changes in human perception and odorant dilution, in that for a given odorant, a dilution that was judged to be more intense had higher values as measured by PID. As would be expected, the relationship did not operate in a linear fashion and was not comparable between odorants. The ability to equate empirically measured odor stimuli with psychophysical function is a valuable contribution to the study of olfactory function.

Supported by DC6369 (L.H.).

Poster Session Sun AM

Sensory and Analytical Evaluations of Complex Mixtures: Effects of prior Knowledge

Michelle Gallagher¹, Laura Sitvarin¹, George Preti^{1,2} and Pamela Dalton¹

¹Monell Chemical Senses Center and ²Department of Dermatology

Water-based paints are known to emit both volatile and semi-volatile organic compounds. These paints have been extensively studied for their possible health hazards and impact on indoor air quality. In comparison, relatively little is reported on the human sensory experiences encountered by the odor of paint. Texanol™ ester alcohol (2,2,4-trimethyl-1,3-pentanediol monoisobutyrate) is an additive that is commonly associated with the “characteristic odor” of water-based paint. This study describes the analytical and sensory evaluation of various formulations of paint coatings among subjects who were either naïve to or familiar with the components of paint, including Texanol. Painted wallboards used in this study were analyzed using solid-phase microextraction (SPME), gas chromatography/olfactometry (GC/O) and gas chromatography/mass spectrometry (GC/MS) to investigate whether individuals having multiple years of professional experience with paint coatings and their components, including Texanol, would be better able to identify the components of the mixtures. Results showed that prior knowledge of paint formulations biased the sensory evaluations. This work was supported in part by the Eastman Chemical Company.

Poster Session Sun AM

Flavor Adaptation: Effects of Ortho- versus Retro-nasal Delivery

Dennis Coleman, Christopher Maute, Ryan McDermott and Pamela Dalton

Monell Chemical Senses Center

Olfactory cues are one of the most important components in the perception of food flavor. Exposure to flavor volatiles can occur prior to ingestion as well as during and following the mastication and swallowing of food or liquids. Because food volatiles can be present for extended periods of time in the mouth, significant opportunity exists for adaptation to affect the flavor perception of food. This study

compared the rate and degree of adaptation for 2 flavor extracts (banana and peppermint) when each were presented both ortho- and retro-nasally for 5 min. To evaluate the degree to which orthonasal adaptation would affect retronasal stimulus intensity (and vice versa) we also obtained intensity ratings for a test concentration of each flavor using each presentation mode before and after adaptation. Ortho-nasal delivery produced more rapid and significant self-adaptation than did retronasal delivery. However, for both banana and peppermint, the degree of adaptation following 5-min of exposure was more pronounced when the adapting and test stimulus were presented via the same pathway (ortho or retro).

Supported by NIH-NIDCD P50 DC 006760.

Poster Session Sun AM

Odor Memory: The Importance of Verbal Labeling

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Investigators of episodic memory for flavors have reported that consistently labeling flavors is associated with accurate recognition of the flavors during recall. They concluded that giving the flavor the same name over 2 exposures was more predicative of accurate recall than label veracity. In the current experiment, 2 groups (label Prompted and Unprompted) were administered an odor memory test that required them to smell and label/identify 10 odors during a “target” trial. The Prompted group received 4 odor label choices to facilitate accurate verbal labeling. The Unprompted group received no cues. After a 10 min break participants were administered a recognition test during which they were asked to accurately distinguish the target odors (experienced previously) from 10 new “distracter” odors. The Prompted group was more accurate at naming odors and was also better at distinguishing the target odors from the distracters. Both accuracy and consistency in labeling between the target and recognition trials were strongly correlated with correct recognition of old versus new odors. However, a linear regression showed that consistency alone accounted for 70% of the variance in recognition performance. Accuracy in labeling did not significantly contribute to the model. Episodic memory for odors was affected by verbal label consistency irrespective of labeling accuracy.

NIH grant DC004139 to R.G.

Poster Session Sun AM

Taste is Abnormal in Parkinson's Disease and Suggests Cortical Spread

Mossadiq Shah, Jacquie Deeb, Marina Fernando, Alastair Noyce, Leslie Findley, Elisa Visentin and Christopher Hawkes

Queen's Hospital

Background: Parkinson's Disease (PD) spares the first and second order taste neurones (solitary tract and nucleus; VPM, thalamus). Sienkiewicz-Jarosz et al. found normal taste threshold in PD. We wished to verify this and determine whether there was a correlation between any taste and smell defect. **Methods:** Taste threshold was estimated from the tongue tip (fungiform papillae) with a Rion

electrogustometer and olfaction by University of Pennsylvania Smell Identification Test (UPSIT) in 75 patients and 74 controls. PD patients were in Hoehn and Yahr stages 1–3 and scored 27 or more on the Minimal Status Examination. **Results:** Patient taste thresholds were greater than controls by 6.9 dB (95% CI: 3.9, 9.9; $P < 0.001$), allowing for age, gender and smoking. 20/75 scored outside the 95% reference range. Taste threshold increased marginally with age in patients but not controls ($P = 0.04$). PD-UPSIT score was severely impaired (PD mean 19.5; range 4–35; Control mean 33, range 13–40; $P < 0.001$). There was no correlation between taste and smell, adjusted for subject status, age and gender (partial correlation coefficient -0.056 ($P = 0.505$)). **Conclusion:** Taste threshold is significantly and independently impaired in PD, in about one quarter patients. Age and smoking have minimal effect. Pathological studies suggest that the first taste structure to show Lewy pathology is the frontal operculum. Hence taste impairment may indicate disease that has advanced into the frontal lobes (Braak stage 5).

Poster Session Sun AM

Characterization of Odor-Active and Volatile Organic Compounds (VOCs) in Human Milk, Vaginal Secretion, and Saliva

Andrea Buettner

Fraunhofer IVV

Odor active compounds play various roles in physiological and psychosocial context for humans. They are believed to serve as chemoattractants between follicle and sperm, but also, to act as communicative agents in mother–infant relationships. Previous studies showed that human infants are selectively attracted to their individual mother's milk, and that food preferences are affected by the mothers' eating habits during the breast-feeding period. However, the underlying molecular principles are predominantly unknown. In the present study, a specific sensitive and selective analytical tool was developed for the characterization of trace volatile and odorous substances in body fluids and human skin emanations, respectively. The methodology was successfully applied for identification of more than 40 characteristic odorants in human milk, as well as the key smelling constituents of salivary and vaginal secretions of healthy human subjects. The technique comprises a modified stir bar sorptive extraction system in combination with 2-dimensional gas chromatographic separation and parallel mass spectrometric and olfactometric characterization of the analytes. The tool can be used both for direct extractive sampling, but also for headspace analysis. Due to its applicability for small sample volumes, even day-to-day physiological VOC variations, or fluctuations within, for example, one breastfeeding episode can be monitored.

Financed by the Deutsche Forschungsgemeinschaft (BU1351-1), the Deutsche Forschungsanstalt fuer Lebensmittelchemie and the HWP II-program for excellent junior researchers.

Poster Session Sun AM

Relationship between Olfactory and Emotional Competencies

Denise Chen and Wen Zhou

Rice University

Competencies in olfaction and emotion are often considered to be essentially unrelated. Competencies in olfaction are associated with genetic and physiological factors (e.g., number of functional olfactory receptor genes, olfactory receptor type, nasal airflow rate and volume), whereas competencies in emotion are associated with social and psychological ones. Here we explore the connections between the two. We measured olfactory competencies by examining how well 44 women identified a familiar same-sex individual, and by measuring their ability to name and their threshold to detect common smells. We measured emotional competencies by examining their cognitive awareness of emotions in others, and their accuracy at reading facial emotions. We found that the olfactory identification of a familiar same-sex individual is significantly and positively related to the cognitive awareness of emotions of others ($P < 0.01$), and to accuracy of reading facial emotions ($P < 0.05$). Our findings suggest that olfactory competencies are related to emotional competencies, and that the 2 may share common underlying mechanisms.

This research was supported by NIH NIDCD R03 DC4956.

Poster Session Sun AM

If You Do Not Like It Now, You Will Not Like It Later: Self-adaptation Does Not Have an Effect on Hedonic Valence of Some Odors

Claudia Damhuis and Charles J. Wysocki

Monell Chemical Senses Center

Olfactory adaptation results in a stimulus-specific decrease in perceived intensity as well as a reduced responsiveness at suprathreshold levels. Post-receptor levels also are suggested to be involved in olfactory adaptation: Perceived intensity can influence hedonic ratings. The decrease in perceived intensity of an odorant through adaptation suggests a decrease of liking and dislike, respectively, in relation to initial ratings after individuals have adapted to the odorant: The effect of the odorant ceases to exist when adaptation is complete. The aim of the present investigation was to ascertain possible correlations between the rate of olfactory adaptation and hedonic valence of odors across-individuals. Stimulation was performed with odorants of the quality musky, fruity, and flowery. Detection thresholds were first obtained. Pleasantness ratings on a scale from “extremely pleasant” (+11) via “neutral” (0) to “extremely unpleasant” (–11) were obtained during baseline before a 5 min adaptation period of continuous exposure to the stimulus as well as 3 min after prolonged exposure to the odorant. Results revealed a strong correlation between hedonic valence at baseline and hedonic valence subsequent to adaptation: Indeed, for some odorants, adaptation had little effect on hedonic valence; baseline estimates were not significantly different from pleasantness ratings taken after prolonged exposure. These data suggest involvement of the post-receptor processes for some odors to be broader than hitherto thought.

Poster Session Sun AM

Parkinson's Disease and Aging: Same or Different Process?

Christopher Hawkes

Queen's Hospital

Background: Parkinson's disease (PD), which probably commences in the olfactory bulb and nuclei of IX and X, may represent accelerated normal aging or a disease specific process. The olfactory and motor disorders are associated in PD, so that smell loss should reflect the underlying disease course. Information on age-associated decline can be obtained by single measurement from cross-sectional analysis of patients and controls of different ages, because the observed between-subject picture may reflect a similar within-subject pattern. **Methods:** University of Pennsylvania Smell Identification Test (UPSIT) scores were examined for aging effects in 263 controls and 266 PD patients. Three models were applied a) simple linear regression b) quadratic term in age: i) assuming 2 parallel curves ii) allowing for 2 separate curves. **Results:** Both groups declined with age. All 3 models were fitted successfully to the cross-sectional data and none suggested that the lower mean UPSIT score in patients compared to controls was a premature ageing effect. The mean PD-UPSIT score even at age 40, was lower than the control mean at the upper life-span limit, implying that the decline in olfaction in PD is faster than simple aging. Extrapolation back in time suggested onset before birth (unlikely), inferring that at some point there is a more rapid decline than observed in this sample. **Conclusion:** On the basis of olfactory measurement it is suggested that PD starts as an acute event followed by further disease progression more rapid than simple aging.

Poster Session Sun AM

Predicting Odorant Pleasantness from Odorant Structure: Cross-cultural Validation

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The rules governing the link between physico-chemical features of molecules and their perceived odor remain unknown. We applied principal component analysis to reduce dimensionality in both odor percepts and physico-chemical descriptors for a large set of molecules. This allowed us to create a perceptual space for odorants, and a corresponding physico-chemical space for their molecular structure. We found that the primary dimension of perceptual space was odorant pleasantness, and the primary dimension of physico-chemical space was molecular compactness. Strikingly, the primary axis of physico-chemical properties predicted the primary axis of olfactory perception. This allowed us to predict the pleasantness of 27 novel molecules by their physico-chemical properties alone ($r = 0.55$, $P < 0.004$). Considering that odor pleasantness is in part culturally specific, we tested the power of our predictions across cultures. Using a new batch of novel odorants, we predicted odorant pleasantness equally in Americans ($r = 0.53$, $P < 0.004$), Muslim Arab Israelis ($r = 0.57$, $P < 0.002$) and Jewish Israelis ($r = 0.49$, $P < 0.018$). Pleasantness is traditionally thought to reflect an internal state, with no obviously linked parameter in nature. Our findings, in contrast, suggest that pleasantness is an olfactory perceptual metric that is lawfully linked to nature, corresponding to an axis of maximal discriminability amongst biologically relevant molecules.

Poster Session Sun AM

Genetic Contribution to Androstenone Anosmia

Antti Knaapila^{1,2}, Hely Tuorila¹, Karri Silventoinen¹, Kaisu Keskitalo^{1,2}, Lynn F. Cherkas³, Tim D. Spector³, Jaakko Kaprio^{1,2} and Markus Perola²

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Specific anosmia for androstenone and iso-valeric acid has been suggested to be genetically determined. We modeled components of variation of intensity rating data from monozygotic (MZ) and dizygotic (DZ) twins. A total of 163 British (156 f, 7 m; 60 MZ and 20 DZ pairs; mean [SD] age 54.9 [12.2] years) and 178 Finnish (118 f, 60 m; 39 MZ and 40 DZ pairs; aged 21–23 years) twins rated scratch-and-sniff stimuli of androstenone, iso-valeric acid, and lemon (control) using 9 categories (endpoints: no odor, extremely strong odor). Mean (SD) intensity ratings were 4.3 (2.3) and 3.4 (2.1) for androstenone, 5.5 (2.2) and 5.5 (1.8) for iso-valeric acid, and 5.9 (1.8) and 6.4 (1.5) for lemon in British and Finnish samples, respectively. Rating of 'no odor' was given to androstenone by 12.3% and 22.0%, to iso-valeric acid by 2.5% and 1.1%, and to lemon by 1.2% and 0% of British and Finnish subjects, respectively. In British and Finnish samples, respectively, additive genetic component (95% CI) contributed 24% (0–47%) and 29% (0–52%) of variation in ratings of androstenone, 0% and 27% (0–49%) of iso-valeric acid, and 0% and 0% of lemon. In the pooled data, the corresponding value for androstenone was 29% (11–45%). Results from both samples are consistent in supporting moderate genetic contribution to androstenone perception, but larger samples are needed to confirm the results.

Supported by the Academy of Finland (206 237) and Quest International (odor stimuli).

Poster Session Sun AM

Nasal Airflow and Odorant Transport Modeling in Patients with Chronic Rhinosinusitis

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Our goal in this 5-year multi-center study is to quantitatively characterize the underlying conductive mechanisms contributing to olfactory loss in chronic rhinosinusitis (CRS) patients, in addition to other inflammatory causes. As yet, the functional impact of various nasal obstructions as sequelae to CRS and their treatment outcomes have not been successfully indexed by any existing tools, such as acoustic rhinometry (AR), or rhinomanometry (RM), the measurements of which correlate poorly with subjective symptoms. Computational fluid dynamics (CFD) techniques have shown great promises to simulate nasal airflow and predict odorant delivery rates to the olfactory epithelium for each patient based on CT scans. In an earlier report, we preliminarily supported the hypothesis that the CFD calculation is a better predictor of olfactory sensitivity

among CRS patients than are conventional methods. In this updated report, 16 additional CRS patients (total $n = 21$) were evaluated using AR, RM and CT, and their olfactory function were characterized with odor ID and thresholds (to *l*-carvone, *d*-limonene and phenethyl alcohol). Correlations between measured olfactory sensitivity and CFD predictions versus those of rhinometric measurements were examined. In the future, we envision the CFD techniques may provide quantitative evaluation of treatment outcomes for CRS and an important pre-treatment guide to optimize airflow and odorant delivery in human nose.

NIH P50 DC006760.

Poster Session Sun AM

Ability of Gum Flavors to Distract Participants from Painful Stimuli: Differential Effects of Retronasal versus Orthonasal Scent Administration

Robert Bayley, Lauren Matthews, Erin Street, Jude Almeida and Bryan Raudenbush

Wheeling Jesuit University

Research has shown that peppermint and cinnamon odors significantly increase physiological arousal, cognitive performance, and physical performance in humans, as well as increase pain tolerance in both mice and humans. More recently, the route of administration of these scents has been found to play a key role in producing such effects. For example, orthonasal (through the nose) scent administration augments level of alertness and leads to increased cognitive processing; however, effects observed during retronasal (through the mouth) administration are significantly less powerful. The present study assessed the efficacy of retronasal odorant administration on pain threshold and tolerance. In a within-subjects design, participants completed the protocol under 5 conditions: cinnamon gum, peppermint gum, flavorless gum, sham chewing, and a control condition. Participants experienced the conditions for 10 min prior to and during a cold pressor test. Following the cold pressor test, participants completed questionnaires related to mood (Profile of Mood States) and workload (NASA-Task Load Index). Despite past research indicating pain tolerance and threshold effects from orthonasal stimuli, the retronasal stimuli in the present study failed to replicate such effects. Thus, route of odorant administration is an important consideration in promoting pain relief.

Poster Session Sun AM

Relationship between Striatal Dopamine Transporter Density and Olfactory Sensitivity

Maria Larsson¹, Lars Farde², Thomas Hummel³, Nina Erixon-Lindroth² and Lars Bäckman²

¹Stockholm University, ²Karolinska Institutet and ³Dresden Medical School

The relationship between age-related losses of striatal dopamine transporter (DAT) density and age-related deficits in olfactory sensitivity was examined in a group of subjects ($n = 12$) ranging from 34 to 81 years of age. The radioligand [¹¹C]-CIT-FE was used to determine DAT binding in caudate and putamen. The results indi-

cated age-related losses of striatal DAT binding from early to late adulthood and a reliable age deterioration in olfactory sensitivity. Importantly, the age-related olfactory deficits were mediated by reductions in DAT binding in putamen but not caudate. Also, DAT binding in putamen added systematic olfactory variance after controlling for age. These findings suggest that in vivo DAT binding in putamen is implicated in sensitivity for olfactory information independently of age.

Poster Session Sun AM

Upright or Supine: Body Position Matters for Weak Odors

Johan Lundstrom, Julie Boyle, Giulia de Prophetis and Marilyn Jones-Gotman

McGill University

We recently demonstrated that a supine position causes a decrease in olfactory sensitivity compared to an upright position. In separate experiments using Sniffin' Sticks, we have explored the extent of, and mechanism underlying, this initial finding. In experiment 1 ($N = 40$), we replicated the decrease in olfactory sensitivity to phenyl ethanol when in a supine compared to an upright position. In experiment 2 ($N = 40$), we determined that there were no position-dependent shifts in ability to discriminate or identify suprathreshold odors, or rate them for pleasantness, intensity, or familiarity. A small but consistent drop in scores ($P < 0.01$) was observed, and performance was slowed ($P < 0.07$), on a cognitive skill while supine. In experiment 3 ($N = 30$), we measured body position dependent shifts in certain physiological variables and their relationships to position dependent changes in odor identification and perithreshold odor intensity discrimination (POID). We also measured sensitivity for *n*-butanol and changes in state anxiety. We found position-dependent changes in sniff magnitude and volume, diastolic blood pressure and heart rate, but not in anxiety. Only men showed decreased sensitivity for *n*-butanol. There was no relationship between these measures and the significant decrease in either POID or sensitivity. These results demonstrate a body position dependent shift in olfactory sensitivity only for perithreshold odors; this does not seem to be mediated by physiological factors.

Poster Session Sun AM

Effects of Peppermint Scent on Diminishing Smoking Cravings and Withdrawal Symptoms

Daniel Felbaum, Jared Bloom, Trevor Cessna, Rosanna Drake and Bryan Raudenbush

Wheeling Jesuit University

A variety of pharmacological methods have been proven effective in alleviating the symptoms of smoking cravings and withdrawals. The present study assessed a more natural approach by using peppermint scent as a potential craving and withdraw symptom inhibitor. In a within-subjects design, participants completed 3 conditions: peppermint inhaler (PI) use in lieu of smoking, control ad-lib smoking (CS), and abstinence from smoking (AS). While undergoing each of these conditions, participants completed a series of surveys 3 times each day. The surveys included the Profile of Mood

States, Cigarette Craving Survey, Smoking Urges Survey, Cigarette Withdrawal Scale, Peppermint Inhaler Use Tally, and Cigarette Use Tally. Results indicated differences in smoking urges, smoking cravings, and smoking withdrawal symptoms, such that CS < PI < AS. This study provides evidence that inhaling peppermint can achieve similar, but not equal, results in curbing smoking cravings withdrawal symptoms when compared to actually smoking.

Poster Session Sun AM

Olfactory Perception in Patients with Eating Disorders

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University of Erlangen-Nuremberg

Olfactory sensitivity and the hedonic evaluation of odors play an important role in the regulation of food intake, but only few studies have been conducted so far investigating olfactory tasks in patients with eating disorders. Thus, the aim of our study has been to analyze olfactory perception including intensity and hedonic estimates in patients with Anorexia nervosa (AN), Bulimia nervosa (BN) and Binge eating disorder (BE). 36 patients with eating disorders who met the DSMIV-criteria for either AN ($n = 25$), BN ($n = 8$) or BE ($n = 3$) were included in the study and compared among each other as well as with an age- and sex-matched control group. Mean age was 30.4, SD 10.4 years. We employed the Sniffin' Stick test to assess olfactory function and registered intensity and hedonic estimates using visual analogue rating scales. Statistical analysis for 13 food-related odors revealed a significantly decreased discrimination ability (11.42, SD: 1.43 vs 12.51, SD: 1.65 discrimination scores) and significantly increased intensity estimates in patients with eating disorders compared to healthy controls (14.13, SD: 2.50 vs 12.76, SD: 2.63 rating units). Comparing patients with AN, BN, and BE, the ANOVA revealed significant differences in the hedonic evaluation of odors between BN and BE, whereas intensity estimates did not differ between the 3 groups. Our results demonstrate an altered olfactory perception, especially concerning food-related odors, in patients with eating disorders. The study might have implications for diet strategies and the sensory pathobiology of eating disorders.

Poster Session Sun AM

Olfactory Functions in First Episode and Chronic Schizophrenia Patients

Claudia I. Rupp, Wolfgang W. Fleischhacker, Georg Kemmler, Thomas Walch, Arne W. Scholtz, Theresa Lechner and Hartmann Hinterhuber

Innsbruck Medical University

There is a huge amount of evidence that schizophrenia patients exhibit olfactory dysfunction. Several studies reported impairments in olfactory identification and quality discrimination. With regard to olfactory sensitivity, the findings are controversial. The aim of the present study was to determine whether patients experiencing a first-episode schizophrenia and patients with chronic schizophrenia demonstrate differences in olfactory deficits. Performance in olfactory functions (sensitivity, discrimination, identification; combined composite score: SDI) were

assessed using the Sniffin' Sticks test battery, and compared between young first-episode patients, young patients with chronic schizophrenia and healthy controls similar in age and gender (all male). Our results indicate that only chronic schizophrenia patients were impaired in all measures of olfactory functions (sensitivity, discrimination, identification, SDI). Compared with healthy controls, patients experiencing their first episode showed significantly reduced olfactory discrimination and identification, as well as a reduced SDI score. No group differences were observed in olfactory sensitivity. Results indicate that both, olfactory discrimination and identification ability are impaired early in the course of illness. Our findings of intact olfactory sensitivity in first-episode patients and impaired olfactory sensitivity in chronic schizophrenia patients suggest either a progressive effect of medication or illness on olfactory sensitivity in schizophrenia.

Poster Session Sun AM

Olfactory Deficits Predict Donepezil Response in Depressed MCI Patients

Matthias Tabert, Gregory Pelton and D.P. Devanand

Columbia University/New York State Psychiatric Institute

This study examined the utility of the University of Pennsylvania Smell Identification Test (UPSIT) for predicting cognitive improvement in elderly depressed patients with cognitive impairment, but not dementia, during treatment with the acetylcholine esterase inhibitor (AChEI), donepezil. Depression (Dep) has been linked to serotonergic abnormalities, and cognitive impairment (CI) to acetylcholine abnormalities. AChEI treatment in mild cognitively impaired (MCI) patients appears to delay the rate of cognitive worsening. The UPSIT has predictive utility in patients with MCI for the follow-up diagnosis of AD and, high specificity for distinguishing major depression from Alzheimer's disease. We hypothesized that a Dep-CI patient with low UPSIT scores would reveal greater cognitive improvement from AChEI (i.e., donepezil) treatment than those with normal UPSIT scores. At baseline evaluation we administered the UPSIT to 13 Dep-CI patients and then entered them in a 12-week double-blind placebo-controlled donepezil drug trial after acute antidepressant treatment. We found that a low UPSIT score predicted improvement in verbal memory ($P = 0.01$) during donepezil treatment. Considering the Dep-CI patient is at an increased risk for AD conversion, targeted treatment to improve cognition is actively being sought. Low UPSIT scores may be an early marker of AD that can help to identify those Dep-CI patients who will be responsive to pharmacological intervention.

Poster Session Sun AM

Recovery from Salivary Habituation Is Similar following Presentation of a Novel Odor via the Same Route and the Same Odor via a Novel Route

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Repeated perception of the same odor is associated with reduced salivation that rebounds following perception of a novel odor “odor switching.” Here we tested whether perception of the same odor via a novel route (“route switching”—ortho to retro or vice versa) would result in a similar rebound in salivation. 24 Ss participated in 2 sessions, each with 9 odor exposure trials. Saliva was collected before and after each trial. On the 7th trial of session 1 the odor but not the route was switched. In contrast, the route but not the odor was switched on the 7th trial of session 2. Intensity and pleasantness ratings were collected after the 6th and 7th trials. Repeated measures ANOVAs were used to analyze the data. As predicted, salivary habituation was observed from trials 1–6 for both sessions and there was a significant rebound in salivary response for both sessions between trials 6 and 7. Further, there was no interaction between session and salivary rebound, indicating a similar effect on salivary response for odor and route switching. Salivary rebound was also associated with increased intensity but not pleasantness perception and this was true for both odor and route switching. Thus, differences in intensity but not pleasantness may contribute to recovery from adaptation. These results demonstrate dissociable physiological responses to ortho and retronasal perception of the same odor providing further evidence for separable neural circuits.

This work was supported by R03 DC006169.

Poster Session Sun AM

Parkinson's Disease: A Dual Hit Hypothesis

Heiko Braak¹ and Christopher Hawkes²

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Background: Lewy body disease (LBD) pathology (Lewy neurites/bodies) in classical Parkinson's Disease (PD) probably commences simultaneously in the medullary vagal motor nucleus and olfactory bulb. We suggest a common pathological mechanism to explain how such anatomically separate structures become involved early in PD. **Literature:** *Olfactory impairment.* There is extensive evidence for this, whether measured by psychophysical, neurophysiological, or pathoanatomical methods. Large prospective studies imply olfaction is damaged well before motor symptom onset. *Vagal disorder.* The vagus is significantly impaired, as shown by heart rate variability, dysphagia or gastric dysfunction, and medullary pathology. Prospective assessment of bowel habit indicates constipation is a premotor sign. LBD pathology occurs early in the gastric enteric plexus, where vagal motor fibres terminate. **Conclusion.** We propose that a pathogen, possibly viral, damages the nasal olfactory neuroepithelium, causing olfactory impairment, and is then swallowed in secretions. After entering the gastric enteric plexus, it travels retrogradely along vagal motor fibres to the medulla. Subsequent rostral progression to the substantia nigra results in classical PD features.

Poster Session Sun AM

Central Presentation of Postviral Olfactory Disorder Evaluated by FDG PET

Jeong-Whun Kim and Yu Kyeong Kim

Seoul National University

The exact location and nature of the damage in olfactory dysfunction after upper respiratory tract infection (URI) are not fully understood, yet. We investigated whether the changes in the cerebral activity occurs in patients with postviral olfactory disorder using FDG PET. Nine patients (mean age: 57 ± 9 years, 8 females) suffering from disability of smelling were enrolled in this study. All patients had neither apparent sinusitis nor rhinitis, however, all recalled causative URI and temporal connection with development of the symptom. Olfactory function tests using butanol threshold test and odor identification task (OI) confirmed that all subjects were anosmic or hyposmic. FDG PET studies during a rest state were acquired after initial evaluation, and the cerebral metabolic abnormality was tested using voxel-wised comparison with those from age and gender matched healthy controls. As comparison with healthy controls, patients showed the significant hypometabolism in the right inferior frontal cortex and bilateral amygdala and parahippocampal area where the olfactory neurons primarily project. Furthermore, hypometabolism was also demonstrated the bilateral insular cortices, medial and lateral temporal cortex where the olfactory information is integrated to produce the sensation. No area with increased metabolism was found. This study clearly demonstrated that the postviral anosmia is typically associated with metabolic suppression in the brain regions where the olfactory information is received and integrated.

Poster Session Sun AM

Effect of Diet on Volatile Profiles of Urines and Sweat in Humans

Jae Kwak¹, Weiguang Yi¹, Alan Willse², George Preti¹, Julie Mennella¹, Allison Steinmeyer¹, Jon Wahl², Kunio Yamazaki¹ and Gary Beauchamp¹

¹Monell Chemical Senses Center and ²Pacific Northwest National Laboratory

Individual people can be recognized by a unique odor that is, in part, genetically determined. In addition, environmental factors such as diet may also affect body odors. Here we test the hypothesis that the genetically-determined odortype is robust against dietary variation. Volunteers were recruited and asked to collect urines and axillary sweat. Collections were made on 10 consecutive days for each person: 4 days of baseline, 6 days of dietary treatment. For the dietary treatment, the subjects consumed capsules containing fish oil, mint, garlic and turmeric. Urines and axillary sweat samples were then analyzed by gas chromatography/mass spectrometry. Preliminary results show that numerous compounds were differentially concentrated between baseline and treatment samples. Most of these compounds appear to be derived from turmeric, garlic or mint. In fact, only few of these compounds were detected in the baseline samples, suggesting that the diet regimen has minimal influence on normally occurring endogenous volatile compounds. These results are consistent with recent animal studies that indicate that dietary variety does not significantly interfere with determination of genetic variation in body odors.

This work is sponsored by DARPA under ARO Contract No. DAAD19-03-1-0109. Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the United States Government.

Poster Session Sun AM

An Epidemiological Study on the Frequency of Smell and Taste Impairment

Thomas Hummel¹, Mechthild Vennemann² and Klaus Berger²

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Aim: Aim of the prospective study (part of the ‘‘Dortmunder Gesundheitsstudie’’) was to investigate taste and smell in a large portion of the general population. **Methods:** The population sample ($n = 1312$; age 25–75 years) was drawn from the central registration office in Dortmund. Following a standardized interview testing of olfactory function was performed with a 12-item odor identification test (‘‘Sniffing Sticks’’); taste function was assessed for 4 basic tastes at suprathreshold concentrations. **Results:** The study yielded the following major results: 1) Over 5% of the investigated population are functionally anosmic, and 2) 24% of the general population appear to have pronounced olfactory deficits. 3) Approximately 9% of the population do not recognize one or more of the 4 basic tastes when presented at suprathreshold concentrations indicating hypogeusia. 4) While the decrease of olfactory function was age-related, no such decrease was seen for taste. 5) Smoking increased the risk for impairment of olfactory function; however, the risk for impairment of gustatory function was not significantly increased. **Conclusions:** The present results suggest that both olfactory and gustatory function are compromised in a significant portion of the general population.

Poster Session Sun AM

Comparison of Block versus Event-Related Design in Olfactory fMRI Studies

Vishwadeep Ahluwalia, Greg Harrington and Birgit Kettenmann

Virginia Commonwealth University

In a multi-run interventional olfaction fMRI study, the results potentially caused by desensitization within and between runs can easily be misinterpreted as a treatment effect. This study aims at comparing 2 stimulation designs, i.e., block and event-related design, to identify the one with the least change in response signal over repeated sessions. 42 healthy volunteers (21 per design) participated in 2 sessions and 2 runs per session (193 volumes per run) using a 3.0 T GE scanner. Banana and Cinnamon odor stimuli of 800 ms duration were applied by an olfactometer (BurghartOM4b) to the left nostril of the volunteers. The 2 designs had the same number of stimuli and total duration. Individual and Mixed Effect Group Analysis was performed using FSL. ROIs used in this study were adapted from the Anatomical Automatic Labeling library. It was observed that the %BOLD signal change in key olfactory areas decreased much more during consecutive runs in the block design (BD) as compared to the event-related design (ERD). For example, piriform cortex: 55% decrease in BD, 13% decrease in ERD. Also certain areas of the orbito-frontal cortex show no %BOLD signal change in BD, but a significant %BOLD signal change in ERD for both odors. The results of this study suggest that event-related olfactory fMRI paradigms are better suited for conducting multi-run intervention studies than block designs. The results also

suggest that habituation and patient fatigue seem to play a bigger role in multi-run block design olfactory fMRI studies.

Poster Session Sun AM

Sex Differences in Neuronal Processing Based on Odor Type

Julie Boyle¹, Johan Lundstrom¹, Bettina Pause², Robert Zatorre¹ and Marilyn Jones-Gotman¹

¹McGill University and ²Heinrich-Heine-University

Sex differences in olfactory processing have been explored extensively in behavioral studies but few imaging studies of potential sex differences exist. Using O-15 PET, 12 women and 12 men were scanned while passively smelling iso-intense concentrations of either a pure (PEA), a bimodal (pyridine), or an endogenous odorant (androstenone). Results are based on statistical contrasts directly comparing women’s and men’s activations to the 3 odor types. Only minor sex differences were observed for the pure odorant: women had greater activation in lateral orbitofrontal cortex (OFC) and men in both middle frontal gyrus (MFG). Larger sex differences were observed for the bimodal odor: women had greater activity in piriform cortex (PIR), MFG and posterior cingulate. Men had greater activity in insula (INS), parietal and cerebellar regions. Perception of the endogenous odorant resulted in clear sex differences. Women showed a greater response compared to men in PIR, both amygdalae, medial OFC, INS and thalamus. In contrast, men showed no activation in traditional olfactory structures but activated a greater number of regions involved in trigeminal and crossmodal processing including the MFG, superior temporal sulcus, and precentral gyrus. Sex differences in olfactory functional neuroimaging studies thus seem to vary greatly depending on the type of odorant. In addition, greater sex differences for the endogenous odor indicate that odor origin may play a role in sex-mediated cerebral functions.

Poster Session Sun AM

Multivariate Pattern Analysis of Odor Quality in Human Piriform Cortex

James Howard¹, John-Dylan Haynes², Jane Plailly¹, Todd Parrish¹ and Jay Gottfried¹

¹Northwestern University and ²Max Planck Institute

It is generally assumed that neural codes of odor quality are spatially distributed across olfactory cortex, but little is known about how these neural codes are organized in the human brain. In conventional functional magnetic resonance imaging (fMRI) analyses, spatial and temporal averaging can obscure potentially important information at the level of individual voxels and scans. Inspired by recent research on human vision, we used olfactory fMRI to ask whether different odor qualities evoke distinct spatial patterns of activity within human piriform cortex. Six subjects were scanned while smelling 4 odorants of distinct quality. Raw fMRI signal was extracted from each activated piriform voxel for each run and subject. We calculated identification accuracies for each subject using comparisons between within- and across-category

correlations. Identification accuracy across all subjects significantly exceeded chance (50%) in posterior piriform cortex (70.1%, $P < 0.001$). In parallel, a separate analysis using a neural network classifier showed above chance classification (25%) of odor-specific patterns in 4 out of 6 subjects in piriform cortex (29.8%, $P = 0.06$). These results suggest that information about odor quality is coded as a spatially distributed pattern of activity in human piriform cortex.

Poster Session Sun AM

A Shock to the Senses: Enhanced Discrimination between Odor Enantiomers via Aversive Learning in an fMRI Paradigm

Wen Li, James Howard, Mark Benton, Emil Davchev, Vess Djoev, Todd Parrish and Jay Gottfried

Northwestern University

It is widely presumed that odor quality is a direct outcome of odorant molecular structure, but increasing evidence suggests that cognitive processes play an equally important role in human olfaction. In this study, we used 2 pairs of odor enantiomers, mirror-symmetric (chiral) molecules that are perceptually indistinguishable, in an fMRI paradigm of classical conditioning ($n = 12$). During the conditioning phase, a target odorant (tgCS+) was repeatedly paired with electric shock, while the other 3 odorants were delivered without shock. Triangle tests administered at the beginning and end of the experiment showed improved discrimination selectively for the tgCS+ and chCS+ pair from pre- to post-conditioning (0.25 to 0.56, $P < 0.05$; chance, 0.33), but unchanged for the non-conditioned pair (0.33 to 0.31). In parallel, pattern analysis on odor-evoked activity in primary olfactory (piriform) cortex indicated differentiation of response patterns for the conditioned pair: voxel-wise spatial correlation between tgCS+ and chCS+ decreased substantially from pre- to post-conditioning whereas the correlation between the other pair showed only a small increase. These preliminary findings suggest that spatial representations of odor quality in human olfactory cortex can be updated through aversive learning, such that odorants initially smelling the same can be made perceptually distinct.

Poster Session Sun AM

Olfactory Discrimination Acuity and Thallium Transport in the Olfactory Nerve of Traumatic Olfactory Disturbance Mice Model

Hideaki Shiga, Yayoi Kinoshita, Koshin Washiyama, Daisuke Ogawa, Ryohei Amano, Toshiaki Tsukatani, Takaki Miwa and Mitsuru Furukawa

Kanazawa University

Objectives: It is worth of assessing the injured olfactory nerve fibers for the prediction of prognosis in patients with traumatic olfactory disturbance. We show the correlation between olfactory discrimination acuity (ODA) and thallium transport rate in the olfactory nerve of mice model. **Methods:** After deprivation of water, mice (ICR, male, 8W) were trained to discriminate between cycloheximide solution and distilled water. The mice were transected with both sides of olfactory nerve fibers under anesthesia (model mice). ODA of the mice

was assessed and 201TlCl solution was administered into the nasal cavity of the mice. The olfactory nerve fibers of model mice were transected at different time points. Radioactivity in the olfactory bulb and nasal turbinate of the dissected mice was measured with gamma-ray spectrometry. Olfactory epitheliums were also assessed with immunohistochemical staining of olfactory marker protein (OMP) after intranasal administration of 201TlCl solution. **Results:** ODA was significantly correlated with the transport rate of thallium in the olfactory nerve. OMP expression in the olfactory epithelium was not different between the administered nasal cavity and untreated side. **Conclusion:** The injured olfactory nerve fibers could be assessed in the model mice with intranasal thallium administration.

Poster Session Sun AM

fMRI Investigation of Central Olfactory Deficit in Early Alzheimer's Disease

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Impaired olfactory discrimination and identification are early signs of Alzheimer's disease (AD). The purpose of this study was to investigate the pathophysiology of olfactory brain structures in early AD with olfactory fMRI. The odor lavender (intensities 0.10%, 0.32%, and 1%) was presented to the nose with an olfactometer. The odor intensities used were determined psychophysically by a previous independent study. Twelve probable AD subjects (74.3 ± 7.8 years, 5 m, UPSIT score 22.2 ± 7.5) and thirteen healthy age-matched controls (67.8 ± 9.8 years, 8 m, UPSIT score 34.1 ± 3.5) received fMRI at 3 T. Region of Interest analyses identified significantly reduced activation with all odor intensities in the primary olfactory cortex (POC), insula and hippocampus in the AD group compared to controls ($P < 0.01$). Olfactory fMRI responses to odor intensities in early AD are distinctively different from age-matched controls, with elevated thresholds and decreased volumes of activation. Although not anosmic, AD patients showed marked reduction of activation in the POC, hippocampus and insula. These results support the feasibility of olfactory fMRI as a bioassay for early detection of AD.

This study is supported in part by Leader Family Foundation for AD Research and NIH R01 EB00454.

Poster Session Sun AM

Brain Activation of Olfactory and Trigeminal Cortical Areas Is Independent from Perceptual Strength—A fMRI Study Using Nicotine Vapor as Chemosensory Stimulus

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Applied to the nasal mucosa in low concentrations, nicotine vapor evokes odorous sensations and at higher concentrations, it produces burning and stinging sensations. The aim of this study was

to compare brain areas activated by nasal stimulation with *S*(-)-nicotine vapor in low versus high concentrations. Olfactory detection thresholds and pain thresholds for nicotine were determined in thirty healthy occasional smokers each. Functional images were acquired using a 1.5 T MR scanner with application of nicotine vapor in concentrations just above the individuals' threshold. In a further session subjects were instructed to evaluate the intensity of the olfactory and trigeminal percept during the stimulation paradigms used before. Although perceptions of nicotine vapor in low and high concentrations completely differed, activations in basically the

same brain areas were found in both fMRI experiments. These brain areas correspond to areas known to be activated following olfactory stimulation of the nasal mucosa, as well as areas specific to processing of painful stimuli. This indicates that the olfactory and trigeminal systems are both activated during chemosensory perception of nicotine vapor and it is not possible to separate olfactory from trigeminal effects by varying the concentration of the applied nicotine vapor.

Research described in this abstract was supported by Philip Morris USA Inc.