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ABSTRACTS

List of Abstracts from the XXVIIth Annual Meeting of the Association for Chemoreception Sciences

Givaudan-Roure Lecture

Leptin and the regulation of body weight

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In mammals, a precise physiologic system that maintains energy balance at an optimum level has evolved. With the identification of leptin and its receptors, two of the molecular components of this pathway have been identified. Leptin is a hormone secreted by adipose tissue that modulates energy balance via effects on the hypothalamus and other tissues. In mutant obese and diabetic mice, the absence of leptin or its receptor results in massive obesity together with most of the abnormalities generally observed during starvation. Leptin levels fall after weight loss resulting in a potent stimulus to eat. In addition to inducing hyperphagia, a reduced leptin level is also associated with abnormalities in reproductive function (in females), hypothalamic and pituitary function, autonomic function, alterations of immune function, decreased insulin sensitivity and hepatic steatosis. These data indicate that decreased leptin concentrations contribute to the biologic response to starvation and further suggest that leptin is a key means by which alterations in nutritional state modulate other physiologic systems. Weight gain leads to an increase in plasma leptin. In animals, physiological infusions of leptin reduce weight and body fat content indicating that leptin also acts to restrain weight gain. Thus leptin signaling maintains body weight and adipose tissue mass within a finite (optimal) range and plays a general role in effecting many of the physiologic responses observed with changes in nutritional state. In this presentation, the role of leptin in the control of body weight and animal physiology, its mechanism of action and its relevance to the pathogenesis of obesity will be reviewed.

Slide: Cellular & Molecular

Characterization of mouse trigeminal neurons identified by viral tracing

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The trigeminal nerve is the major mediator of somatosensory perceptions from the mammalian head and comprises neurons that transduce mechanical, thermal and chemical stimuli. Thereby single neurons mediate sensory input from selective areas of the head (meninges, cornea and conjunctiva of the eyes, facial skin, mucous membranes of the oral and nasal cavities). Physiological features of peripheral neurons depending on their function and area of innervation remain largely unclear. Viral tracing using a neurotropic alpha-herpesvirus, a recombinant Bartha strain of the Pseudorabis virus (PrV), was performed to identify trigeminal neurons mediating information from the murine nasal cavity. Twenty-four hours after intranasal application of high titered GFP expressing PrV, green fluorescent ganglionic neurons could be identified in cryo sections of the gasserian ganglion. Additional subcutaneous injection of an RFP expressing PrV variant into the facial skin allowed identification of red fluorescent cells innervating the facial target and revealed an exclusive separation of both subpopulations within the trigeminal ganglion. Both populations could be identified after dissociation and plating and allowed activity measurements of individual identified neurons in primary cell culture. Relevant cells were characterized by Ca imaging as well as electrophysiological recordings and were compared with *in vitro* infected as well as to un-infected control neurons. Data indicate that basic electrophysiological properties of traced neurons were not altered by PrV infection compared with control neurons and demonstrate the benefit of PrV (Bartha) for life-cell tracing studies and the investigation of individual cellular chemosensory properties.

Slide: Cellular & Molecular

Manipulating the developing olfactory and vomeronasal systems through the use of ultrasound-guided retrovirus injection

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The olfactory and vomeronasal systems are attractive model systems for studying neuronal development, regeneration and pharmacology. However, *in vivo* analysis of gene function has thus far been technically challenging, primarily relying on gene targeting of mice or transient overexpression using adenovirus. Here we report a technique that allows for stable mosaic overexpression of genes in the developing rodent olfactory and vomeronasal systems. Using high resolution ultrasound-guided retrovirus injection, we infected the developing olfactory system *in utero* (between E10 and E14 in mouse or E13 and E15 in rat). Since the retrovirus preferentially infects dividing cells, this time window allowed us to infect cells that give rise to clonal patches of OE and VNO. Within 24 h after injection we found widespread transgene expression that continued

into adulthood. This stability is likely due to the ongoing replacement of neurons and support cells by the infected basal cells. Thus, we believe this technique provides a rapid, stable and effective way to study gene function in both the developing and mature OE and VNO. Through the use of an IRES-tauGFP construct the effect of overexpressed genes on both the pharmacology and the development of chemosensory neurons was assayed. We are now beginning to explore the use of retrovirus to study the developing olfactory bulb.

Supported by NIH.

Slide: Cellular & Molecular

Expression of TRPM5 in the main olfactory epithelium

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Mice defective for the cyclic nucleotide-gated channel (CNGA2) are responsive to odors suggesting involvement of alternate transduction pathways (Lin *et al.*, 2004, *J. Neurosci.*, 24:3703). To investigate potential involvement of the phospholipase C (PLC) pathway, we examined the expression of TRPM5, a channel involved in the PLC pathway in taste transduction. In mice where the TRPM5 promoter drives expression of a green fluorescent protein (GFP), two populations of cells were found labeled in the main olfactory epithelium (MOE). Regularly spaced, short cells (<15 μm long), reminiscent of microvillar cells, were scattered throughout the MOE. Most of these cells did not have axons, and were closely associated with trigeminal nerve fibers. In addition, GFP-positive cells with the morphology of olfactory receptor neurons (>15 μm long) were located in the ventrolateral zones of the epithelium, sending axons to discrete glomeruli in the olfactory bulb. Most of these cells coexpressed olfactory marker protein and CNGA2, suggesting a colocalization of PLC and cAMP signaling pathways. Antibodies against TRPM5 labeled cell bodies and apical processes of these cells. Our data suggest that the TRPM5/PLC signaling pathway is involved in signal transduction in cells of the MOE.

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Slide: Cellular & Molecular

Olfactory imprinting alters gene expression in the olfactory epithelium

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The molecular mechanisms of olfactory imprinting are not well understood, particularly in the peripheral olfactory system. We have shown that the zebrafish (*Danio rerio*) is able to form and retain olfactory memories of odorants experienced as juveniles. We exposed juvenile zebrafish to the odorant phenyl ethyl alcohol (PEA) during the first 3 weeks of life. Our behavioral tests showed that fish raised in PEA prefer to be in the presence of PEA as adults while unexposed siblings show a neutral response to the odor. Through expression array experiments using RNA isolated from adult olfactory epithelia we identified 48 genes that were up-regulated in imprinted fish when compared with controls. By *in situ* hybridization we demonstrate that one of these genes, the transcription factor *otx2*, is expressed in a small subset of cells in the olfactory placode. We find *otx2* expressing cells in the ventral–medial olfactory placode and they often span the basal (where the olfactory sensory neuron precursors are found) to apical regions of the olfactory organ at 2–3 days of age. The number and exact pattern of cells expressing *otx2* is variable within and between fish. The number of cells expressing *otx2* is significantly increased in 1- to 3-day-old odor exposed fish when compared with controls. This increase appears to be odorant specific because no increase was observed when fish were exposed to L-cysteine or vanilla. We are also exploring the expression patterns of other genes identified on the microarray.

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Slide: Chemosensory Development

Specificity, sensitivity, and host shifts: variation in peripheral chemoreception and sympatric speciation in *Rhagoletis* flies

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The *Rhagoletis pomonella* species complex has been at the center of the sympatric speciation debate for much of the last 50 years. Studies in our laboratory have shown that flies from sympatric populations infesting hawthorn, apple and flowering dogwood fruit can distinguish among unique volatile blends identified from each host. This odor discrimination acts as a premating barrier to gene flow between flies infesting each fruit. We have also discovered significantly reduced olfactory preference for host blends in *R. pomonella* hybrids which may serve as a post-zygotic barrier as well. Here we use identified host volatiles and single-cell electrophysiology to examine if differences in peripheral chemoreception could contribute to divergent host preference and fidelity in the *pomonella* group. Olfactory receptor neuron (ORN) response characteristics were compared from the three populations of *R. pomonella* and *R. mendax* (an outgroup), as well as F1 and F2 hybrids and backcrosses between the populations. Dose–response trials revealed similar chemical specificity in all parent populations, but unique response profiles in hybrid offspring as well as significant variability in ORN sensitivity and temporal firing pattern among all populations. These results support the hypothesis that variation in peripheral chemoreception can influence host preference and contribute to the host shifting process in *Rhagoletis* flies.

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Slide: Chemosensory Development

Odor receptor gene choice in *Drosophila*

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We have found evidence that a combinatorial code of *cis*-regulatory elements is critical to the process of receptor gene choice in *Drosophila*. The *Or* gene family in *Drosophila* consists of ~60 members, most of which are expressed either in the antenna or the maxillary palp, but not both. We have identified both positive and negative *cis*-regulatory elements that act together to dictate the organ-specific expression of individual receptor genes in the maxillary palp. Within this organ, individual *Or* genes are expressed in only one of 6 functional classes of neuron. We have identified *cis*-acting regulatory sequences that act in specifying expression in five of the six neuron types. The first of these neuron-specific elements was identified by comparison of sequences flanking two *Or* genes that are coexpressed in the pb2A neuron. The other neuron-specific elements were identified by comparisons of sequences flanking *Or* orthologs in two distantly related species, *D.melanogaster* and *D. pseudoobscura*. *In vivo* analysis of these elements shows that within the maxillary palp, both positive and negative regulatory mechanisms are required in the process of receptor gene choice. Interestingly, mutational analysis of these elements reveals a hierarchy in the selection network of *Or* gene expression. Finally, we have identified transcription factors that are required for this highly regulated process of *Or* gene choice.

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Slide: Chemosensory Development

Wnt5 and its receptor, derailed, patterns the *Drosophila* olfactory map

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Although numerous guidance molecules have been found to be expressed by the olfactory system, the molecular mechanisms underlying the formation of the olfactory topographic map are unknown. To unravel the molecular mechanisms governing the orderly termination of olfactory receptor axons (ORN axons) in the brain, we searched for genes that disrupt the precise anatomy of the *Drosophila* antennal lobes (ALs). In the fly, ORN axons expressing a given receptor synapse on projection neuron (PN) dendrites within one of 43 unique glomeruli in the ALs. From a screen of 3996 fly lines, we identified 233 genes that, when forcibly expressed in the ORNs, altered the stereotyped wiring pattern of the ALs. One of these genes encodes *Wnt5*, a member of the *Wnt* family of secreted proteins. Overexpression of *Wnt5* in the ORNs induces the striking development of numerous ectopic glomeruli. Loss-of-function mutations in *Wnt5* on the other hand, disrupt the stereotyped arrangement of glomeruli. Loss-of-function mutations in *derailed* (*drl*), which encodes a receptor for *Wnt5*, also disrupt the glomerular map. Epistatic analyses show that *Wnt5* down-regulates the activity of *drl*. Cell-type specific gene expression studies and mosaic analyses show that *Wnt5* functions in the ORNs while *drl* acts in the PNs. We hypothesize that *Wnt5* is expressed in particular axonal arbors where it provides positional cues that pattern the targeting of *drl*-expressing dendrites.

Slide: Chemosensory Development

Taste progenitor cells are specified by BMP and Shh signaling

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Like epithelia, taste cells are continuously renewed from basal stem cells, yet like neurons, taste cells form synapses and respond electrophysiologically to stimuli. Thus, signaling pathways important in both epithelial and neuronal development may specify taste bud progenitor cells. Previously, using cultured axolotl pharyngeal endoderm (PE) destined to give rise to taste buds, we identified a critical period early in development when taste bud progenitor cells are specified by cell contact-dependent signals; transient interruption of cell contacts resulted in more and larger taste buds (Parker *et al.*, 2003). To determine the molecular mechanisms responsible, we tested candidate signaling pathways, including BMP, Shh, and Notch/Delta. First, via RT-PCR, each of these signaling cascades was expressed in axolotl PE during the critical period. Second, to test for function, BMP, Shh or Notch signaling was manipulated in PE explants during the critical period. Blocking Notch did not alter taste bud development, while disrupting Shh induced more taste buds. However, blocking BMP fully phenocopied the results previously observed after physical disruption of cell contacts; block of BMP increased both taste bud number and size. We are now testing if Shh and BMP actions are restricted to the critical period, and assessing whether all cell lineages within taste buds are affected similarly.

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Symposium: Coding in the Taste System: New Perspectives on an Old Problem

Coding in the taste system: new perspectives on an old problem

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For decades, there has been controversy about how taste information is coded in the peripheral gustatory system of mammals. On the one side are proponents of a labeled line code, and on the other side are those that favor a more ensemble pattern code, such as found in

color perception. Recent physiological and molecular biological studies have done little to quell the argument. Calcium imaging and patch clamp studies at the cellular level indicate that many taste cells respond to multiple chemical modalities (Gilbertson *et al.*, 2001, *J. Neurosci.*, 21:4931; Caicedo *et al.*, 2002, *J. Physiol.*, 544:501), while the molecular expression data for G protein coupled taste receptors (Zhang *et al.*, 2002, *Cell*, 112:293) indicate that individual taste cells respond to only specific modalities. This symposium re-examines these issues from new perspectives, and seeks to resolve the apparently conflicting data on information processing in the mammalian gustatory system.

Symposium: Coding in the Taste System: New Perspectives on an Old Problem

Taste recognition in *Drosophila*

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The gustatory system in *Drosophila* is crucial for detecting food, selecting sites to lay eggs and recognizing mates. Taste neurons are distributed on many parts of the fly's body surface and they detect sugars, salts, acids, alcohols and noxious chemicals. A large family of candidate taste receptors is selectively expressed in taste neurons, providing essential molecular markers to examine taste recognition. We examined whether the features of taste location and taste quality are mapped in the fly brain using molecular, genetic, calcium imaging and behavioral approaches. Transgenic axon labeling experiments demonstrate that gustatory projections are segregated based on their peripheral location. We also find that projections are segregated by the category of tastes that they recognize. A combination of behavioral and calcium imaging studies reveal that segregated projections represent different taste qualities: neurons that recognize sugars project to a region different from those recognizing noxious substances. These studies demonstrate that taste quality and position are encoded in anatomical projection patterns.

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Symposium: Coding in the Taste System: New Perspectives on an Old Problem

Division of labor in mammalian taste buds

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Mammalian taste buds contain cell types that subserve distinct specialized functions. Separate subsets of polarized (differentiated) cells have been shown to express receptors of the T2R family or combinations of T1Rs. Such cells, with sensitivity to only bitter or sweet or umami compounds, are presumed to synapse directly on taste afferents. This cellular organization, which would produce a 'labeled line' code for taste information, is apparently at odds with many functional data that suggest that individual taste cells can respond to multiple types of taste stimuli. A possible resolution of this conundrum is that sensory signals from specialized receptor cells may converge on separate cells that synapse onto sensory afferent fibers. Consistent with this view, our molecular profiling data suggest separate populations of cells express receptors and synaptic proteins. Further studies to examine information processing within taste buds will illuminate the cellular interactions and mechanisms underlying such processing.

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Symposium: Coding in the Taste System: New Perspectives on an Old Problem

Discriminatory performance of central gustatory neurons

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Current models of taste quality coding incorporate 'patterns,' representing quality by the response of a neuronal population, or 'labeled lines', requiring independent neuronal types that can discriminate among qualities. Here we explored the discriminative capacity of central gustatory neurons within the framework of signal detection theory. Gustatory neurons ($n = 222$) recorded from the nucleus of the solitary tract of anesthetized rats were segregated into types that responded (spikes/s) most effectively ('best') to sweet, salty, sour or bitter stimuli. Receiver-operating-characteristic (ROC) curves were compiled to compute discriminatory performance among responses to best and non-best stimuli within each cell type. The area under the ROC curve (a) is an index of the expected detectability of a signal, in this case the correct taste quality. This measure ranges from 0.5, when the probabilities for a 'hit' (response to stimulus of the same quality as the best stimulus) and 'false-alarm' (response to stimulus of a quality different than the best stimulus) are equal (i.e. chance performance), to 1.0, indicating perfect discriminatory performance. Results show that rate of firing in individual neuron types alone is not sufficient to reliably impart taste quality information. For example, neurons most effectively activated by sweets poorly discriminate between sweet and salty stimuli ($a = 0.60$). Bitter-best neurons fail to discriminate between bitter and salty ($a = 0.54$) or sour ($a = 0.51$) stimuli. These findings have important implications for understanding the neural code for taste.

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Symposium: Coding in the Taste System: New Perspectives on an Old Problem

Sweet, bitter and umami tastes are mediated by distinct cell types and dedicated sensory pathways

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The mammalian taste system can detect and respond to a diverse repertoire of chemical entities. Mice show a strong preference for

sweet substances (sugars and artificial sweeteners) as well as many amino acids (particularly when combined with purine nucleotides) while they are vigorously averse to bitter-tasting compounds. A number of reports have suggested that individual taste receptor cells are responsive to multiple taste modalities, including cells that respond to both aversive as well as appetitive stimuli. These studies led to the proposal of broadly tuned taste receptor cells as mediators of taste, and the belief that sweet and bitter taste are encoded at the periphery by an across-fiber pattern. Instead, we will present data demonstrating exquisite functional segregation of attractive (sugar/amino acid) and aversive (bitter) tastes, and prove that dedicated (hard-wired) taste pathways mediate sweet and bitter responses.

Poster: Computational Approaches

Morphological classification and morphometric modeling of antennal lobe local interneurons in *Bombyx mori*

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The antennal lobe (AL) of insects, the structural and functional analog of the olfactory bulb of mammals, is the first order olfactory center in the brain. The AL consists of glomerular structures. Odor information is represented spatially and temporally on a topographic map of the glomeruli. AL local interneurons (LNs) play critical roles through intra- and inter-glomerular computations to shape the output from the AL to the higher brain centers. However actual dynamics of LNs have been hardly elucidated. As a first step towards understanding the functions of LNs, it is important to reveal the variety of LNs taking part in the AL neural circuits. We adopted the intracellular staining under visual control method and comprehensively sampled neurons in the lateral cell cluster of the AL. We stained intracellularly 126 LNs of the silkmoth *Bombyx mori* and closely analyzed them with a confocal microscope. We revealed the existence of various types of LNs and classified them into four types (seven subtypes) based on morphological differences such as connecting glomeruli, dendritic profiles within a glomerulus. Furthermore, we made a program automatically reconstructing single neurons from the confocal serial images. We applied it to the different types of LNs and investigated the electrical dendritic properties by computer simulation.

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Poster: Computational Approaches

Changing roles of temporal representation of the odorant during the oscillatory response in the olfactory bulb

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It has been hypothesized that the brain uses combinatorial and temporal coding strategies to represent stimulus. The mechanisms and properties of the temporal coding remain undetermined, although it has been hypothesized that synchronization can underlie binding between different stimulus features and thus promote formation of neural representation of stimuli. Here, with a reduced model of olfactory bulb that includes a self-inhibitory property of mitral/tufted cells, we recover some of the basic features of the experimentally observed oscillatory behavior in turtle olfactory bulb and possibly uncover the basis for temporal coding within this oscillatory response. We show that the specific roles of oscillatory response may change through out odorant identification process. The initially observed oscillation combines and resolves features of the presented odorants, while the later one combines the activities of neurons coding identity of the odorant. The former shows that slow self-inhibition mediates formation of a temporal sequence of simultaneous activations of glomerular modules associated with specific odorant features within the oscillatory response and it depends on relative properties of the activated odorant features and thus may mediate discrimination of odorants activating overlapping glomeruli. The latter one involves feedback from coincident modulators and thus can separate odor identities when odor mixture is present. Furthermore we show that the experimentally observed period doubling transition in turtle olfactory bulb may be driven through excitatory feedback from a coincidence mediator.

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Poster: Computational Approaches

Consensus structure–function determinants for olfactory receptors and odor ligands

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The lack of an experimentally determined structure is the primary impediment in the elucidation of OR function. OR structure determination involves building models from low-resolution diffraction data of rhodopsin (Singer, 2000, *Chem. Senses*) and using ab initio positioning of amino acid sidechains (Floriano *et al.*, 2000, *Proc. Natl Acad. Sci. USA*) or through homology modeling using a highly resolved structure of rhodopsin (Man *et al.*, 2004, *Protein Sci.*). In order to arrive at a structural construct for functional olfactory receptors, we identified four olfactory receptors—rat (Buck and Axel, 1991, *Cell*) and mouse I7 (Krautwurst *et al.*, 1998, *Cell*), s19 (Malnic *et al.*, 1999, *Cell*) and OR912–93 (Roquier *et al.*, *Mammalian Genomics*, 1999)—known to be activated by odor ligands. We study the electronic character of the binding pocket to identify facets that promote specificity of ligand binding. Our methodology consisted of mapping the atomic coordinates of a model of the binding pocket of the OR onto a two-dimensional plane and calculating the electrostatic potential of this plane on an electron density surface that mimics the Van der Waals surface. This provides a view of the electronic character of the binding pocket. Results indicate that a strongly negative region on the planar surface will likely match a positive charged ligand, and a receptor with a strong positively charged region will be attached by a ligand with a negatively charged

functional group. Non-polar odorants are likely to be stabilized in a binding pocket by Van der Waals interactions from non-polar amino acid residues. Our methods will aid experimental efforts in identifying rational panels of potential odor ligands.

Poster: Computational Approaches

Functional representation of olfactory receptors: a framework for visualizing odorant space

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Olfactory receptor (OR) genes constitute the basis for the sense of smell, and are the largest gene family in mammalian genomes. Mice and dogs have roughly three times as many putatively functional OR genes compared with humans. At face value, this observation suggests that humans have lost the ability to detect a subset of the odorant space accessible to mouse or dog. However, psychophysical data indicate that odorant discrimination capabilities do not differ markedly between the three species. This apparent paradox might be resolved by invoking a non-random loss of OR genes in humans, in which the intact human olfactory repertoire retains the capability to detect most of the odorant space. To examine this hypothesis, we used the available OR gene repertoires of human, mouse and dog, and inferred the odorant space of the three species by using Grantham's scales for amino acid composition, polarity and volume as proxies for functional differences in binding site properties. We show that this odorant space map is functionally relevant and provides information not obtainable through phylogenetic methods. A comparison of the inferred odorant space of the three species shows great overlap and reveals marked species specific differences.

This work was supported by the NIDCD.

Poster: Computational Approaches

A probabelistic model for discriminating between functional and non-functional olfactory receptors

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Olfactory receptors (ORs), the largest mammalian gene superfamily (900–1400 genes), has >50% pseudogenes in humans. While most of these inactive genes are identified via coding frame disruption(s), seemingly intact genes may also be inactive due to other deleterious mutations. Accurately telling intact genes from pseudogenes is essential for functional and genetic studies. Therefore we developed a probabilistic model for precise pseudogene classification, by assessing the protein's deviation from its functionally crucial consensus. Sixty highly conserved positions were characterized, by comparing mouse and dog OR homologs. These underwent analysis via a logistic regression model aiming to distinguish between functional (mouse) and pseudogenes (human) ORs. A good separation was obtained, correctly characterizing 95% of the functional ORs and 67% of the pseudogenes. Applying this algorithm to seemingly intact human OR genesrevealed that 127 of them (~33%) are likely encoding non-functional proteins, thus bringing the total potential pseudogene count of human ORs to ~70%. Interestingly, another 39 purportedly intact genes were found to be segregating between active and inactive forms due to single-nucleotide polymorphisms, thereby more than doubling the known count of such important human genetic variation loci. Our algorithm is also applicable to a better definition of inactive members in other gene families.

Poster: Computational Approaches

Comparative genomics of odorant- and pheromone receptor genes in rodents

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We applied a comprehensive data mining strategy to examine the repertoires of rat and mouse odorant receptors (ORs) and type 1 pheromone receptors (V1rs) using the mm5 and rn3 genome respectively. In total, 1576 rat OR genes were identified, including 292 pseudogenes. The rat V1r repertoire is composed of 115 intact genes and 72 pseudogenes. We have updated the mouse OR and V1r database using the newest assembly mm5, from which 1380 mouse ORs and 308 V1rs were identified, with more than 100 putative pseudogenes from mm2 becoming intact because of the higher sequence quality. With these new data we have conducted a series of genomic analysis of the OR and V1r genes from mouse and rat at three incremental levels: families, coding sequences and motifs. At the family level, we found that V1r genes have more species-specific families than OR genes. About 20% of intact V1r genes have no orthologous counterparts in the same family, whereas <1% of intact ORs are alone. At the coding sequence level, OR genes are more conserved than V1r genes. OR genes are more similar with their orthologous counterparts than with their closest neighbor, whereas V1r genes are the opposite. Motifs were discovered to obtain biological insights. All of the identified motifs were classified into four categories in terms of the conservation in the repertoire and homology between two species. We further searched for modification sites of the motifs to specify the slight difference of conserved motifs between mouse and rat. Numerous species-specific motifs and special modifications were found, which may result in the differential perception of odors and pheromone-dependent behaviors between mouse and rat.

Poster: Computational Approaches

Molecular modeling sweet taste receptors

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The sweet taste receptor is a heterodimer of two G protein coupled receptors, T1R2 and T1R3. From our experiments with mutant and

chimeric forms of the sweet receptor we have identified three potential ligand binding domains: (i) the amino terminal domain of hT1R2, required for the sweetness of aspartame; (ii) the transmembrane helix domain of hT1R3, required for the sweetness of cyclamate and the sweet antagonism of lactisole; and (iii) the cysteine rich domain of hT1R3, required for the sweetness of brazzein. Although crystal structures are not available for the sweet taste receptor, useful homology models can be developed based on appropriate templates. The amino terminal domain, cysteine rich domain and transmembrane helix domain of T1R2 and T1R3 have been modeled based on the crystal structures of metabotropic glutamate receptor type 1, tumor necrosis factor receptor and bovine rhodopsin, respectively. We have used homology models of the sweet taste receptors, molecular docking of sweet ligands to the receptors and directed mutagenesis of the receptors to identify potential ligand binding sites of the sweet taste receptor. These studies have led to a better understanding of the structure and function of this heterodimeric receptor, and may lead to a rational structure-based design of novel non-caloric sweeteners.

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Poster: Olfaction—Central Anatomy

NR2B gene expression is altered in mouse piriform cortex after peripheral deafferentation

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Olfactory receptor neurons (ORNs) in the olfactory epithelium (OE) project to the olfactory bulb (OB), where they synapse with target neurons such as mitral and tufted cells that convey olfactory information to higher order olfactory structures, including piriform cortex (PC). Although the functional organization of the OB to PC network has been studied electrophysiologically, the molecular mechanisms underlying neuronal plasticity in PC remain elusive. To learn more about the influence of the periphery on gene expression in the PC, we investigated the effect of olfactory deafferentation in the mouse. Intranasal irrigation with zinc sulfate was used to produce total disruption of functional connections from the OE to the main OB with resultant anosmia. Immunostaining for *c-fos*, an immediate early gene, was virtually eliminated both in the main OB and in the PC within 1 week confirming the efficacy of the lesion. We also investigated the effect of this deafferentation on the laminar distribution of glutamate receptors in pyramidal cells in PC using immunocytochemistry. Normally, NMDA receptor 2B (NR2B) is found in layer II where most of the pyramidal neurons reside. These pyramidal neurons extend their apical dendrites to layer I and make synaptic connections with terminals of either mitral/tufted cells, or with ipsi- and contralateral olfactory cortical association inputs. After lesion, this pattern of NR2B expression was shifted from the deep layer to the superficial layers of II. Similar results were observed following nares closure. This study demonstrates that transsynaptic alterations in gene expression in the PC are dependent on peripheral activity.

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Poster: Olfaction—Central Anatomy

α-Bungarotoxin binding in the olfactory system of select inbred mouse strains

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Characterization of the relationship between genetic background and tissue-specific expression will be useful in understanding the functional role of α7-nicotinic acetylcholine receptors (α7-nAChRs). Inbred mouse strains contain polymorphisms in the α7 promoter region that may affect tissue- and stage-specific control of transcription. Inbred mice can also differ in the splicing of α7 mRNA. Because of these differences, inbred mouse strains may demonstrate tissue-specific differences in α7 expression. We characterized the differences in α7 protein between various olfactory tissues in multiple strains of inbred mice. Using [^{125}I]α-bungarotoxin (α-BGT) autoradiography, we quantified α7 protein in olfactory bulb. Here we report the results from C57BL6/J, DBA2/J and C3Hhen mice. These data should provide us the ability to correlate α7 expression with various olfactory parameters and identify putative roles of α7 in the olfactory system.

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Poster: Olfaction—Central Anatomy

Sex differences in the number of tyrosine hydroxylase immunoreactive periglomerular cells surrounding P2 glomeruli

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Differential behavioral responses of female and male rodents to various chemosensory signals have been described. To illustrate, female and male mice exposed to urine from a dominant male mouse are attracted and repelled respectively. Currently, there is evidence that sexually dimorphic glomeruli in the main olfactory bulb may contribute to sex-differentiated behaviors. Here we provide data showing a correlation between the sex differences in the volume of P2 glomeruli and the number of tyrosine hydroxylase (TH)-immunoreactive (IR) periglomerular (PG) cells surrounding the P2 glomeruli. Specifically, we examined the PG cells surrounding the urine responsive sexually dimorphic glomeruli associated with the P2 odorant receptor in both male and female P2-IRES-tauGFP mice. The lateral P2 glomeruli are sexually dimorphic: female mice have a larger glomerular volume compared with male mice. Using immunohistochemical methods, we show that a significantly higher number of PG cells surrounding the lateral P2 glomeruli are stained positively for TH protein in female mice compared with male mice. These results suggest that the TH-IR PG cells surrounding the P2 glomeruli contribute to the sex differences in the volume of those glomeruli. Since others have shown that the TH-IR PG cell

population is activity dependent, our data suggests that the sex differences in P2 glomerular volume may also be activity dependent.

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Poster: Olfaction—Central Anatomy

A new, open source software package for mapping the glomerular layer of the olfactory bulb

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We have developed a suite of informatics software designed to integrate several techniques for mapping glomeruli in an olfactory bulb. This suite provides the necessary tools to process high-resolution digital images and map glomeruli to within a statistically significant region of the olfactory bulb. In addition, the suite can generate contour maps based on glomerular activity, density or area and can further aid in the statistical analysis of these maps. We offer both a plug-in for ImageJ, a public domain Java image processing program, and a toolbox for the cross-platform MATLAB environment (The Mathworks, Inc.). We present our software in an effort to consolidate the disparate techniques that are currently being employed in the field and in the hopes of establishing a universal mapping technique. As such, we have made our software freely available under the GNU public license for noncommercial and open source development, and have included the ability to export data sets to several public domain, web-based repositories for odor maps. Finally, to illustrate the usefulness of our software, we present a brief study on the molecular changes in the murine olfactory bulb that underlies operant conditioning in adult mice responding to odors.

Poster: Olfaction—Central Anatomy

Influences of sensory activity on the development of or specific glomeruli

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The axons of olfactory sensory neurons expressing the same odorant receptor (OR) project to spatially conserved regions of the bulb and coalesce into glomeruli. This process utilizes molecular determinants. The roles of sensory activity have just begun to be appreciated. In a previous study, we examined the maturation of M71 and M72 glomeruli in OR gene targeted mice. ORs M71 and M72 are highly homologous and their glomeruli are within close proximity. Our data showed that the absence of sensory activity perturbs the maturation of glomeruli during distinct sensitive periods, and preserves the heterogeneous glomeruli that normally disappear with age. To examine whether these are common features for glomerular maturation, we are investigating whether sensory activity influences the formation of MOR23 and P2 glomeruli. MOR23 and P2 glomeruli have many features that clearly differ from M71 and M72 glomeruli. Unlike the high homology between OR M71 and M72, OR MOR23 and P2 are not related to each other or to M71 and M72. Unlike the postnatally formed M71 and M72 glomeruli, P2 glomeruli begin to form prenatally. In addition, MOR23 glomeruli are in the dorsal–anterior bulb, while P2 glomeruli in the ventral–medial bulb, which are both different from the dorsal–posterior positioned M71 and M72 glomeruli. Together these selected glomeruli display a variety of features that are likely to represent to a large extent the entire glomerular array. Results from studies on the maturation of MOR23 and P2 glomeruli will begin to reveal not only the common characteristics of glomerular development, but also to address whether the glomerular location and the onset of its formation are critical for the dynamics of glomerular maturation.

Poster: Olfaction—Central Anatomy

Glutamate transporter and NMDA receptor expression in the canine olfactory bulb

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The dog's nose has been used as a chemical detector for centuries, yet little is known about the structure and function of the canine olfactory system. We examined the dog's olfactory bulb, concentrating on the glomerular area, for expression of glutamate transporter proteins and NMDA receptor subunits. Using LM, EM, immunohistochemistry and gel electrophoresis/Western blotting, we found that the glutamate transporter GLT-1 (EAAT2) was the major glial glutamate transporter and that GLAST (EAAT1) was barely detectable in the canine bulb. This contrasts with the results obtained in the mouse olfactory bulb where GLAST was the major glutamate transporter. GLT-1 labeling was heaviest in the periglomerular region of the dog bulb and, by comparison, much lighter within the glomerulus. NMDAR1 and 2A/B were both expressed in the canine bulb. NMDAR1 was present in the perikaryon and proximal dendrites of mitral/tufted and granule cells, but was only scarcely expressed in the glomerulus. Electron microscopy showed this glutamate receptor subunit was mainly found in the cytoplasm of small, dendrite-like profiles, some of which contained clear vesicles. NMDAR 2A/B labeling in the glomeruli was more circumscribed, with clusters of gold particles found on or near the plasma membrane of small profiles, some with, some without vesicles. These results suggest that glutamate concentrations within the canine glomerulus are reduced primarily by diffusion, with uptake concentrated at the periphery. However, the detrimental effects of lingering low concentrations of glutamate in the glomerulus may be mitigated by the relatively sparse expression of NMDA receptors.

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Poster: Olfaction—Central Anatomy

GFAP expression in the diabetic rat olfactory bulb

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Many diabetic individuals develop anosmia but the site(s) of dysfunction is (are) unknown. We showed earlier that the mitotic rate in the diabetic rat main olfactory epithelium (OE) is suppressed compared with that of normal individuals. The OE mitotic rate is regulated by the olfactory bulb (OB) and is dependent on the state of the OB neurons and glia. Astrocytes modulate neuronal function in many respects including the production of neurotrophic agents. It was recently shown that, in other brain regions, astrocytes express glial acidic fibrillary protein (GFAP) at lower levels in diabetics compared with controls but the numbers of S100B(+) expressing astrocytes do not differ between the groups. We used immunohistochemistry to examine astrocyte GFAP expression and S100B expression to count astrocytes in the OB external plexiform layer of male Wistar rats after 8 weeks of STZ-induced type 1 (insulin dependent) diabetes. Qualitative analysis indicates that diabetic rat GFAP expression in the external plexiform layer is lower compared with controls. Quantitative analysis of S100B(+) cell counts showed no difference in astrocyte numbers between or within the groups.

Poster: Olfaction—Central Anatomy

The central chemosensory system of mosquitoes

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Mosquito behavior is heavily dependent on olfactory and gustatory cues, which are detected by receptor neurons on the antennae, maxillary palps and on specialized mouth parts. Recent progress in mosquito sensory genomics has highlighted the need for an up-to-date understanding of the neural architecture of the mosquito brain. Here we reveal, for the first time, the neural structures residing within the primary olfactory and gustatory centres, the deutocerebrum and the suboesophageal ganglion, of two species of mosquitoes, the African malaria mosquito, *Anopheles gambiae*, and the yellow fever mosquito, *Aedes aegypti*. Furthermore, we present three-dimensional models of the glomerular organization of the antennal lobes of males and females of these two species.

Poster: Olfaction—Central Anatomy

The projection of output neurons from the olfactory bulb of the sea lamprey, *Petromyzon marinus*

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Olfactory stimuli elicit a broad range of behavioral responses, including feeding and mating. These behaviors are dependent upon activation of locomotor control areas by olfactory stimuli. For example, in *Petromyzon marinus*, ovulated females respond to a pheromone, 3-keto petromyzonol sulfate, released by spermiated males, by increasing their swimming activity towards the odor source (Li *et al.*, 2003, *Steroids*, 68:297–304). Our overall goal is to determine the neural pathway that governs this response. Research in other species of lamprey *Icthyomyzon unicuspis* (Northcutt and Puzdrowski, 1988, *Brain Behav. Evol.*, 32:96–107) and *Lampetra fluviatilis* (Polenova and Vesselkin, 1993, *J. Hirnforsch.*, 34: 261–279) has revealed secondary olfactory fibers that project to higher order regions in the telencephalon and diencephalon that are associated with locomotor control, including the ventral thalamus and the striatum. We applied a biotinylated dextran into small foci in the ventro-medial olfactory bulb of lampreys. Our preliminary data show labeling of secondary olfactory projections that travel medially and terminate in the telencephalon as seen by Polenova and Vesselkin (1993) and Northcutt and Puzdrowski (1988). These preliminary data support the idea that lamprey olfactory bulb outputs project to neural structures associated with locomotor control.

Poster: Olfaction—Central Anatomy

The localization of terminal nerve fibers in the olfactory bulb of round goby (*Neogobius melanostomus*)

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The nervus terminalis, cranial nerve (0), which modulates peripheral chemosensory neuron activity, is likely active during reproductive behaviour (e.g. reviewed by Wirsig-Wiechmann *et al.*, 2002, *Progr. Brain Res.*, 141:45–58). In this study, we investigate the possibility that the terminal nerve modulates spatially selective regions of the olfactory bulb of the round goby, a percid teleost fish which displays pheromone directed reproductive activity. Terminal nerve processes, containing the molluscan cardioexcitatory peptide FMRFamide and gonadotropin-releasing hormone (GnRH), were recognized through immunocytochemistry, and the axons of olfactory sensory axons were visualized through anterograde labeling with diI. The terminal nerve ganglion was located ventrally, in the transitional region between the olfactory bulb and the telencephalon, adjacent to olfactory sensory neuron extrabulbar fibers that extended from the olfactory epithelium to the diencephalon. Terminal nerve fibers were prevalent in the mitral cell layer and the granule cell layer of the olfactory bulb, and very fine processes were located within inter-glomeruli areas. In the round goby, glomerular territories were abundant ventrally, rostrally and laterally, and very few glomeruli were observed medially or caudally. This spatial relationship between terminal nerve fibers with primary and secondary neurons of the olfactory pathway, suggests

modulation of specific anatomical regions of the olfactory pathway, which may be associated with olfactory sensory activity during reproduction.

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Poster: Olfaction—Central Anatomy

Characterization of three distinct types of output neurons in the adult zebrafish olfactory bulb

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In our continued investigation of mitral cells in zebrafish, we have found that these output neurons should not be identified solely based on their size, their distribution in the olfactory bulb or presence of their axons in the olfactory tract. Using retrograde tract tracing with a biotinylated dextran, we were able to label several cell types including mitral cells, ruffed cells, and ganglion cells of the terminal nerve. Mitral cells were by far the most numerous output neurons in the bulb. They had variable-shaped somata and generally possessed a single dendrite with a discrete dendritic tuft. Ruffed cells were distinguished by an obvious membranous protrusion surrounding the initial part of the axon, and terminal nerve cells could be identified by their distribution in large, amorphous clusters. Ruffed cells and mitral cells showed substantial overlap with regard to their cellular localization, and these cells were similar in distribution to a class of bulbar interneurons, as well. We found that the terminal nerve cells were the largest cells in the olfactory bulb, followed by ruffed cells then mitral cells. Subsequently, future studies examining mitral cell function should use combined methods of recognition to support the identification of this cell type in zebrafish. We are continuing our exploration of output neurons by focusing on activity-dependent morphology in mitral cells in order to establish if there is a connection between afferent input and dendritic structure in this key cell type.

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Poster: Olfaction—Central Anatomy

Distinguishing between periglomerular and granule cells in the mouse olfactory bulb

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The predominant types of olfactory bulb (OB) interneurons are periglomerular (PG) and granule cells. Both are derived throughout life from stem cells in the subventricular zone (SVZ) and migrate to the OB in the rostral migratory stream (RMS). In the RMS of neonatal mice, we showed expression of transgenes [either GFP or LacZ reporters driven by a tyrosine hydroxylase (TH) promoter] that mark the dopamine (DA) phenotype. These findings suggested that DAergic differentiation begins prior to progenitors reaching a PG position despite evidence that TH protein expression occurred only after PG cells received olfactory afferent stimulation. Previously we have shown that calcium calmodulin-dependent protein kinase IV (CaMKIV) labeled a subpopulation of deep granule cells with no overlap between expression of TH/LacZ or TH/GFP and CaMKIV. Recently, expression of the ETS transcription factor, ER81, was found primarily in PG and superficial granule cells (Stenman, 2003, *J. Neurosci.*, 23:267) suggesting a role in DA differentiation. The current study confirmed that ER81 occurs in PG and superficial granule cells, the RMS and SVZ, as well as in cortex, cerebellum and superior colliculus. Expression of ER81 and CaMKIV did not overlap even in the granule cell layer where both were found. While most TH-stained cells coexpressed ER81, not all ER81-containing cells coexpressed TH and some PG cells did not contain either marker. Odor deprivation produced by adult unilateral naris occlusion decreased TH, but not ER81, expression. These data suggest that ER81 and CaMKIV distinguish distinct subsets of OB interneurons, but neither plays a role in the regulation of the DA phenotype.

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Poster: Olfaction—Central Anatomy

Long-term effects of acute social stress on neurogenesis in the male golden hamster dentate gyrus and olfactory bulb

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Since the discovery of neurogenesis in the brains of adult mammals in 1965, researchers have attempted to understand the signals that regulate proliferation and the factors that control and mediate migration and survival, and to understand the functional significance of these cells in the adult animal. One of the most interesting findings to come out of this research is that levels of neurogenesis are intimately tied to hormonal changes associated with stress, changes in circulating gonadal hormones and environmental manipulations such as enrichment. The goal of this experiment was to replicate and extend findings reported in the tree shrew, demonstrating that stress induced through social subordination results in decreased neurogenesis in the dentate gyrus. For our study, we utilized the thymidine analog BrdU to identify newly dividing cells in the brains of adult hamsters. We took advantage of the well-characterized aggressive behavior of the golden hamster to address the question of whether or not acute bouts of social and agonistic interactions have an impact on neurogenesis in animals that lose, win or receive comparable levels of non-agonistic social experience. We further attempt to correlate changes in levels of neurogenesis with behavioral measures of stress as well as agonistic behaviors. We describe here an extension of previous research in our laboratory and report physiological and anatomical changes in these animals, as well as report levels of neurogenesis observed in the dentate gyrus and olfactory bulb. In the future we hope to expand upon these findings to begin to understand the functional role of these cells and their regulation by stimuli in the environment.

Poster: Olfaction—Central Anatomy

Bulbar synaptic targets maintain olfactory sensory neurons in adult mice

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Axons from olfactory sensory neurons (OSNs) expressing the same odor receptor converge to topographically fixed glomeruli in the olfactory bulb. Odor receptors govern axon guidance and convergence, as shown by receptor swap and gene deletion experiments. Bulbar cues do not control convergence, as this occurs even in the absence of the bulb. However glomerular targeting is disrupted in adults when the OSN population regenerates after axotomy or chemical lesion of the epithelium. With these methods, mature OSNs die and are replaced by newly developing OSNs that reinnervate the bulb with less precise topography. In the present study, we examined the organization of mature sensory axons expressing the P2 odor receptor in adult mice after chemically ablating bulb neurons with *N*-methyl-D-aspartate (NMDA); this lesion leaves mature OSNs, their axons and ensheathing glia, intact. Rapid degeneration of bulb neurons was followed by shrinkage of glomeruli into smaller, irregular compartments. Within these loci, sensory axons continued to converge and maintained their approximate topographic locations for up to 2 weeks after bulb lesion. However, with increasing time, there were progressively fewer P2 axons in the lesioned bulb and fewer P2 neurons in the ipsilateral nasal cavity. By 3 weeks, the targetless sensory epithelium was thinner and olfactory marker protein mRNA levels were reduced. These results support evidence from other studies indicating that bulbar synaptic contacts do not maintain sensory axon convergence but do regulate neuronal turnover in the olfactory epithelium.

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Poster: Olfaction—Peripheral Anatomy

Mitral cell survival, regeneration of olfactory tract and recovery of olfaction following neonatal olfactory tract axonal damage in rats

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Olfactory bulb projection neurons were disconnected from the piriform cortex by olfactory tract transection (OTS). This tract damage initially produced degeneration; but following longer survival, regeneration of olfactory tract occurred (Singh *et al.*, 1997, *Exp. Neurol.*, 144:174–182). Here the main focus was to assess mitral cell survival and recovery of olfaction after OTS. The olfactory tract was transected in newborn (P0) rat pups and bulbs were obtained at P15, P30, P45 and P70 days. Coronal sections of bulbs were cut and alternate serial sections were Nissl stained. Mitral cells were counted, there was significant mitral cell loss after OTS at all ages (ANOVA, $P < 0.001$). More mitral cell loss was found at P15 (43%) than other age groups; however, there was a steady and gradual reduction in mitral cell loss, with greater mitral cell survived by P70 (57 versus 68%). Rats were trained to locate and retrieve chocolate hidden in random locations in a wooden testing cage (P22–28). Normal rats learned to retrieve chocolate within 5 min criterion, but no OTS rats were able to do the task in 5 min criterion. During test sessions (P31–69), the normal rats performed well reaching a 30 s latency at 6 weeks. OTS rats showed severe deficit in the first test sessions, with a profound improvement afterwards, from a latency of 4.93 ± 0.05 min at 2 week to 0.82 ± 0.28 min at 6 week, indicating a significant recovery. Reorganization of higher order olfactory connections is exceptional due to inherent growth properties of the mitral cells and their axons in the olfactory tract.

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Poster: Olfaction—Peripheral Anatomy

Tenascin-C and its receptors and binding partners in the developing olfactory system

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Glomerular development follows a distinct spatiotemporal timeline. Olfactory sensory neurons (OSNs) extend axons to the telencephalon where they form a presumptive olfactory nerve layer (pONL). Axons remain restricted to the pONL for up to 4 days before growing in deeper into the olfactory bulb (OB) and inducing glomerular development. We have identified the extracellular matrix (ECM) molecule Tenascin-C (TNC) as a candidate boundary molecule that prevents OSN axon ingrowth prior to glomerular formation. To test this hypothesis we utilized an *in vitro* assay of OSN neurite outgrowth. We demonstrate that OSN explants plated on purified TNC show a dose-dependent inhibition of neurite outgrowth, providing strong evidence that TNC acts as an inhibitory guidance cue to OSN axons *in vivo*. TNC is a large alternatively spliced multi-domain protein, which contains both adhesive as well as counter-adhesive activities that coexist in different domains that are mediated by different interactions with binding partners and receptors (both integrin and non-integrin). Thus far, we have localized one receptor, Contactin/F3/F11, and one binding partner, phosphacan, to the developing olfactory system. Moreover, preliminary PCR screens of both the integrin family of ECM receptors and the matrix metalloproteinase (MMP) family of ECM degrading enzymes have revealed dynamic expression patterns in the developing olfactory system. Glomerular formation likely involves a coordinated response of alternative splicing, selective TNC degradation and/or modulation of TNC receptor expression to allow axon ingrowth and synaptic contacts to form.

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Poster: Olfaction—Peripheral Anatomy

Onset of olfactory sensory neuron molecular phenotype in the early embryonic mouse

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Olfactory sensory neurons (OSNs) have a number of molecular markers which distinguish them from non-sensory cells in the olfactory epithelium (OE). The mechanisms that influence the earliest differentiation of OSNs are not fully understood. As a first step toward defining their molecular differentiation in the nascent OE, beginning at embryonic day 8.5, we used a panel of 11 markers characteristically found in mature OSNs. In most cases, Theiler staging (TS) was used as a more reliable index of development than gestational age. The earliest marker to appear was beta-tubulin III at TS15/E8.5, indicative of the neuronal nature of the OSNs. By TS16–17/E9–E9.5 PSA–NCAM appeared. These two markers, the most precocious we studied. Of interest, expression of PSA–NCAM preceded NCAM expression by ~12 h. GAP-43, normally thought to identify immature axons, was first detected in subpopulations of OSNs at TS18/E10, the same age at which NCAM was first detected. Onset of OMP and OCAM expression was first detected in subsets of OSNs at TS20/E11, at least 12 h after axon outgrowth is initiated. By TS20/E11, a definitive subset of OSN axons encapsulated the nascent olfactory bulb, and most remaining markers, including TAG1, contactin, peripherin and N-cadherin, are detected in the OSN axons. These data demonstrate that each of the markers associated with OSNs has a unique temporal sequence of expression which is likely to correlate with the morphological aspects of OSN maturation.

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Poster: Olfaction—Peripheral Anatomy

Olfaction and olfactory epithelium in zinc gluconate treated mice

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The fact that intranasal administration of zinc sulfate can produce transient degenerative changes in olfactory epithelium in a variety of species has raised concerns about the safety of using zinc compounds in non-prescription nasal formulations. Recently, Jafek *et al.* (2004) provided anecdotal reports of patients who claim to have suffered a loss of smell function after using an intranasal cold medication containing zinc gluconate. Further, press reports and websites describe similar concerns and even lawsuits from users of zinc gluconate nasal products. We used anatomical and behavioral methods with mice to assess effects of a commercial nasal formulation containing 1.6% zinc gluconate. Bilateral nasal injection of 2 µl of product (or more than 5 times the recommended equivalent human dose) had no discernable effect on a psychophysical test of odor sensitivity or odor discrimination. Unilateral injections had no discernable effect on anterograde transport of HRP from OE to OB glomeruli although partial deafferentation was produced by 2 µl of 5% zinc sulfate, indicating that this volume was sufficient to produce anatomical changes. Much larger volumes (50 µl) of product did cause widespread deafferentation in 2 day survival mice but recovery was largely complete in 20 day survival cases. Our results fail to support the contention that appropriate use of zinc gluconate containing nasal sprays could damage olfactory epithelium or produce hyposmia or anosmia.

Poster: Olfaction—Peripheral Anatomy

Olfactory ensheathing cells and target tissue influence primary olfactory axon trajectory.

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During development of the primary olfactory system, numerous guidance molecules are known to contribute to axon sorting and targeting; however, we still do not understand how axons follow discrete peripheral trajectories to reach the olfactory bulb. For example, axons arising from the ventral nasal cavity make a distinct dorsal turn at the rear of the septum to reach the olfactory bulb. We investigated the growth of axons *in vitro* to assess (i) whether the behaviour of axons and olfactory ensheathing cells (OECs) contributes to fasciculation; and (ii) whether the target tissue influences axon trajectories. We generated reporter transgenic mice that express dsRed under the control of the S100B promoter so that OECs are easily identified. Time-lapse microscopy of axon fascicles revealed that OECs display dynamic behaviours and rapidly moved through this tissue. In addition, OECs respond to axon contact with fast extension and retraction of processes. We then examined axon trajectories using a co-culture system of nasal septum and brain. In the absence of brain tissue, axon fascicles fail to turn dorsally at the caudal septum. In comparison, co-culture with olfactory bulb placed at the caudal septum caused the axon fascicles to turn dorsally. Together, these results reveal that development of axon fascicles involves highly complex and motile interactions between axons and OECs and that their trajectories are influenced by the presence of the olfactory bulb.

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Poster: Olfaction—Peripheral Anatomy

Morphological analysis of crayfish (*Orconectes rusticus*) major chelae sensory hairs

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It has been shown that the major chelae of crayfish have both mechano- and chemosensory abilities. Our previous investigations have shown that reproductive male crayfish (*Orconectes rusticus*) use their major chelae and respond significantly with an increased handling time to a female conditioned water source when compared with those crayfish whose chelae have been sensory lesioned. Non-reproductive males did not respond significantly to female odors. These results suggest that it is important to investigate the morphology and neuroanatomical attributes of the sensory hairs on the major chelae of male crayfish that correlate to chemosensory function. We used scanning electron microscopy to visualize hair pockets on the dorsal surface of the chelae and compared number of sensory hairs (simple and plumose setae) for reproductive and non-reproductive males. In addition, we performed a permeability assay

using crystal violet and determined that simple setae readily take-up and hold the dye after clearing with xylene, while plumose setae do not. This suggests that simple setae are potential chemoreceptors and that odorous substances can permeate the cuticle to the underlying neural cells. To confirm this observation, transmission electron microscopy was used to determine investigate the ultrastructure of simple and plumose setae, investigating structures containing microtubules. Also, retrograde labeling of the chelae nerve at the merus–carpus joint using DiI and DiO was completed and it has been shown that only the simple setae and not the plumose setae show innervation. These morphological findings suggest that the simple setae on the chelae are putative chemoreceptors that may be used for perception of female odours.

Poster: Olfaction—Peripheral Anatomy

Evidence for endogenous neurosteroid production in the mammalian olfactory mucosa: immunocytochemical localization of cytochrome p450scc

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Steroid hormones and their metabolizing enzymes have previously been identified in the olfactory mucosa. Enzymes associated with different types of cytochrome p450s, which are thought to play a role in the metabolism or activation of airborne toxins, have also been identified in mammalian olfactory mucosae. In the synthesis of steroid hormone, cholesterol is transported to the mitochondria where side-chain cleavage enzymes (cytochrome p450scc) convert cholesterol into pregnenolone. Conversion of cholesterol to pregnenolone is an obligate step in steroid hormone production. The specific aim of this study is to identify cytochrome p450scc in the mammalian olfactory mucosae. Using polyclonal antibodies to p450scc, we found immunoreactivity for cytochrome p450scc in the rat olfactory mucosa. Within the olfactory epithelium, the supranuclear region of sustentacular cells was immunoreactive for cytochrome p450scc. Olfactory neurons, basal cells, olfactory nerve axons and acinar cells of Bowman's glands were unstained. In positive control tissue (adrenal glands), staining for p450scc was seen in all layers of the adrenal cortex. The localization of cytochrome p450scc to sustentacular cells is consistent with a functional role for mitochondrial cytochrome p450scc in the production of olfactory mucosa-specific neurosteroids. This study provides evidence that endogenous neurosteroids are involved in the modulation of olfactory function.

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Poster: Olfaction—Peripheral Anatomy

Morphological analysis of two types of receptor neurons in goat olfactory epithelium

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Most mammals have two nasal chemosensory epithelia that are olfactory epithelium (OE) and vomeronasal epithelium (VNE). Olfactory receptor neurons (ORNs) detect odor molecules and have cilia in their apical surface, whereas vomeronasal receptor neurons (VRNs) detect pheromone molecules and have microvilli. It still remains unclear whether these findings in rodents are common in other mammals such as goat. In previous our study, we identified goat pheromone receptor genes and one of them (gV1ra1) was expressed in not only VRNs but also ORNs. In this study, we investigate morphological characteristics of goat ORNs using transmission electron microscopy (TEM) and immunocytochemistry. TEM observation revealed that there were microvillous cells in OE. Microvillous cells were located in the supporting cell layer. Although supporting cells in OE also have microvilli, there were morphological differences between cytoplasmic structure of microvillous cells and that of supporting cells. To determine whether these microvillous cells were neuron, we performed immunocytochemistry for olfactory marker protein (OMP). Most OMP (+) neurons were located in the receptor cell layer. Some OMP (+) cells were located in the supporting cell layer, suggesting that OMP (+) cells located in the supporting cell layer possess microvilli. Although the function of the microvillous cells is unknown, the present results imply that two subsets of receptor neurons that have either cilia or microvilli may exist in goat OE.

Poster: Olfaction—Peripheral Anatomy

Sodium channel distribution in the mouse main olfactory epithelium

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Voltage-gated sodium channels (VGSCs) produce the action potentials in olfactory sensory neurons (OSNs). Hormones (epinephrine, GnRH) and neurotransmitters (dopamine, 5-HT) can modulate these channels, potentially affecting both odor sensitivity and perception. In neural tissues nine primary isoforms of VGSCs occur with subtle differences in their physiology and regulation. Previous RT-PCR experiments detected mRNA for five of the nine isoforms (Nav1.2, Nav1.3, Nav1.5, Nav1.6 and Nav1.7). We have examined the location in the main olfactory epithelium (MOE) of three isoforms in a study of their contributions to signal transduction and neuromodulation. Immunocytochemistry was performed using isoform-specific, commercially available antibodies on coronal slices of mouse MOE. Nav1.5 was strongly expressed near the basement membrane, in OSN dendrites, dendritic knobs, somata and axon bundles in the lamina propria. The kinetics of this isoform may affect repetitive firing. Nav1.3 was present in some OSNs; its staining was primarily in OSNs in the basal layer with very faint staining of dendrites through the sustentacular cell layer, but no staining of dendritic knobs or axon bundles. By contrast, Nav1.2 was found in axon bundles, in OSN somata and dendrites, and sustentacular cell somata; it is unclear whether Nav1.2 is present in the dendritic knobs. Nav1.2 and Nav1.3 showed little overlap, suggesting that the OSNs expressing these isoforms are largely distinct; this may be a consequence of neuronal development, for in the brain Nav1.3 is found in immature neurons while Nav1.2 is found in mature neurons. All three VGSC isoforms were also detected at low levels in ciliated, non-neuronal respiratory cells, and Nav1.5 was present in smooth muscle cells.

Poster: Olfaction—Peripheral Anatomy

Differential distribution of aquaporins in main and vomeronasal olfactory epithelia, and in vallate taste buds; a freeze-substitution fine-structural study in the rat

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Aquaporins are transmembrane proteins that regulate cell volume and thus salt and protein balance. At least 10 such aquaporins are known. We studied the fine-structural immunocytochemical distribution in the rat's main and vomeronasal olfactory epithelia, and in vallate taste buds, of aquaporins 1–9 (excluding 7). In main and vomeronasal olfactory epithelia, none of the antibodies labeled sensory cell and so-called microvillar cell structures (the latter only in the main system). Instead, aquaporins 1 and 2 are specifically expressed in microvilli of supporting cells of the vomeronasal sensory epithelium. Anti-aquaporin 3 immunoreacts with lateral cell membranes of olfactory epithelial supporting cells. Aquaporin 5 is found in supporting cell microvilli of this epithelium, and more sparsely, in supporting cell microvilli of the vomeronasal sensory epithelium. Aquaporin 4 is expressed in the lateral cell membranes of ciliated cells of the non-sensory part of the vomeronasal organ, and of respiratory epithelia. Unlike the situation in olfactory systems, sensory did label in vallate taste buds. Aquaporin 1 immunoreacts with microvilli of cells that appear similar in morphology to gustducin(+) cells, but that do not label with anti-gustducin. Antibodies to aquaporin 2 label epithelial cells that surround vallate taste buds. Those to aquaporins 6, 8 and 9 did not immunoreact in all cases. Thus, sites of volume and salt regulation in main and vomeronasal olfactory epithelia seem to be mainly located in supporting cells; in vallate taste buds the sensory cells themselves could play a role in such functions.

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Poster: Olfaction—Peripheral Anatomy

Lectin application to the olfactory epithelium supports the multiple profile-multiple receptor site model for vertebrate olfaction

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Odorants are postulated as having numerous mutually independent or overlapping odor active molecular profiles (a, b, c, etc.). Each profile is able to interact physically and reversibly with a corresponding receptor site (A, B, C, etc.) on or in an olfactory receptor cell, resulting in individual interactions (aA, bB, cC, etc.). A variety of receptor sites and cells must sense an odorant's multiple profiles before information about its quality is completed. Rats trained to detect low odor concentrations of only ethyl acetate (EA) do not respond to EA presentation after application of the lectin Concanavalin A (ConA) to the olfactory epithelium (OE) while rats trained to detect several different odorants still respond to EA in spite of the preceding ConA application. It is assumed that the lectin modifies the odor quality by selectively binding to a receptor site involved in EA recognition leaving others free to still interact with other molecular profiles of the EA odorant. Rats trained to several odorants learned to generalize and therefore will respond to any odorant offered. The behavioral data are supported by Fos immunoreactivity. The distribution pattern of active cells in the olfactory bulb is odorant dependent. After treatment of the OE with ConA the distribution pattern is modified. Similarly, application of the lectin WGA to the OE also results in a modified activity pattern, however, different from that obtained after ConA application.

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Poster: Pheromones/VNO

A putative social chemosignal elicits different cortical responses than perceptually similar odorants

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The endogenous compound androstadienone is by some considered to be a putative human pheromone. To test whether the odor of androstadienone is processed differently than perceptually similar odors, chemosensory event-related potentials (ERPs) were recorded in 15 healthy right-handed women. The three compounds androstadienone, androstenone, and H2S were presented 20 times each monorhinally in blocks of four. The odors were deemed to be iso-intense and of a similar hedonic valence. Moreover, there were no differences between the three odorants in amplitudes. However, ERP analyses indicated that androstadienone was processed significantly faster than the other odorants. Androstadienone produced shorter latencies on all ERP components in comparison to both androstenone and H2S but most so for the late positivity. There were no differences in latencies between androstenone and H2S. The large differences in latency in all ERP components suggest that androstadienone is processed by a neural subsystem different from the main olfactory system. Such a sub-system has previously been proposed for emotional visual stimuli.

Poster: Pheromones/VNO

Medial amygdala categorizes species-specific chemosensory input in both male hamsters and male mice

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In previous work with male hamsters, we have shown that both anterior and posterior medial amygdala (MeA, MeP) express increased immediate early gene proteins (Fos and FRAs) when exposed to conspecific, socially relevant, chemosensory input from females [hamster vaginal fluid (HVF) and female flank gland secretion (fFGS)] and from males [male flank gland secretion (mFGS)]. When exposed to heterospecific stimuli (male or female mouse urine), the hamster MeA, but not the

MeP showed increased levels of immediately early gene expression. The present studies demonstrate that the medial amygdala of male mice also responds categorically to species-specific chemosensory input. The mouse MeA responded to conspecific stimuli (male mouse urine and female mouse urine) and to heterospecific stimuli (HVF, fFGS and mFGS), but MeP responded only to the conspecific (mouse) stimuli. In hamsters, another part of the amygdala, the largely GABAergic intercalated nucleus (ICN) is activated when MeP was not activated with heterospecific stimuli, suggesting inhibition of MeP by ICN. Preliminary data suggests the same reciprocal relationship between ICN and MeP in mice. There were no significant differences in attention to the stimuli (on swabs) that could account for differences in amygdala Fos or FRAs expression between groups.

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Poster: Pheromones/VNO

Modulation of main olfactory input to the medial amygdala by gonadotropin-releasing hormone (GNRH)

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Mating in male hamsters is dependent on chemosensory input from the vomeronasal organs or main olfactory system, whose central pathways contain cell bodies and fibers of gonadotropin-releasing hormone (GnRH) neurons. In sexually naive males, mating is impaired by vomeronasal organ removal (VNX), but not by olfactory lesions. Intracerebroventricular (icv-) GnRH restores mating behavior in naive VNX males and enhances activation of the medial amygdala by chemosensory stimulation, perhaps by enhancing olfactory access to the amygdala. However, initial results suggest GnRH suppresses amygdala activation from electrical stimulation of the main olfactory bulb (MOB) in naive VNX males. We analyzed FOS-protein expression in the anterior (MeA) and posterior medial (MeP) and anterior cortical nucleus (ACN) of the amygdala, piriform cortex (PC) and medial preoptic area (MPOA); with icv-GnRH (50 ng) or saline infusion into the lateral ventricle and electrical stimulation (250 μA) of the MOB to activate main olfactory input. Initial results in naive VNX males indicate an interaction between icv-GnRH and electrical stimulation, decreasing FOS activation in the medial amygdala and PC below levels with electrical stimulation or GnRH alone. These results suggest an action of GnRH in the amygdala that affects transfer of information between the two olfactory systems, but may differ depending on the pattern of input.

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Poster: Pheromones/VNO

Desensitization in the mammalian vomeronasal organ

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Pheromones are necessary for the reproductive and social behavior of most mammals and are detected through the vomeronasal system. The initial events of pheromonal detection require the activation of G-protein receptors and the transduction of this stimulus into an electrical signal. Once transduced, this signal can be desensitized by several mechanisms. Receptor desensitization is induced by activation of the receptors via their respective agonists. One such mechanism of desensitization is due to the actions of G-protein-coupled receptor kinases (GRKs), which specifically phosphorylate only the agonist-occupied form of receptors, and hence, cause uncoupling of the G-protein from the receptor. Phosphorylation of receptors by GRKs subsequently promotes the binding of regulatory arrestin proteins, such as Beta-arrestins, that are thought to prevent further receptor coupling to G-proteins. To investigate desensitization in the vomeronasal organ (VNO), microvillar membranes from mouse VNO were prepared. Through Western blot analysis we were able to show the presence of GRK 2, GRK 3 and Beta-Arrestin 1 and 2. We further investigated whether the proteins are localized to the microvillar surface of the VNO. Immunohistochemical staining of coronal sections through the VNO with antibodies against GRK 2 and 3 revealed staining of microvillar tufts at the surface of the vomeronasal lumen.

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Poster: Pheromones/VNO

Sex-specific responses to urinary chemicals by the mouse vomeronasal organ

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The vomeronasal organ plays an essential role in the detection of pheromones; the chemical cues secreted by animals that elicit genetically programmed sexual and aggressive behavior among conspecifics. Previous experiments have shown that behavioral responses to the same pheromone differ depending on the sex and endocrine status of the respondent. Possible contributors may be sexually dimorphic receptors or differences in central processing within the brain. Two urinary compounds (2-heptanone and 2,5-dimethylpyrazine) were used to stimulate the production of inositol (1,4,5)-trisphosphate (IP_3) in microvillar membrane preparations of the vomeronasal organ as an indirect measurement of pheromonal stimulation between male and female mice. Incubation of VNO membrane preparations from prepubertal mice with urine from the same sex or opposite sex results in an increase in production of IP_3. This stimulation is mimicked by GTPγS and blocked by GDPβS. We found that 2-heptanone present in both male and female urine was capable of stimulating increased production of IP_3 in the female VNO but not the male VNO. Finally, 2,5-dimethylpyrazine present only in female urine was also only capable of stimulating increased production of IP_3 in the female VNO.

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Poster: Pheromones/VNO

Chemical assessment of fighting risks: the fine-tuned responses of the lizard *Liolaemus monticola*

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A crucial aspect for an animal's fitness is its ability to make 'good' decisions. What remains unclear in most ecological domains of chemoreception, particularly in vertebrates, is how individuals assess the characteristics of their conspecifics, such as the fighting abilities of potential opponents (e.g. body size). It also unclear if animals can process chemical information from continuous variables, or whether they have specific thresholds for the perception of different chemical signals which determine on/off responses. I tackled these questions by studying the behavior displayed by *Liolaemus monticola* males in the territory of an unknown conspecific male (resident), during the owner absence. The range in difference between body size of intruder and resident (asymmetry index) was 36 mm. This index was negatively correlated to variables associated to chemoreception (latency to the first tongue flick, number of tongue flicks and motion time) and social interactions (number of headbobs and number of different displays). Additionally, the asymmetry index was positively correlated to the latency to the first headbob. These data indicate that males of *L. monticola* can process and assess continuous information from chemical signals. In the context of fighting risk, males appear to behave according to a fine balance between their own intrinsic characteristics and those of a potential opponent, suggesting that self-reference phenotype matching should be the mechanism employed for these types of decisions.

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Poster: Pheromones/VNO

Homer, a family of adaptor proteins, is expressed in the vomeronasal organ and olfactory bulb

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The roles of protein–protein interactions in the generation of an electrical signal in the rodent vomeronasal organ (VNO) are not clear. The transient receptor potential channel 2 (TRPC2) is important for the response of the VNO to chemical stimuli, as its genetic ablation results in nonspecific mating and lack of aggressive behavior towards intruders. TRPC2 also physically interacts with a member of the putative scaffolding apparatus, namely the type 3 IP_3 receptor (IP_3R3). Here we show that Homer, a family of adaptor proteins, may be important in regulating the activity of the VNO. The Homer family is composed of long forms (H1b/c, H2a/b/c and H3) which dimerize and interact with proteins at each end of the dimer, and a short form, H1a, which is incapable of dimerization. SDS–PAGE and Western analysis of VNO tissue reveals expression of H1b/c and H3, but not H2, in the VNO, whereas all three isoforms are found in the olfactory bulb. The short form, Homer 1a, is a product of an immediate–early gene and expression is activity-dependent. Here we demonstrate that Homer 1a protein is upregulated in the VNO within one h of investigation of stimuli by the VNO. In addition, Homer co-immunoprecipitates with TRPC2 and IP_3R3 from VNO lysates. Therefore, Homer may serve as a stimulation-dependent linker between TRPC2 and IP_3R3, and this complex may be associated with a larger protein scaffold whereby IP3 and/or adaptor proteins could subserve regulatory functions of the primary transduction current in the VNO.

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Poster: Pheromones/VNO

Identification of a sex-specific peptide that stimulates vomeronasal sensory neurons in behaving mice

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In mammals, social and reproductive behaviours are modulated by pheromones, which are chemical signals that convey information about sex and strain. Although numerous molecular and physiological findings suggest that the vomeronasal organ (VNO), located at the base of the nasal septum, is responsible for mediating pheromone information in mice, the nature of pheromones and their action has remained elusive. In this study, we aimed to identify male-specific compounds secreted from the facial area. The three-step column purification of active fractions resulted in the resolution of active peaks. The N-terminal sequence analysis and the mass spectrometry revealed that the peaks had a single male-specific peptide, named ESP1, that was encoded by a gene from a previously unrecognized large family. The recombinant ESP1 elicited an electrical response in the VNO by activating sensory neurons that expressed a specific V2R-type pheromone receptor. Conversely, female mice secreted distinct peptides that, in turn, stimulated a different type of V2R receptor neurons. These results indicate that mice respond to sex-specific peptides via an accessory olfactory pathway during direct contact in behaving mice.

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Poster: Pheromones/VNO

The ontogeny of sexually dimorphic reproductive signals and responses by wild and captive Asian and African elephants

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Complex behaviors of sexually dimorphic elephants are interlocked with an extensive chemical communication system. Wild and captive Asian males use (*Z*)-7-dodecenyl acetate pheromone levels to precisely assess female periovulatory status. These males undergo a two-decade-long maturation process; fully adult males sustain long musth periods, and demonstrate a high level of chemosensory responsiveness. In particular, the pheromone frontalin, indicative of adult musth, facilitates dominance ascension and access to females. Our inchoate wild studies on the African species have demonstrated that olfactory responses divaricate between sexes during calfhood and exhibit differential patterns as maturation occurs. Concurrent with maturesence and initial appearance of musth, males exhibit elevated chemosensory exploration. This more widely

ranging interest in odor fields continues to develop as they reach full maturity, with especially intense chemosensory responses and behaviors toward a primary disperser of chemical signals—the urine. Mature captive males respond with a high frequency of chemosensory responses to both preovulatory urine and its solvent extracts. Known functionalities of albumin and odorant binding proteins in the Asian species are being explored in our studies of African elephant chemosignalling.

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Poster: Pheromones/VNO

Androgen receptor expression in female olfactory epithelium

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Androgens act via nuclear androgen receptors. Although, androgens are present in females as a metabolic intermediate, they appear at a low concentration. In neuronal tissue, which is influenced in growth by androgens, more cells for example in the visual cortex of males express androgen receptors than in females, resulting in a visual cortex structure bigger in males than in females (Nunez *et al.*, 2000, 2003) because of less cell death in males. In the rat, males are bigger than females and so is the olfactory sheet of the nose. Thus we were interested, first, if any expression of androgen receptor (AR) is present in female olfactory epithelium (OE), and second, if so, if there is a difference in expression level comparing male and female OE. Using RT-PCR we show, that AR expression is found in adult female OE and that the expression level does not differ from male OE, either in the septal or in the turbinate mucosal fraction. The expression of ARs in approximately equal amounts in the olfactory epithelium of males and females suggests the possibility that androgen-dependent genes might be active; thus they can influence and regulate processes that are not gender-related, such as the cell cycle cascade. This observation, plus two other observations, namely that (i) the androgen-induced proliferation inhibitor, APRIN, is also expressed approximately equally in males and females at the same ages and (ii) the proliferation density is the same in males and females at the same age, raises the possibility that androgens play a role in olfactory proliferation in both males and females.

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Poster: Pheromones/VNO

Kinetic studies on ligand binding to insect pheromone-binding proteins

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Insects have remarkable sensitivity and selectivity towards species-specific odorants (pheromones) used for communication. We are studying pheromone detection in the gypsy moth, *Lymantria dispar*. The moths have sensory hairs (sensilla) on various body parts. The sensilla known to detect the pheromone in gypsy moths are located on the antennae. These sensilla contain the sensory neuron, protected within the chitin structure of the hollow hair. Odorants enter the lumen of the hair through pore canals, which end in a protein-rich fluid (lymph) in the lumen. The major protein in sensillar lymph is the pheromone-binding protein. These proteins are known to bind hydrophobic ligands, and it has been hypothesized that they aid in solubilization of water-insoluble compounds in the aqueous lymph. We have hypothesized that the binding proteins contribute to two aspects of odorant detection in a sensory hair: (i) the concentration window the hair responds to; and (ii) the molecular selectivity of the hair. Previous studies have used the equilibrium constants of lignad binding as a measure of binding protein selectivity. However, the binding protein/ligand system typically requires 30 min to reach equilibrium. This is much longer than the time a hair spends in an odor plume (from ms to s). We hypothesize that the rate of ligand binding and release amplify the inherent ligand binding selectivity of the binding proteins and thereby significantly contribute to the selectivity of a sensory hair. We present a new assay, in which we probe fluorescent tags (covalently attached to the binding protein) to follow slow and rapid kinetic processes. The rates of the rapid and slow process vary with ligand and also with conditions (salt, pH).

Poster: Pheromones/VNO

Sex, strain and individual variation in the major urinary proteins (MUPs) expressed by inbred laboratory mice

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Major urinary proteins (MUPs) are abundant in the urine of house mice and play a number of important roles in communication. The MUPs of male mice bind several volatile pheromones that are species- and sex-specific, including 2-s-butyl-4,5-dihydrothiazole (thiazole) and 3,4-dehydro-exobrevicomin (brevicomin). These ligands are attractive to both sexes, stimulating competitive signalling and aggression in males and priming female reproductive physiology. MUPs are also highly polymorphic, such that individual genetically heterogeneous wild mice each express a different pattern that is used to identify the individual scent owner. Given their importance in mouse communication, we undertook a quantitative and qualitative survey of the urinary protein output from a wide range of inbred and MHC-congenic laboratory mouse strains using a variety of proteomic approaches. We examined strain differences according to their genetic lineages in addition to sex differences and variability within a strain. Sex differences in MUP expression were not as pronounced as previously suggested by the literature. MUP complexity was considerably more limited among inbred mice compared with their wild counterparts, with most strains expressing one of two major patterns. However, closely related strains differed in relative expression of specific MUPs, including strains that were MHC-congenic.

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Poster: Pheromones/VNO

Relative roles of the main and accessory olfactory systems in behavioral responses to MHC class I peptides: Bruce effect

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Selective pregnancy termination (the Bruce effect) depends on the formation of a social memory. Small peptide ligands of the MHC class I molecules represent the first molecularly defined sensory cues by which the outcome of this memory process can be switched in a predictable manner (Leinders-Zufall *et al.*, 2004, *Science*, 306). Although we showed previously that MHC peptides are detected by neuronal populations in the VNO, our new results (Spehr *et al.*, this meeting) demonstrate that these ligands are also detected by sensory neurons of the main olfactory epithelium (MOE). These new findings make it necessary to assess the relative roles of the main and accessory olfactory systems in the peptide-induced pregnancy block. To begin to address this question, we have used inbred mice in which the VNO was surgically removed (VNX). In sham mice, a pregnancy block could be induced by the addition of MHC peptides to urine. By contrast, this effect was not observed in VNX mice. These results strengthen the notion that the pregnancy block effect depends critically on signaling via the accessory olfactory system and that recognition of MHC peptides by the MOE does not replace VNO sensory input in this context. Experiments are underway to investigate effects of specific VNO gene deletions on the peptide-induced pregnancy block.

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Poster: Pheromones/VNO

MHC-related odorprints in mice

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We describe the application of GC/MS to identify volatiles from murine urine samples obtained from two groups of inbred mice that differ only in the genes of the major histocompatibility complex (MHC). The MHC genes have been shown in behavioral studies to contribute to an individual's unique urinary odor. Singer *et al.* (1997, *Proc. Natl Acad. Sci. USA*, 94:2210–2214) demonstrated structural differences in volatile urinary profiles of two mouse strains but only used manually quantified peak heights derived from GC with flame ionization detection; 32 relatively abundant, visually identifiable components were screened. GC/MS identified compounds in a few samples, but was not used to compare MHC types. Complex mixtures from biological samples might comprise several hundred volatile compounds. Because the number and location of compounds we seek are unknown, and because components overlap in complex chromatograms, the statistical problems offer significant challenges beyond traditional two-group screening procedures. We employed a novel statistical procedure to compare the GC/MS profiles between two MHC groups and identified several dozen regions of significant differences. This procedure enormously increases the analyst's ability to identify differential marker compounds in complex mixtures. Our results suggest that several dozen compounds are potentially involved in MHC chemosignaling, including two known mouse pheromones, 2,5-dimethylpyrazine and 2-s-butyl-4,5-dihydrothiazole.

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Poster: Pheromones/VNO

HLA-related odorants in humans

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We have recently reported upon the application of a unique statistical approach to the analysis of GC/MS data of volatiles from the urine of congenic strains of mice. These analyses demonstrate that more than 3 dozen compounds constitute the MHC-related chemosignal. Human odortypes, like those in rodents, are hypothesized to be regulated by the genes of the MHC (HLA in humans). Our approach to the identification of human HLA chemosignals uses parallel mouse bioassays in conjunction with statistical treatment of analytical data from multiple analyses of human urine and axillary volatiles. To obtain odorous samples, human volunteers are typed for HLA; urine and underarm sweat are collected for 10 days while donors refrain from use of fragranced soaps/deodorants and spicy, aromatic foods. Samples are stored at −30°C until needed for analytical or bioassay studies. Volatile organic compounds (VOCs) in urine and axillary sweat are collected (by solvent extraction and SPME) and analyzed by several analytical methods including GC/MS, GCxGC/MS and GC/FPD. Preliminary results are available from a small number of subjects ($n = 3$ of one HLA super-type and 4 of another). Our techniques yield low intra-subject variability in the amounts of VOCs recovered. We have identified large numbers of volatile molecules in both urine and sweat. We do not find compounds that are present in one HLA super-type, but absent in others. However, our preliminary data indicates that the relative amounts of some compounds vary according to HLA super-type. Some compounds are of exogenous origin (e.g. diet, medications); many of these are easily distinguishable from endogenous compounds and ignored.

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Poster: Pheromones/VNO

Comparison of volatile components of mice and their urine

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Behavioral studies suggest that the major histocompatibility complex (MHC) influences the composition of volatile components in mouse urine (Beauchamp and Yamazaki, 2003, *Biochem. Soc. Transact.*, 31:147). MHC-dependent differences in urine odor types can be detected by glomerular activation patterns in the main olfactory bulb (Schäfer *et al.*, 2002, *J. Neurosci.*, 22:9513) or by arrays of gas sensors (Montag *et al.*, 2001, *Proc. Natl Acad. Sci. USA*, 98:9249). The chemical basis in urine odor composition for these differences appears to be of quantitative rather than qualitative nature (Schwende *et al.*, 1984, *J. Chem. Ecol.*, 10:1603; Singer *et al.*, 1997, *Proc. Natl Acad. Sci. USA*, 94:2210). Analysis of mouse odor has so far not been reported. We have studied the volatile components produced by mice and their urine, for comparison, from C57BL/6 mice (wild type) and isogenic mutants lacking class I or class II MHC molecules by gas chromatography/mass spectroscopy. A set of 127 chromatograms were evaluated for a total of 65 components allowing a detailed comparison between mouse and urine volatiles and their MHC dependence. The ongoing analysis of the data shows (i) a qualitatively a high reproducibility but (ii) large variations in abundance of some components, (iii) characteristic differences between mouse and urine volatiles and (iv) only limited overlap in components found in this and earlier studies. The data will be discussed with regard to the influence of MHC on odor composition and the relationship between odor production and odor recognition in mice.

Poster: Pheromones/VNO

Water-soluble alarm pheromone induced autonomic stress response in male rats

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We previously reported that alarm pheromone released from the perianal region of male Wistar rat aggravated the stress-induced hyperthermia in recipient rat without any effect on behavioral responses in the novel environment paradigm. In the present study, we assessed the alarm pheromone's effects under home cage condition. An anesthetized donor rat was given electrical stimulation to the perianal region for releasing alarm pheromone in a small box containing water droplets on the ceiling. After the stimulation, the aqueous fraction of pheromone trapped in water droplets was collected using two sheets of filter papers. The water collected from the box in which no animal was placed was used for control group. After the water collection, the filter papers were placed on the wall of recipient's home cage, and behavioral and autonomic responses were monitored for the subsequent 30 min. Fos expression in the mitral/tufted cell layer of the accessory olfactory bulb was also examined. The pheromone-containing water significantly aggravated stress-induced hyperthermia in recipient rats ($P < 0.05$, ANOVA) as compared with those seen in control group, whereas there was no effect on behavioral responses (MANOVA). In addition, the pheromone exposure significantly increased Fos expression in the accessory olfactory bulb ($P < 0.05$, ANOVA). These results suggest that the alarm pheromone is water-soluble and that it can exert the same effects in the home cage condition.

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Poster: Pheromones/VNO

Male blue crab pheromone originates in semen

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The origin and basic properties of a male blue crab pheromone are reported. Females mate once after their terminal molt and are attracted to males by pheromones several days before the molt. Fishermen catch 'peeler females' by baiting crab traps with males. A bioassay was developed by watching peeler females in a tank with an entrapped male. Peeler females walk continuously. When responding to male pheromones females stop walking and perform a meryl spread. In the dark, females maintain the meryl spread posture for several min. In the light, females maintain the meryl spread posture, approach crab-sized objects and attempt to back under them. In the bioassay a positive response was recorded if a walking female stopped showed a meryl spread within 10 s of stimulus presentation. Male pheromone was generated predictably during initial interaction between a male and a premolt female. Pheromone found in macerates of mature seminal vesicles is <10 000 daltons, stable to freezing and boiling. Stressed males and males multiply mated until sperm store depletion do not release pheromone and do not attempt to mate. We used Superglue® to seal the male telson so semen could not be released. Pheromone was not released. Over time, Superglued males became very aggressive and if presented with a recently molted female psuedocopulated for 36 h. Copulation is normally for 8 h. Male pheromone represents a form of 'truth in advertising'.

Poster: Pheromones/VNO

Divergent V1R repertoires in five species

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The V1R gene family comprises one of two types of putative pheromone receptors expressed in the mammalian VNO. We have compiled a near-complete repertoire of V1R genes in the mouse, rat, dog, chimp and human genomes. The three non-rodent genomes have fewer than ten intact V1Rs compared with >100 intact V1Rs in the two rodent genomes. We also provide a description of the diversity of V1R pseudogenes in these species. Primate and dog pseudogenes are distributed among almost all V1R subfamilies seen in rodents, indicating that the common ancestor of these species had a diverse V1R repertoire. V1R genes were subject to strikingly different fates in different species and in different subfamilies. In rodents, some subfamilies underwent roughly equivalent expansion in mouse and rat; other subfamilies expanded in one

species but not the other. The small number of intact V1Rs in the dog genome is unexpected given the presumption that dogs have a complex pheromonal system. We identify an intact transient receptor potential channel 2b in the dog genome, consistent with a functional VNO in dogs. We consider three possible implications of the vastly different functional repertoire sizes in rodents and dogs: (i) V1Rs might indeed provide a greatly expanded range of specialized functions in rodents, or even allow the VNO to recognize additional non-pheromonal ligands; (ii) the many duplication events in rodents could have produced a largely redundant and overlapping set of functions, with the extra genes making only an incremental difference in the range of encoded functions; (iii) other gene families, such as V2Rs in the VNO or olfactory receptors in the nose, might be much more important than V1Rs in pheromone perception in dogs and perhaps other mammals.

Poster: Olfactory Bulb: Neurophysiology

Stimulus specific spatio-temporal Ca^{2+} dynamics of moth olfactory projection neurons

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We studied the Ca^{2+} dynamics of odour-evoked glomerular patterns in the antennal lobe (AL) of the moth *Spodoptera littoralis* using optical imaging. Here we selectively stained a large population of AL output neurons, projection neurons (PN), by retrograde filling with FURA–dextran from the inner antennocerebral tract (IACT) in the protocerebrum. Different plant-associated odorants evoked distributed patterns of activated glomeruli that were odour-dependent and repeatable. These patterns were, however, dynamic during the period of odour exposure. Time courses differed across glomeruli due to differences in latency, amplitude and duration. This caused a temporal change of patterns, which was repeatable across stimulations. Next we examined how the correlations between patterns evoked by different odorants changed with time. Within the period of odour exposure (300–900 ms after stimulus onset) we found a significant reduction in similarity of responses evoked by different odours. Our results suggest that olfactory information is contained in a code with both spatial and temporal components and that discrimination ability may improve with time.

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Poster: Olfactory Bulb: Neurophysiology

Effects of bond type, position, number and stereochemistry on glomerular responses to hydrocarbon odorants in the rat olfactory bulb

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We investigated the possible influence of double and triple bonds on the representation of odorants using a systematic set of hydrocarbons, including the 8-carbon octane (saturated), octenes (double-bonded) and octynes (triple-bonded). In prior studies, we had identified the stimulation of a cluster of glomeruli both laterally and medially by a number of 8-carbon aliphatic odorants, including octane. The presence of a single double bond in the *trans* configuration, regardless of its location along the carbon chain, produced similar responses there, whereas odorants possessing a double bond in the *cis* stereoconfiguration did not. Furthermore, the presence of a triple bond at the 1-carbon position evoked responses in the same region, but this response was lost when the triple bond was located elsewhere. The presence of either two double bonds or two triple bonds in a molecule also prevented activity from being evoked in those glomeruli. Finally, all of the octenes and octynes, but not octane, stimulated a dorsal glomerular region that had shown reliable responses to both aliphatic ketones and some aromatic odorants. These data, together with previous results, indicate that the nature of bonds between carbon atoms of an odorant molecule can have a profound effect on glomerular representations.

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Poster: Olfactory Bulb: Neurophysiology

Spiking properties of EPL interneurons

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Spiking properties can be used to classify functionally distinct cortical interneurons, which appear to form separate synaptic networks for synchronizing neural activity. The external plexiform layer (EPL) of the mouse main olfactory bulb contains morphologically heterogeneous interneurons, the functions of which are unknown. We have examined the spiking properties of these interneurons. In 14 of 18 EPL interneurons, depolarizing current pulses evoked action potential trains after a significant delay, as seen in late-spiking (LS) cortical interneurons. In 10 of the 14 interneurons, suprathreshold pulses evoked regular, prolonged action potential trains (coefficient of variation 0.09 ± 0.02), as seen in fast-spiking (FS) cortical interneurons. The spike characteristics and time constant were also similar to those of FS interneurons, but the input resistance was higher, closer to that of LS interneurons. In four of the 14 interneurons, depolaring current pulses evoked brief trains of truncated spikes, and these interneurons had higher input resistances, longer time constants and lower EPSC frequencies. They might have been damaged or another cell type. Three of the four briefly-firing interneurons exhibited autocorrelated EPSC bursts, and all four were located in the superficial EPL. By contrast, only three of the 10 prolonged-firing interneurons exhibited EPSC bursts, and the prolonged-firing interneurons were located throughout the EPL. Morphological properties of the briefly firing and prolonged-firing interneurons overlapped. These results indicate that mouse EPL interneurons exhibit distinctive electrophysiological properties, and that many share properties with both LS and FS cortical interneurons.

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Poster: Olfactory Bulb: Neurophysiology

The bursting of olfactory bulb external tufted (ET) cells is coordinated by synaptic and gap junction currents

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In rat olfactory bulb slices, ET cells spontaneously generate spike bursts. Bursting among ET cells of the same glomerulus is synchronous. The mechanism underlying their synchrony is unknown. Although ET cell bursting is intrinsically generated, its strength and precise timing are regulated by synaptic input. Using dual extracellular and patch clamp recordings from ET cell pairs of the same glomerulus, we found that the bursting of ET cells is synchronized by several mechanisms. First, ET cells receive coincident fast excitatory input that probably originates from olfactory nerve axons. Second, they exhibit correlated slow excitatory synaptic currents that can be triggered by stimulation of individual ET cells. These slow currents may reflect the recurrent release of glutamate via spillover from the dendritic tufts of other ET or mitral/tufted cells affiliated with the same glomerulus. Third, ET cells exhibit correlated bursts of inhibitory synaptic activity that usually follow immediately the synchronous fast and slow excitatory input. The bursting feature of this inhibitory activity was eliminated by CNQX and may therefore reflect correlated feedback inhibition from periglomerular cells that are excited by ET cells via non-NMDA receptors. Fourth, in the presence of fast synaptic blockers, ET cells exhibit rhythmic spikelets or burstlets associated with slow membrane current oscillations that were sensitive to the gap junction blocker, carbenoxolone. These findings suggest that coordinated synaptic transmission and gap junction coupling synchronizes the spontaneous bursting of ET cells of the same glomerulus.

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Poster: Olfactory Bulb: Neurophysiology

Activation of metabotropic glutamate receptors (mGluRs) enhances bursting in external tufted cells of the olfactory bulb

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Activation of mGluRs contributes to synchronized oscillations in many brain areas. ET cells exhibit rhythmic spike bursts and membrane potential oscillations, and also abundantly express mGluRs. In the presence of synaptic blockers (CNQX, APV and gabazine), mGluR agonists from group I (DHPG, 30 μM) or group II (L-CCGI, 3 μM), but not from group III (L-AP4, 10 μM), potentiated bursting in external tufted (ET) cells by enhancing membrane potential oscillations and increasing the number of spikes/burst. The latter effect was mimicked by application of the calcium channel blockers, cadmium (100–300 μM) and nickel (1 mM). In the presence of TTX (1 μM), TEA (10 mM) and synaptic blockers, DHPG, and to a lesser extent L-CCGI, reduced calcium currents evoked by depolarizing voltage steps in voltage clamp recordings, and enhanced an outward calcium-activated potassium current. These effects were prevented by including BAPTA (10 mM) in the pipette solution, suggesting that they are mediated by release of calcium ions from intracellular stores. In the presence of synaptic and calcium channels blockers, DHPG enhanced a TTX-sensitive persistent sodium current and activated a TTX-insensitive, putative non-specific cation current. In the presence of synaptic blockers, DHPG induced membrane current oscillations (1–2 Hz) associated with burstlet-like events that are possibly due to gap junctions among ET cells. We propose that mGluR activation enhances bursting by modulating gap junction coupling and several currents implicated in burst generation.

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Poster: Olfactory Bulb: Neurophysiology

Serotonin modulation of external tufted cells in mouse olfactory bulb glomeruli

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External tufted (ET) cells—one of three types of juxtaglomerular neurons—have unique physiological properties that distinguish them from periglomerular (PG) and short axon (SA) cells. All ET cells spontaneously generate periodic bursts of action potentials that are generated by intrinsic currents. ET cells receive monosynaptic olfactory nerve (ON) input, are readily entrained by repetitive ON stimulation of <10 Hz and appear to play an important role in the amplification and synchronization of glomerular input-output functions. Mouse ET cells have a prominent I_h current and a persistent sodium current, I_{NaP}, which is essential to burst generation. Both of these currents are potential targets of modulatory transmitter systems (ACh, 5-HT, DA), which innervate the glomeruli. Therefore, we are investigating the ability of modulatory transmitters to alter ET cell bursting and/or coupling. Our findings show 5-HT does not alter I_h or I_{NaP}. However, 5-HT acting via 5-HT2 receptors decreases an outward K^+ conductance active at rest resulting in a depolarization of the membrane potential. This depolarization significantly increases the spontaneous bursting frequency of the cell. 5-HT2 activation may also increase an inward (Ca^{2+}) current at depolarized potentials; this action is under investigation. ET cells provide excitatory input to PG and SA cells; PG cells inhibit ON terminals and provide feedback inhibition onto mitral and tufted cells. By increasing ET cell burst frequency, 5-HT may increase the spatial and temporal contrast of ON inputs.

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Poster: Olfactory Bulb: Neurophysiology

Cellular and synaptic properties of dopaminergic juxtaglomerular neurons in the mouse olfactory bulb

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Olfactory receptor neurons in the nasal cavity project axons through the olfactory nerve (ON) to terminate in olfactory bulb glomeruli. These axons form glutamatergic synapses with the apical dendrites of mitral/tufted cells and juxtaglomerular interneurons. There are three types of juxtaglomerular (JG) cells: external tufted (ET), periglomerular (PG) and short axon cells. The glomerular layer contains the largest numerical population of dopaminergic (DA) neurons in the brain but it is unknown which of the three types of JG cells express DA. The goal of this study was to characterize the cellular and synaptic properties of DA-JG cells in a transgenic mouse expressing GFP under control of the tyrosine hydroxylase promoter (TH-GFP). Using whole cell patch-clamp techniques, we found that TH-GFP positive JG cells exhibited low frequency spontaneous spiking (2.2 ± 0.6 Hz), high input resistance (IR = 663 ± 59.1 MΩ) and small hyperpolarization-activated current (I_h = 22.0 ± 4.9 pA) as compared with the ET cells (IR = 301.7 ± 11.6 MΩ; I_h = 318.9 ± 22.6 pA). Most TH-GFP cells exhibited recurring bursts of spontaneous EPSCs; ON stimulation evoked either mono- or polysynaptic input. Paired-pulse stimulation to the ON produced a long-lasting depression of the second evoked EPSC. The maximum paired-pulse depression, 38% of the conditioning EPSC, was achieved at an inter-stimulation interval of ≥100 ms. However, significant depression could still be observed for up to 2 s. These findings indicate that: (i) the cellular and synaptic properties of DA-JG cells are consistent with that of PG cells; (ii) DA-JG cells receive mono- or polysynaptic ON input; and (iii) ON input to DA-JG cells is subject to presynaptic inhibition.

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Poster: Olfactory Bulb: Neurophysiology

Cholinergic modulation in the zebrafish olfactory bulb

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Acetylcholine (ACh) functions as a neurotransmitter and neuromodulator in the CNS. We have previously reported that ACh modulates neurotransmission at the mitral cell synapse in the zebrafish olfactory bulb (OB; Edwards and Michel, 2001, *Chem. Senses*, 26). Using an ion channel permeant probe (agmatine, AGB) to label bulbar neurons in activity-dependent manner, we established that nicotinic ACh receptor (AChR) activation stimulated labeling of approximately one-fifth of all projection neurons. Neuronal labeling could be blocked with nicotinic ACh receptor (nAChR) antagonists and with calcium-free artificial cerebrospinal fluid or iGluR antagonists. These data are consistent with activation of nAChRs expressed presynaptically on excitatory neurons, which results in glutamate release and AGB labeling via activated postsynaptic iGluRs on mitral cells. We also noted that the muscarinic AChR agonist, oxotremorine, stimulated labeling of a single glomerulus located in the ventral OB. This labeling was blocked by the muscarinic AChR antagonist, atropine. Three new lines of evidence suggest that the muscarinic receptors are localized to the olfactory sensory neurons. First, labeled fibers can be traced into the olfactory nerve layer of the bulb. Second, unilateral ablation of the olfactory epithelium eliminates oxotremorine stimulated labeling in the ipsilateral bulb. Finally, messenger RNA for M2, M5 and a putative muscarinic AChR were isolated from the olfactory rosettes of adult zebrafish using RT-PCR. Our findings suggest that activation of muscarinic AChRs affects a very focal region of the olfactory bulb, while presynaptic nicotinic AChR activation more generally affects projection neuron activity.

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Poster: Olfactory Bulb: Neurophysiology

The influence of stimulus duration on olfactory event related potentials

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Olfactory event-related potentials (OERP) are mainly determined by stimulus characteristics like stimulus concentration and stimulus onset. It is not clear how the duration of a stimulus influences OERP and olfactory perception. The aim of the present study was to investigate the relationship between stimulus duration and OERP parameters. A total of 20 young, healthy subjects (10 male, 10 female) participated in the study. OERP recordings were performed in four sessions. Subjects were presented with PEA and H2S stimuli lasting 100, 200 or 300 ms. OERP were analyzed at recording site Pz with regard to latency and amplitude of the major peaks N1 and P3. There was a clear effect of stimulus concentration on amplitude and latency P3 ($P < 0.002$). Stronger stimuli evoked larger and earlier responses. In addition, there was a statistical interaction between stimulus duration and stimulus concentration for amplitude P3 ($P = 0.002$). Whereas weak stimuli showed no effect of stimulus duration ($P = 0.5$), strong stimuli were clearly influenced by stimulus duration ($P = 0.01$) with regard to amplitude P3: short stimuli evoked responses of smaller amplitude when compared with longer lasting stimuli. These data indicate that, similarly to the trigeminal system, information about stimulus duration is integrated into OERP.

Poster: Olfactory Bulb: Neurophysiology

Slice electrophysiology and histological evidence of calcium-fluxing AMPA receptors in the olfactory bulb

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AMPA receptors (AMPARs)— ion channels comprised of the subunits GluR1-4—contribute to olfactory processing. Studies in other brain regions have shown that AMPARs lacking GluR2 are highly permeable to Ca^{2+} (CaAMPARs) and generate inwardly rectifying currents. The latter property is due to the effects of internal polyamines (e.g. spermine), which block channels lacking GluR2. We previously found that 1-naphthylacetyl spermine (NAS), a selective antagonist of CaAMPARs, inhibits AMPAR-mediated currents in cultured olfactory bulb (OB) to various degrees. Here, we used whole-cell recording and histology to examine whether CaAMPARs are also present in adult rats (OB slices) and at synapses. In OB slices, olfactory nerve stimulation elicited excitatory responses in juxtaglomerular (JG) and mitral cells. Bath application of 10 μM

NAS + 100 μM AP5 (to isolate AMPARs) variously suppressed these synaptic responses compared with responses obtained in AP5 alone. Also consistent with synaptically activated CaAMPARs, NAS inhibited EPSCs and miniature EPSCs ('minis') isolated in some cultured OB neurons. Addition of spermine to the electrode yielded current–voltage plots with inwardly rectifying currents in some cells. Cobalt staining, which is specific for CaAMPARs, produced diverse labeling patterns in OB slices from adult rats. These results suggest that OB neurons in adult animals express functional CaAMPARs, which could influence odor encoding via activation of K^+ channels or CaMKII, modulation of NMDA receptors, or influencing synaptic vesicle fusion.

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Poster: Olfactory Bulb: Neurophysiology

Odorant response property of the mOR-EG glomerulus in transgenic mice

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Since the discovery of the olfactory receptor (OR) gene superfamily, several ORs have been deorphaned using various functional expression techniques. Detailed pharmacological information, however, has been obtained for only a few ORs. The mOR-EG, encoded by the MOR174-9 gene, is a mouse OR for eugenol (EG), and its ligand profile has been well characterized using a mammalian cell line. In this study, we generated mOR-EG-IRES-gapEGFP transgenic mice to explore the response property of mOR-EG glomerulus in the olfactory bulb. Using Ca^{2+} imaging and intrinsic signal imaging techniques, we analyzed the following pharmacological properties of the mOR-EG glomerulus: (i) the responses to various odorants at the mOR-EG glomerulus in comparison with a ligand profile that had been obtained in mOR-EG-expressing HEK293 cells; (ii) the EC_{50} value of the mOR-EG glomerulus to EG that was applied to the nasal cavity in air phase; and (iii) the inhibition by methyl isoeugenol, an antagonist of mOR-EG. The current study describes an odorant response property of a glomerulus that receives olfactory neurons expressing a defined OR, and provides further understanding of ligand–OR interactions under the physiological condition.

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Poster: Olfactory Bulb: Neurophysiology

Pharmacological analysis of the physiological functions of GABAergic intra- and inter-glomerular inhibition in the antennal lobe of a moth

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Much is known about the pharmacological profiles of different types of GABA receptors (GABA-Rs), but few studies have characterized the functional roles of the GABAergic inhibition in olfactory information coding. Owing to the commonly observed distinct time course of intra- and inter-glomerular inhibition in the antennal lobe of the moth *Manduca*, it is possible that these inhibitions are mediated by different GABA-R subtypes. Using a juxtacellular recording method, we examined the responses of glomerular output neurons to their known odor inputs. We tested a range of stimulus concentrations, and examined olfactory responses before, during and after bath application of agonists and antagonists of mammalian GABAA and GABAB -Rs. Our results show that: (i) muscimol (GABAA agonist) reduced the rate of spontaneous firing as well as response magnitude; (ii) bicuculline methiodide (competitive GABAA antagonist) induced a change in spontaneous activity from a random to a regular pattern of firing, while also potentiating and prolonging intra-glomerular excitatory odor responses, but in sharp contrast, this treatment did not affect inter-glomerular inhibitory odor responses; (iii) picrotoxinin (non-competitive GABAA antagonist) did not alter spontaneous firing, but lowered the threshold for intra-glomerular excitatory responses; and (iv) 2-hydroxysaclofen (GABAB antagonist) broadened the molecular receptive ranges of some neurons. In summary, our data support the hypothesis that intra- and inter-glomerular inhibitory synaptic interactions are mediated by different subtypes of GABA-Rs.

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Poster: Olfactory Bulb: Neurophysiology

Modulating levels of nitric oxide and soluble guanylyl cyclase affects antennal lobe neuron processing in the moth *Manduca sexta*

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Nitric oxide (NO) and other gaseous messengers are beginning to emerge as important modulators in many physiological processes including olfaction. In the moth *Manduca sexta*, NO synthase (NOS) is found in olfactory receptor neurons (ORNs), while a well-characterized target of NO, soluble guanylyl cyclase (sGC) is found in a subset of neurons that are post-synaptic to ORNs in the antennal lobe (AL). Further, NO-sensitive dye imaging reveals that odor stimulation of the antenna produces NO in odor-specific regions of the AL. These data revealed the possibility that NO is released focally when odor is present, and then modulates signaling in an activated glomerulus by acting on sGC-containing neurons or possibly other NO targets in the AL. This hypothesis was tested with multi-channel and intracellular recording methods coupled with pharmacological manipulation of NO and sGC levels in the AL. Blocking NO production with two different NOS inhibitors resulted in an overall decrease of spontaneous activity in AL neurons, and this effect was mimicked reversibly by an sGC inhibitor. Odor-evoked responses were also diminished when NO-dependent signaling was blocked. Addition of NO caused an increase in activity in some neurons. To extend and confirm these results, we are using RNAi to knockdown NOS mRNA expression. These results suggest that NO is an important modulator of odor responsiveness, and that sGC-dependent signaling is at least partially responsible for the observed changes in AL neuron activity levels.

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Poster: Olfactory Bulb: Neurophysiology

Learning-induced oscillatory activities correlated to odor recognition: a network activity

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In the mammalian olfactory system, oscillations of field potential activities related to odor representation have been described. Previous data showed that in olfactory bulb (OB) and piriform cortex (PC) of awake rats engaged in an olfactory learning, odor presentation induced a power decrease of gamma oscillations (60–90 Hz) followed by a power increase in beta range (15–40 Hz). Both phenomena were strongly amplified after training. The aim of this work was to further characterize under which conditions this oscillatory activity could emerge. Local field potentials (LFPs) were recorded through chronically implanted electrodes in the OB and PC of freely moving rats performing an olfactory discrimination.

Two different methods were used to investigated the possible role of the OB–PC feedback loop in the expression of beta activity following learning. In one group of animals, unilateral section of the olfactory peduncle was made before training, and LFPs were symmetrically recorded in OBs along the acquisition of the learning task. On the other hand, animals chronically implanted with intracerebral cannula were trained in the same task. LFPs were recorded under unilateral inactivation of the peduncle (lidocaine infusion) in expert animals.

Data showed that deprivation of centrifugal feed-back led to an increase of spontaneous gamma activity, and abolished the typical beta activity observed in response to learned odors. As a whole, emergence of beta oscillatory response in OB and PC appears modulated both by the odor representation in this structure and odor processing at a higher level.

Poster: Olfactory Bulb: Neurophysiology

Intercorrelation of olfactory bulb spikes during local field potential oscillations is low

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We investigated the relationship between olfactory bulb field potential oscillations and single unit activity using Michigan multiple electrode arrays (Neuronexus Technologies). Single units were recognized by correlation of their shape and profile across electrodes with a template. The quality of isolation was assessed by the variance and by interspike interval analysis. This preliminary report is based on analysis of external plexiform layer units isolated in 11 urethane anesthetized rats with odorants presented over a concentration range of four log steps in mineral oil dilution. These stimuli produced strong field potential oscillatory in the gamma range at higher concentrations. Contrary to some reports in the literature, we have seen only very low correlations of single units with the local field potential correlations and low cross correlations between pairs of units. We seen evidence with current density analysis that the field potential oscillations are locally produced, and analysis of multiple unit recordings gave evidence of at least weak correlation between groups of units in different layers. We conclude that, at best, individual projection cell spikes are only weakly entrained by the oscillatory activity that seems to exist in granule cells.

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Poster: Olfactory Bulb: Neurophysiology

Modulation of receptor neuron input to the olfactory bulb mediated by feedback versus lateral presynaptic inhibition

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Olfactory bulb afferents (ORNs) converge onto glomeruli that correspond to individual odorant receptors. Afferent input to a glomerulus can be modulated via mechanisms mediated, at least in part, by $GABA_B$ receptors. We asked how this presynaptic inhibition affects odorant-evoked input to glomeruli by imaging odor representations with an optical reporter of ORN transmitter release and blocking $GABA_B$ receptors *in vivo*. A $GABA_B$ antagonist greatly increased odorant-evoked transmitter release in each responsive glomerulus, but did not change the spatial distribution of responsive glomeruli. Co-activation of neighboring glomeruli with odorant mixtures did not suppress transmitter release relative to the corresponding single-odorant presentations. Experiments in olfactory bulb slices revealed that single olfactory nerve shocks profoundly inhibited subsequent transmitter release from all fibers innervating a responding glomerulus, including afferents entering the glomerulus from separate, unstimulated bundles. This intraglomerular feedback inhibition was long-lasting and reduced by glutamate receptor blockers and by $GABA_B$ antagonists. We also found lateral inhibition of transmitter release between neighboring glomeruli in slices, but this inhibition was much weaker than feedback inhibition and limited at physiological temperatures. We conclude that feedback inhibition of transmitter release from ORNs can strongly modulate the magnitude of afferent input to the bulb, but that lateral inhibition plays a relatively small role.

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Poster: Olfactory Bulb: Neurophysiology

Basic characteristics of neuron network components in the antennal lobe of the silkmoth

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Conventional intracellular recording and staining methods revealed physiological and morphological characteristics of antennal lobe (AL) neurons in male silkworm moths, *Bombyx mori*. Pheromonal processing in the macroglomerular complex of the AL is well understood. However non-pheromonal odor processing in ordinary glomeruli is still unknown. For analyzing general properties of the AL neurons, aliphatics, monoterpenoids and pheromone were used as odorants. We observed reproducible olfactory responses in several different types of interneurons. Most of local interneurons (LNs)

showed brief excitation. Projection neurons (PNs) showed five types of response patterns. Phasic excitation and phasic inhibition were most frequently observed. Tonic excitation, tonic inhibition and other temporal patterns were also observed. Stained neurons were carefully investigated using confocal microscopy. We classified PNs into three groups, uniglomerular, oligoglomerular and multiglomerular type by innervation patterns in the AL. We also classified PNs by their soma positions and neural tracts. The cell clusters of PNs showed strong correlation with antenno-cerebral tracts (ACT). This is consistent with immunocytochemical observations in the silkmoth. Glomeruli innervated by PNs were identified using the method that we previously established. We had classified the AL into 6 regions and identified 39 glomeruli of total 60 ± 2. Some morphological differences of PNs among regions were found. For example, PNs innervating the posterior region had unique characteristics. These PNs had their soma in the lateral cell cluster, ran through the medial or outer-ACT and showed non-specific response to odors.

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Poster: Olfactory Bulb: Neurophysiology

Olfactory bulb responses are altered by regional blockade of epithelial regions by exposure to UV light

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Differences in the regional sensitivity of the olfactory epithelium (OE) to odorants have been observed using the electro-olfactogram (EOG). Olfactory sensory neurons (OSNs) expressing a given olfactory receptor (OR) message has been shown to lie dispersed within the OE, suggesting it contributes to differences in response intensities in different areas of OE. OSNs form synapses with mitral/tufted (M/T) cells in the olfactory bulb (OB). The M/T cells form a prominent lateral inhibitory network with granule cells. This finding suggests that the OB can enhance the contrast in activity between OSNs that respond to a given odorant, leading to a suppression of weakly responsive OSNs. We propose that exposing spatially defined regions of OE to UV light can selectively block regions of OE from responding to odorants (Cheung and Kauer, 2002). We selected odorants with identified peak responses in anterior, posterior, and middle thirds of the tiger salamander OE. We show in this study that suppressing the posterior half of the OE led to: attenuation of subsequent odorant evoked responses in OB, few to no changes in OB response patterns in middle-third stimulating odorants, altered OB response patterns in odorants that stimulated peak responses in the posterior-thirds of OE, and altered OB response patterns of an anterior-third OE stimulating odorant. This last finding suggests that areas away from the peak OE responses can contribute to the pattern of activity evoked by whole OE stimulation.

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Poster: Olfactory Bulb: Neurophysiology

Representation of odors in the mouse olfactory bulb

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We recorded action potentials of mitral cells and local field potential (LFP) in the olfactory bulb of the behaving mouse. The mouse was trained to discriminate two odorants in a go/no-go paradigm. Two prominent components of the LFP oscillations are observed: the theta (2–3 Hz) and the gamma (50–60 Hz) rhythms. We found that both firing rate and gamma oscillations are modulated by behavioral events and correlated with the odor stimulus. We examined the time-course of the amplitude of gamma oscillations and mitral cell firing rate. In multiple recording sessions, both of these functions display an increase during anticipatory period, while the animal was waiting in the odor port for the stimulus delivery. During the stimulus presentation the amplitude of LFP oscillations shows statistically significant correlation with the odorant's quality (36% of recorded cases). Firing rate displays correlations with odorant only in 20% of recorded cells. We applied nonlinear time-series analysis to the LFP during odor delivery. We observed no significant differences between two odors in the nonlinear signatures of the oscillations other than the differences in the overall amplitude. We also observed the spike timing relative to the phase of the gamma oscillations. Spikes have a tendency to occur at the positive peak of the gamma oscillations. However, no statistically significant differences were detected for different stimuli during the odor presentation. These findings yield clues into mechanisms of information processing in olfactory bulb.

Poster: Olfactory Bulb: Neurophysiology

Screening for plasma membrane expression of fluorescent protein-voltage sensitive sensors

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A genetically encoded fluorescent protein (FP)-voltage sensitive sensor would enable optical recording of the activity of specific neuronal populations. A shaker potassium channel based GFP construct, Flash (Siegel and Isacoff, 1997) and a skeletal muscle sodium channel based, GFP construct, SPARC (Ataka and Pieribone, 2002) are able to optically transduce changes in membrane potential in frog oocytes. However, when these constructs are expressed in HEK 293 cells, they are retained in the endoplasmic reticulum (ER) and are unable to report changes in the plasma membrane potential. A FRET-based voltage sensitive protein, VSFP-1 (Sakai *et al.*, 2001) also shows high internal expression consistent with substantial ER retention. To screen for membrane expression of potential FP-voltage sensors, we developed a double labeling protocol. Cells transfected with a fluorescent probe are first imaged and then stained with a hydrophobic, voltage-sensitive dye to estimate the plasma membrane expression of the FP-voltage sensors. Because di8-ANEPPS has a long wavelength emission spectra that can be easily separated from the FP emission of the voltage sensor, a direct comparison of membrane expression can be made using confocal microscopy. Analyses of HEK cells transfected with SPARC constructs show a clear distinction between the plasma membrane stained with di8-ANEPPS and the FP signal residing mainly in the ER. The Flash variant FLARE, a Kv1.4 GFP construct, also shows an ER staining pattern. Because HEK cells may lack the proper machinery to correctly traffick these channels, we have begun expression studies in primary rat hippocampal neurons.

Poster: Olfactory Bulb: Neurophysiology

Dendrites of juxtaglomerular neurons in rat olfactory bulb express Ca^{2+}-permeable AMPA receptors

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The glomerular layer of the olfactory bulb contains a mixed assemblage of juxtaglomerular (JG) interneurons—external tufted (ET), short axon (SA) and periglomerular (PG) cells. Dendrites of these cells project into glomeruli, where they branch and elaborate tufts which receive glutamatergic input from olfactory nerve terminals or apical dendritic tufts of mitral cells. We investigated the pharmacology of functional glutamate receptors in JG tufts by perforated-patch recording and calcium imaging in rat olfactory bulb slices. Tuft stimulation by caged glutamate photolysis evoked currents mediated by AMPA/kainate and NMDA receptors. A large AMPA/kainate current was isolated in 1 μM TTX, 150 μM Cd^{2+}, 50 μM bicuculline and 30 μM dichlorokynurenate. In ET and most PG/SA cells, this current exhibited strong inward rectification and use-dependent block by 1-naphthylacetylspermine (NAS), a polyamine blocker of Ca^{2+} permeant (CP) AMPA receptors. The percentage of current blocked (−60 mV) was: ET, 59 ± 4% (n = 12); SA, 48 ± 5% (n = 15); PG, 49 ± 5% (n = 15); only five PG cells had an NAS-insensitive current. Fluorescent imaging of dendrites after uncaging revealed an NAS-sensitive component of Ca^{2+} influx. Thus, a majority of JG neurons express CP-AMPA receptors. Calcium influx through these receptors could facilitate GABA release, or mediate synaptic plasticity. Our results extend recent studies implicating functional diversity of AMPA/kainate receptors in the olfactory bulb. The CP-AMPA receptors may play important roles in shaping activity patterns of glomeruli during initial stages of olfactory information processing.

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Poster: Human Olfactory Performance

Effects of odors on the exercise performance of subjects at different training levels

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This study evaluated the effect of the odors sandalwood and lavender on the exercise performance of varsity collegiate athletes compared with the performance seen in subjects not currently participating in a varsity sport. During a test session, subjects were instructed to peddle as fast as they could for 20 min on a stationary bike. While being tested, subjects were exposed to the smell of sandalwood, lavender, or to an odorless control. Each experimental condition (sandalwood, lavender, odorless control) was repeated three times for a total of nine trials per subject. The subject's heart rate, speed and distance traveled were recorded every 2 min. Although somewhat dependent on the training level of the subjects, exposure to both odors showed an improvement in exercise performance. In athletes, exposure to the odors was associated with an increase in speed during the first 6 min of the exercise compared with the first 6 min of exercise with the odorless control. Additionally, exposure to the odors increased heart rate during the last 6 min of exercise. Somewhat in contrast, with odor exposure subjects not currently participating in a varsity sport experienced a decrease in heart rate during the first 6 min of exercise. Like athletes, exposure to the odors was associated with an increase in speed in these subjects, but this effect was not seen until the last 6 min of the exercise. So, although these data demonstrate exposure to sandalwood and lavender can increase performance in a maximal effort exercise in all subjects, the timing of the effect seems to vary depending on the training level of the subject.

Poster: Human Olfactory Performance

Episodic odor memory: influences of handedness, sex and side of nose

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Unlike other major sensory systems, the vast majority of fibers within the olfactory system project ipsilaterally from the receptors to the cortex, bypassing the thalamus. Presently it is not known whether, or to what degree, odor memory is influenced by lateralized brain processes. In this study we administered a standardized 12-item match-to-sample odor memory test separately to the left and right sides of the nose of 30 left- and 30 right-handed subjects of equivalent age and overall general smell ability. Each group was comprised of half of each sex. Three delay intervals were tested: 10, 30 and 60 s. Women, but not men, performed significantly better on the left than on the right side of the nose. Subjects who received the first test on the right side of the nose outperformed those who received the first test on the left side of the nose. In general, sinstrals tended to outperform dextrals. As in previous work, an age-related decrement in odor memory test scores was present. Overall, this study suggests that performance on a match-to-sample odor memory task is influenced in complex ways by lateralized brain processes that are related to the sex of the subject.

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Poster: Human Olfactory Performance

Postmenopausal hormone replacement: do estrogen and progesterone differentially affect smell function?

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Although controversial, a number of research studies suggest that some forms of hormone replacement therapy (HRT) may improve

cognitive function in postmenopausal women. Since smell loss is among the first signs of Alzheimer's disease, the question arises whether HRT protects against such loss. As part of a large multidisciplinary study, we are testing olfactory and cognitive function in a projected cohort of 600 postmenopausal women. To date, 374 women between the ages of 52 and 89 years have been tested. Data include a detailed history of HRT, circulating levels of E_2, E_3, FSH, dHEAS, cortisol and testosterone, and the results from comprehensive olfactory and neuropsychological test batteries. Preliminary findings, based on disproportionately more women in the bottom half of the aforementioned age range, suggest that current HRT users perform better than past users on a standardized test of odor memory, but not on tests of smell identification or detection. Women who were currently receiving estrogen replacement therapy alone performed more poorly on the smell identification test than past users, never users, and current users of progesterone. During the next 2 years, the focus of recruitment will be on women 70 years of age and older. Additionally, we plan to determine whether an association exists between ApoE4 genotype and estrogen's influences on the test measures.

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Poster: Human Olfactory Performance

Relationship between individual odor threshold, odor quality perception and odor identification

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In a study on the effect of air pollution on olfactory function, individual thresholds for the odors of orange juice and instant coffee were determined. As has been reported previously, subjects living in highly polluted Mexico City had significantly higher thresholds than those living in unpolluted but geographically similar Tlaxcala. In this study comprising 168 healthy volunteers, balanced for age and gender, supra-threshold concentrations of the diluted odorants were presented in ascending order in sniff-bottles after completion of the threshold test. Dilution steps differed by factors of 50.8 and 61.0 for orange and coffee, respectively. Subjects were required to describe odor quality and to identify the odorant. At threshold, 80% of subjects reported to perceive nothing, or just air, and only 3% identified odorants. At steps 1 and 2 above threshold ~50% of subjects reported an odor quality, and 75% were able to do so at step 3 above threshold. Correct identification was possible for 25% of subjects at step 2, for 50% at steps 3 and 4, and for 75% at step 5 above threshold. Although the number of dilution steps between threshold, quality perception and odor identification decreased slightly with increasing threshold, no significant differences in this pattern across groups were found. Thus, a 100- to 1000-fold increase above individual threshold concentration seems generally necessary for odorants to carry behaviorally relevant information.

Poster: Human Olfactory Performance

Time and intensity patterns of orthonasal and retronasal smelling

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Retronasal (retro) smelling can differ from orthonasal (ortho) in threshold, identification ability, and suprathreshold intensity (Halpern, 2004, *ChemoSense*, 6(3):1–7); responses over time require further study. For six odorants (anise, cinnamon, coffee, orange, peppermint and strawberry, all alcohol-free food-grade extracts), with concentrations selected by each subject to match a common standard and presented vapor-phase-only either in the oral cavity for retro smelling (exhalation through anterior nares) or to the anterior nares for ortho smelling, 20 subjects judged intensity over 30 s trials on a digital computer using a Labeled Magnitude Scale. We found that intensity reaction times (RT) were 4.044 s for odorants in the retro location and 4.008 s in the ortho (means), with RT for ortho anise and cinnamon < retro ($P < 0.04$) but not for the other odorants ($P > 0.09$). Times of final intensity judgements were 25.30 s for retro; 23.479, ortho, with final ortho < than retro for anise and coffee ($P < 0.042$). Ortho initial judged intensity was > retro for all odorants ($P < 0.02$); final intensity, ortho > ($P < 0.01$) except for coffee ($P = 0.2$). For ortho odorants, mean final intensity exceeded initial (4% to 10%) except for cinnamon (−1%), but not significantly ($P > 0.05$). For retro odorants, mean final intensity exceeded initial (2% to 13%) except for anise and strawberry (−2%, −8%) but not significantly ($P > 0.05$). It appears that ortho intensity RT is faster than retro, and that for both ortho and retro loci, judged intensity shows little decrease and may increase over the first 30 s of exposure.

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Poster: Human Olfactory Performance

Smell identification declines from age 36 years and mainly affects pleasant odours

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Objective: to determine the effect of age on smell identification ability and on pleasantness, strength and irritation attributes. Design/methods: recognition was tested in 106 controls below 50 years old compared with 105 controls over 50 years old. Identification was measured using the 40-odorant UPSIT, each of which has an intensity, pleasantness and irritation rating. Differences in odour identification for the two age groups were analysed according to gender and odour attribute. Results: a best-fitting multiple regression model was used to predict UPSIT score in terms of age and gender. At all ages women had an UPSIT score greater than men by 1.8. Significant decline was detected at age 36 years and accelerated with advancing age. Proportions of correct responses were derived for each odour in the two age groups. As hedonic score increased there was worsening of older compared with younger people. Those under 50 years old outperformed those over 50 years old for odours of low intensity or low irritation. In the over 50 years group odours with a moderate–high

hedonic rating and low–moderate intensity score that were less affected by age were: chocolate; liquorice; grass; coconut; strawberry; rose; and melon. Conclusions: our data confirm the overall decline in odour identification with age and the superiority of females. Physiological decline in smell identification begins at age 36 years. Those under 50 years old outperformed older subjects in all three components. Intensity predicted age effects better than hedonic score but the two components behaved independently. This general rule was broken for seven odours all of which have higher hedonic score but low intensity and therefore less affected by age. These would be more suitable for smell testing in elderly subjects.

Poster: Human Olfactory Performance

Modeling odor mixture perception

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Four concentrations of pyridine, *n*-butanol and amyl acetate were mixed into 16 binary mixtures for each pairwise combination. The three sets of mixtures were presented along with their single constituents to participants by means of a dynamic constant-flow olfactometer. After learning the single constituents of the mixtures, the participants judged perceived odor intensity, quality and valence of each stimulus six times. Intensity was judged using the method of free magnitude estimation. Quality was assessed by asking the participants to identify whether the one, the other, or both constituents were in the mixture. Valence was judged on a seven-step category scale ranging from very bad to very good. Odor intensity, quality, and valence of mixtures of the three pairs of substances were compared. A model (Olsson, 1998) for the prediction of odor intensity and quality based on the perceived intensity of the unmixed constituents was tested.

Poster: Human Olfactory Performance

Task demands affect sniffing behavior

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It is well established that sniffing, the exploratory behavior associated with olfaction, is reduced when an odor stimulus is encountered. This response is very rapid, and sufficiently reliable to serve as the foundation for the Sniff Magnitude Test (SMT), a recently developed tool for the clinical assessment of olfactory function. Recent studies in our laboratory have demonstrated the minimal reliance of the SMT on cognitive abilities, but have left open the question of how changes in the demands of this olfactory task influence sniffing behavior. This information is useful in designing an SMT procedure that most accurately measures an individual's olfactory abilities. A series of experiments was conducted with the SMT using four different sets of instructions to determine if variations in the demands of an olfactory task influenced sniffing behavior. Two hundred university students completed the SMT, with 50 participants in each instruction group. One group simply was instructed to 'sniff' the odor stimuli, one was told to 'sniff until you smell something', a second group identified which of four odors were presented and another performed a task similar to an odorant reaction time procedure. A between-groups ANOVA performed on sniff magnitude ratios (the SMT measure) showed a significant difference between the instructional groups [$F(3,196) = 14.7$, $P < 0.001$] with the instruction to 'sniff until you smell something' producing the highest level of odor-induced sniff suppression. This result demonstrates that sniffing behavior is sensitive to the demands of the SMT, and that sniff magnitude ratios are not entirely reflexive.

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Poster: Human Olfactory Performance

How big are individual differences in olfaction anyway?

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Groups of 20 subjects sought to detect the odors of ethanol and 2,2,4-trimethyl-1,3-pentanediol diisobutyrate (TXIB). Subjects generated individual psychometric functions that spanned 2–3 orders of magnitude for both odorants. The subjects detected TXIB ~100× better than ethanol. The subjects also generated functions for chemesthesis. These spanned under an order of magnitude. The subjects felt TXIB ~1000× better than ethanol. In theory, the psychometric function reflects instantaneous variation in sensitivity. No one can cogently argue, however, that variation in olfactory receptor functioning exceeds that of chemesthetic receptor functioning by 1–2 orders of magnitude. It would mean that olfaction is inherently quite unstable. Most variation in performance must arise beyond the periphery, in the CNS. There, olfactory input has already undergone the compressive transformation seen in responses of receptors and second-order neurons. This is so strong that it gives olfaction great stability in the intensity dimension. The apparent variation comes, it seems, from small differences in performance reflected back into the variable of concentration. So, a 3 to 1 difference in performance shows up as a 10 to 1 difference in 'sensitivity'. For chemesthesis, which shows no such compression, a 3 to 1 difference in performance shows up as ~3 to 1 in sensitivity. When psychometric functions are constructed across subjects, the same illusion occurs: people seem differ greatly from one another when their performance differs much less so. The metric used to assess individual differences creates an illusion of variation.

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Poster: Human Olfactory Performance

The influence of mecamylamine on trigeminal and olfactory chemoreception of nicotine

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Nicotine presented to the nasal cavity at low concentrations evokes 'odorous' sensations, and at higher concentrations 'burning' and 'stinging' sensations. Mecamylamine was able to block painful responses following chemical stimulation of the tongue and of the ethmoidal nerve. The aim of our study in man was to investigate the effects of Mecamylamine on the olfactory and the trigeminal chemoreception of nicotine enantiomers. In order to achieve this aim, before and after mecamylamine we (i) determined olfactory and trigeminal thresholds and subjective intensity estimates and (ii) recorded the negative mucosal potential (NMP) following nasal stimulation with nicotine in a placebo-controlled double-blind fourfold crossover study (n = 15). CO_2 was used as a trigeminal and H_2S as an olfactory control stimulus. Mecamylamine significantly increased trigeminal thresholds of S-(–)-nicotine and reduced intensity estimates and NMPs following stimulation with nicotine enantiomers, but did not influence NMPs and trigminal intensity estimates following stimulation with CO_2. Mecamylamine did not influence olfactory thresholds nor olfactory intensity estimates registered following stimulation with olfactory stimulus concentrations. These results demonstrate that the trigeminal nasal chemoreception of nicotine enantiomers is mediated by nicotinic acetylcholine receptors and give evidence that the olfactory chemoreception of nicotine is independent from nAch receptors.

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Poster: Human Olfactory Performance

Cognitive effects of human emotion semiochemicals

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In an investigation of the effects of human emotion semiochemicals on reaction time, 53 participants (n = 24 males, 29 females) were exposed to three such semiochemicals: fear, anger and happy, and a no odor control condition. The objective measure was response times to an at-threshold, rapid serial visual presentation task (RSVP). The RSVP consisted of 50 every day photos including snakes and spiders as well as flowers, landscapes, photos of people and animals. Participants judged each photo as pleasant or unpleasant. Stimuli were presented in a random, counter-balanced order in a repeated measures experimental design. There was a significant main odor effect [Wilk's lambda, $F(3,50) = 3.45$, $P < 0.03$]. Post-hoc tests revealed a significant difference between three pairs of odor conditions: happy/anger (n = 53, t = 2.37, $P < 0.03$), happy/control (n = 53, t = 2.17, $P < 0.04$) and happy/fear (n = 53, t = 2.80, $P < 0.01$). No significant effects were observed in the gender × odor interaction. In all cases, participants had the fastest response times in the happy odor condition. Overall, females responded more quickly than males in all emotion odor conditions except anger. Response times to visual stimuli vary as a result of exposure to emotion semiochemicals. Humans not only produce mood odors but also respond to them.

Poster: Human Olfactory Performance

Perception of freshness of food by humans: sensitization effects shown in a chemosensory evoked related potential paradigm

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Effects of olfactory learning on odor discrimination performances have been reported. However, applications in the domain of food odors are very unusual. In this study we seek for physiological effects in the perception of freshness of food. Chemosensory evoked related potentials (CSERP) were recorded while subjects were submitted to stimuli using an olfactometer. Four odors were delivered, a butter aroma and three binary mixtures of the aroma and butyric acid at three concentrations (10^{-2}, 10^{-3} and 10^{-4}) and three mixtures of butter aroma and vanillin (10^{-1}, 10^{-2} and 10^{-3}) were used as controls. Eight subjects divided in two groups were tested on three consecutive days: on day 1, CSERP baselines were recorded in response to stimulations of butter aroma and to the three rancid butters. On day 2, subjects were sensitized by repetitive stimulations while completing a set of psychophysical assessments (intensity, pleasantness, familiarity and edibility). The Test group was trained with butter and rancid butter mixtures, the Control group was trained with butter and vanilled butters. On day 3, CSERP were recorded while subjects had to compare pairs of butter/rancid butters and evaluate similarities or differences. In the Test group, subjects were able to detect the rancid hint in mixtures at a lower concentration than subjects in the Control group suggesting a sensitization effect. CSERP recordings from the two training groups showed significant differences in the cortical brain activity.

Poster: Human Olfactory Performance

Effects of intermittent peppermint odor administration on alertness, mood, mobility and sleep patterns

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Prior research has shown that peppermint odor administration significantly improves attention, motivation, mood, alertness and vigor. The present study extended those findings by evaluating alertness throughout the day and subsequent sleep patterns at night following the intermittent daily administration of peppermint odor. Utilizing a within-subjects design, participants completed two 24 h sessions. The experimental condition consisted of the inhalation of pharmaceutical grade peppermint vapors every other hour from 9 a.m. to midnight, while the control condition consisted of sham odorant administration. Participants continually wore an ActiWatch movement sensor to assess their waking-hour activity patterns, circadian rhythms and sleep patterns. During their waking hours, participants also made ratings of vigor and mood throughout the day. Results supported previous research indicating that intermittent peppermint odor administration increased mood and alertness throughout the day, and resulted in lower ratings of fatigue. This effect was more pronounced for the male participants. In addition, level of sleep

movement was less following the peppermint odor condition, indicating a more restful night of sleep despite no differences in the actual amount of sleep received. Implications relate to maintaining alertness and productivity during the day and increasing sleep quality.

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Poster: Human Olfactory Performance

Measurements related to odor perception are modulated by looking at emotionally evocative images

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Objectives: data from clinical studies indicate that odor perception is changed in patients suffering from endogeneous mood disorders. We were interested whether mood changes in healthy subjects also lead to measurable changes in olfactory perception. Methods: olfactory thresholds and olfactory discrimination were assessed in 15 healthy subjects (Sniffin' Sticks). Data obtained of two conditions, i.e. before (baseline, BL) and after looking at either positive (POS) or negative (NEG) emotionally evocative pictures (international affective picture system, IAPS) were compared. In addition, subjects rated their actual mood with reference to pleasure and arousal, and the pleasantness and intensity of the odorant phenyl ethyl alcohol (PEA). Results: looking at the IAPS pictures changed the mood of the subjects. Subjective ratings of pleasure changed accordingly (BL: 5.3; NEG: 3.9; POS: 7.0, $P < 0.001$), while both conditions increased the arousal (BL: 3.0; NEG: 6.1; POS: 5.0; $P < 0.001$). Olfactory thresholds increased after looking at the IAPS images (BL: 10.4; NEG: 8.1; POS: 9.0; $P < 0.05$), whereas olfactory discrimination did not change. Subjects rated the odorant PEA as more pleasant in the POS condition, and less pleasant and more intense in the NEG condition (pleasantness: BL: 4.1; NEG: 2.9; POS: 5.7; intensity: BL: 3.5; NEG: 5.6; POS: 3.3; $P < 0.05$). Conclusions: olfactory perception is not independant of the emotional status of subjects.

Poster: Human Olfactory Performance

Effects of odor administration on driving performance, safety, alertness and fatigue

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Past research indicates the odors of peppermint and cinnamon enhance motivation, performance, and alertness, decrease fatigue, and serve as central nervous system stimulants. Given these results, it is reasonable to expect that the presentation of peppermint or cinnamon odor while driving may produce a more alert and conscientious driver, and minimize the fatigue associated with prolonged driving. In the present study, 30 participants were monitored during simulated driving under three odor conditions (peppermint, cinnamon, non-odor control). Odors were added to low flow oxygen (1.3 l/min) via an oxygen concentrator and presented at the rate of 30 s every 15 min. Subjective measures of cognitive performance, wakefulness, mood and workload were also assessed. In general, prolonged driving led to increased anger, fatigue and physical demand, and decreased vigor. However, fatigue ratings were decreased in the cinnamon condition. Both cinnamon and peppermint administration led to increased ratings of alertness in comparison to the no-odor control condition over the course of the driving scenario. Periodic administration of these odors over long term driving may prove beneficial in maintaining alertness and decreasing highway accidents and fatalities.

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Poster: Human Olfactory Performance

Perception of odors at different stages of age with special regard to sex, puberty and menopause

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This study investigates the odor perception and description of 10 odors from five important categories with special attention to age and sex. Because of the discussion of the variation of oder perception during phases with hormonal changes we also examine subjects at a pre- and post-pubertal as well as at a pre- and post-menopausal age. Referring to the literature and previous experiments, the five odor categories are (i) human (androstenone, isovalerc acid), (ii) musk pentadecalactone, galaxolide), (iii) natural odors (rose, lavender), (iv) food (vanillin, citral) and (v) dangerous odors (ammonia, charcoal). There are two concentrations of each odor (slightly above threshold and a moderate concentration) and six age groups between 6 and 80 years (child, pre pubertal, post pubertal, young adult, pre-menopausal, post-menopausal). There are 30 men and 30 women in each age group. Each subject takes part in a threshold test (CCCRC) in the beginning and at the end of the session. There is a specific test of anosmia concerning androstenone. We also take biographical and health data. Each odor and odor concentration is presented with sniffing sticks in random order. Each odor is presented separately and rated for pleasantness, intensity and familiarity. The odor description for each odor concentration is done on 10 qualitative rating scales.

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Poster: Human Olfactory Performance

Reaction times to sequences of two odours

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Objective: to investigate if reaction times to decide that two sequentially presented odours are the same are faster than if the odours are different. Method: we measured RTs to all combinations of four different odours, two from edible and two from non-edible substances. Ten subjects each produced 120 RTs, 24 in each of five sessions. Iso-intense stimuli were delivered by a computer controlled constant air flow olfactometer. Odour 1 was presented for 1.5 s, followed by a pause of 0.2 s before the presentation of odour 2 for

1.5 s. Subjects pressed a button as fast as possible to indicate if the odours were the same or different. Results: for all subjects mean RTs to different odours are faster than to same odours. Mean over all subjects: RT(diff) = 1141 ms, RT(same) = 1383 ms. ANOVAs reveal significant effects of subject ($P < 0.05$), odour pair (same versus different) ($P < 0.01$) and session number ($P < 0.0004$) as well as significant interactions between subjects and odour pairs. There is no significant effect of edibility of the odours. For all five sessions RTs to different odours are shorter than to same odours, but there is a gradual decline in mean RTs from 1422 ms to 1185 ms. Conclusion: contrary to what is found with visual stimuli, RTs to sequences of different odours are faster than RTs to sequences of identical odours. This fundamental difference between vision and olfaction is in line with recent findings contrasting olfactory and visual memory and illustrates the different functional architectures of two sensory systems serving different ecological purposes.

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Poster: Human Olfactory Performance

Scent tracking in humans

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Scent tracking is a natural and complex olfactory behavior. Unlike chemotaxis in bacteria, moths, ants and robot models, scent tracking in mammals has received little experimental attention. Elucidating factors that influence scent tracking performance might provide important insights about the neural mechanisms that underlie olfactory behavior. Here we asked whether humans can scent track. Sixteen subjects (9F/6M, age 20.1 ± 1.3) attempted to track an 8 m long odor trace on a lawn. Subjects tracked on all fours, with their nose to the ground. All non-olfactory input was prevented (earmuffs, blindfolds, heavy gloves). Spatial trajectories were recorded by aerial digital video, and real-time nostril-specific inhalation rates were recorded via respiratory cannulas linked to a portable spirometer and a data logger. Each subject was tested three times. Four subjects reached the end point once, three twice and three all three times. The mean velocity along the track in successful efforts was 2.30 ± 1.14 cm/s. Comparison of sniff patterns suggested that different sniff strategies are employed by good and poor performers. Good trackers showed more burst-like high-frequency sniffs. These findings suggest that humans have the olfactory and neural mechanisms necessary for scent tracking and that the sniffing behavior of better trackers parallels that of macrosmatic animals.

Poster: Human Olfactory Performance

Neural correlates of attention to pleasant versus unpleasant odors

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Neural mechanisms underlying attention to odors and their properties are poorly understood. The main question addressed in this study was whether or not regional brain activations differ as a function of attention to pleasant versus unpleasant odors. Twelve healthy participants underwent a positron emission tomography (PET) study, in which the distribution of regional cerebral blood flow (rCBF) was measured during three 60 s scans. The experiment consisted of three conditions: Attention to pleasant odors, Attention to unpleasant odors and Baseline. The stimuli presented in the two attention conditions were exactly the same—three different binary mixtures, each consisting of a pleasant and an unpleasant odor that were subjectively isointense. Each mixture was presented three times, for a total of nine stimuli per scan. During Attention to pleasant odors, participants indicated with a mouse click whether each stimulus contained a pleasant odor or not; during Attention to unpleasant odors, the task was to respond whether each stimulus contained an unpleasant odor. During Baseline, the odorless stimulus was presented nine times and participants pressed the mouse button randomly after each one. We found that regions within the right orbitofrontal cortex were activated when participants attended to odorants regardless of their affective valence, while activation within the insula/frontal operculum showed a lateralized response; i.e. right insula activation was associated with attending to pleasant, and left to unpleasant, odors.

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Poster: Human Olfactory Performance

Perithreshold exposure increases odor detectability but not odor recognition

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Repeated exposures to perithreshold, but not suprathreshold, odorants can lead to dramatic sensitivity increases among young women, (Dalton *et al.*, 2002), even when the target odorant is superimposed on a suprathreshold 'background' odorant (Dalton *et al.*, 2003). To evaluate the impact of this phenomenon on odor recognition, two-alternative forced-choice detection thresholds for benzaldehyde were obtained at each of 10 daily sessions from seven females (18–24 years of age). At the beginning and end of the study, psychometric functions for detection and recognition of benzaldehyde and citralva (control) were obtained. As shown in prior studies, threshold sensitivity to benzaldehyde (as measured by a modified staircase method) increased significantly (average >6 orders of magnitude) following repeated testing. Repeated threshold testing of benzaldehyde also shifted the slope of the psychometric function for odor detection, but not for recognition. No change was observed on any measure for the control odorant, citralva.

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Poster: Taste: Human Sensory Performance

Relationship between genotypes of the TAS2R38 bitter taste receptor gene and sweet preferences in children and adults

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The present study aimed to determine how variation in the TAS2R38 taste receptor gene influences the gustatory experience and preferences of children and adults. Genomic DNA was extracted from cheek cells of a racially and ethnically diverse sample of 143 children and their mothers. Forced-choice procedures embedded in the context of a game were used to determine sucrose preferences and food habits. Genotypes at the TAS2R38 locus were significantly related to preferences for sucrose ($P = 0.01$), as well as liking for sweet-tasting beverages ($P = 0.048$) and foods such as cereals ($P = 0.05$) in children. Children who were heterozygous or homozygous for the bitter taste allele (AP and PP) preferred significantly higher concentrations of sucrose solutions than AA children. They were also significantly less likely to include milk or water as one of their top two beverages ($P = 0.006$) and were more likely to include carbonated beverages as one of their most preferred beverages ($P = 0.05$). There were also significant main effects for race/ethnicity on preferences and food habits. As a group, black children liked cereals with significantly higher sugar contents than white children ($P = 0.01$) and were significantly more likely to report that they liked to add sugar to their cereals ($P = 0.00007$). Unlike children, there was no correspondence between TAS2R38 genotypes and sweet preference in adults. Here the effects of race/ethnicity were the strongest determinants, thus suggesting that cultural forces and experience may override this genotype effect on sweet preferences.

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Poster: Taste: Human Sensory Performance

'Active' versus 'passive' tasting: evidence of differential effects across taste stimuli, sensory modalities and oral sites

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Taste and chemesthesis are rarely studied under conditions of normal, 'active' tasting, i.e. when the gustatory surfaces of the tongue are brought into contact with the opposing surfaces of the palate. Contact of this kind, and the mechanical stimulation it produces, has the potential to affect flavor perception in several ways, including spreading the stimulus over a larger area and adding a tactile component to the chemosensory signal. Here we report two experiments that uncovered significant perceptual differences during 'active' versus 'passive' tasting. In Experiment 1, subjects rated the intensity of tastes produced by sucrose, NaCl, and MSG applied to the tongue tip, hard palate, soft palate and circumvallate papillae. Ratings were made while the tongue was held still in the mouth or after it had been touched to the palate in a single, 'smacking' motion. A repeated-measures MANOVA revealed significant effects of mode of tasting and oral site, with the largest differences occurring for the savory taste of MSG. Experiment 2 compared the effect of active versus passive tasting on the intensity of taste and chemesthetic sensations from QSO_4, urea, $MgCl_2$, sucrose, NaCl, citric acid and MSG applied singly and in mixtures. Active tasting tended to enhance mixture interactions (e.g. bitterness suppression) and reduced burning/stinging caused by urea and $MgCl_2$. The varied nature of these effects implies that mechanical stimulation modulates oral chemosensory perception via multiple mechanisms.

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Poster: Taste: Human Sensory Performance

Switching costs asymmetry in pleasantness and intensity in taste

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Task-switching costs are thought to reflect the time it takes to reconfigure the cognitive system to perform a new task. If asymmetrical task switching times are found, this is thought to reflect a difference in strength of the tasks between which switching is required. We wanted to investigate which task would be stronger: intensity or pleasantness judgements of taste. A task switching paradigm, in which subjects had to switch from rating intensity to rating pleasantness and vice versa, was used to investigate which of these two attributes in taste is stronger or more automatically processed. During continuous rating of orange lemonades (a time–intensity procedure), subjects switch between rating intensity and pleasantness at a signal. Task switch times were significantly longer (~300 ms) for the switch from intensity to pleasantness than vice versa [$F(1,11) = 13.143$, $P = 0.004$]. This result is interpreted as indicating that intensity processing is stronger and more automatic than pleasantness processing.

Poster: Taste: Human Sensory Performance

Enhanced responses in the cortical sensory areas to anticipated taste or food stimuli; magnetoencephalographic studies in humans

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When a stimulus occurs at regular intervals or a sensory component of a familiar stimulus occurs, we learn to anticipate those stimuli. To examine brain activities in such a situation, we performed the following two experiments where we measured evoked magnetic field in humans using 122-channel whole head SQUID system (Neuromag-122™, Finland). In the first experiment, to examine whether taste perception is influenced by presentation sequences, we investigated the relation between magnetic responses and reaction times (RT) in presentation sequences. We used 50 mM citric acid and 500 mM sucrose solutions as taste stimuli. Taste stimuli were administered orally to each subject 'alternately' or 'randomly'. In the alternately presented task, subjects can anticipate the next taste stimulus, but they cannot do so in the randomly presented task. In the inter-stimulus interval, the tongue of subject was rinsed with distilled water for 15 s. Each taste stimulus was presented >40 times. The magnetic responses in the alternately presented task occurred at

a shorter latency with a higher magnitude than in the randomly presented task for both taste stimuli. In the second experiment, the odor of banana or strawberry was followed by the visual image of either of these foods. When the odor and the image matched, the evoked magnetic field was larger in the occipital cortex than when mismatched. These results suggest that sensory information processing is affected by anticipation in the cortical sensory areas.

Poster: Taste: Human Sensory Performance

Interactions between cyclamate and msg tastes in human subjects

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On the basis of *in vitro* studies, Xu *et al.* (2004, *Proc. Natl Acad. Sci. USA*, 101:14258–14263) recently proposed that unlike most sweeteners, sodium cyclamate (CYC) acts on the T1R3 subunit of the human T1R2/T1R3 sweet receptor complex, and as a consequence, may also modulate the activity of the human T1R1/T1R3 umami taste receptor. Their data point to specific enhancement of the activity of T1R1/T1R3 by CYC, but not other common sweeteners, in the presence of L-glutamate. Testing the correlation between receptor activity and perception is, in human subjects, complicated by potential cognitive suppressive effects of CYC's sweetness on umami taste. As a first step, however, we contrasted the impact of CYC (8 mM) with that of sucrose (SUC, 160 mM) on the perceived umami taste intensity of MSG (30 mM), both before and after suppression of sweet taste by *Gymnema sylvestre*. At these concentrations there was no indication of enhancement of umami taste by CYC. Umami ratings of CYC–MSG and SUC–MSG mixtures were equivalent and tended to be lower than those of MSG alone, whether or not the perception of sweetness was suppressed. However, MSG appears to enhance specifically the sweetness of CYC.

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Poster: Taste: Human Sensory Performance

PTC non-tasters find the fruit of *Antidesma bunius* bitter, while PTC tasters find it sweet

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There is strong evidence that PTC tasting and non-tasting haplotypes are balanced, but it is not known why. As one suggestion, sensitivity to bitterness from fruit of the *Antidesma bunius* tree was perfectly and inversely correlated with ability to taste bitterness from PTC (Henkin and Gillis, 1977, *Nature*, 265:536–537). All subjects who tasted antidesma extract as bitter found PTC not bitter, and no subject who tasted PTC as bitter found antidesma bitter. Also, less than half of the PTC non-responders were able to detect antidesma as bitter (45%). To replicate these findings with additional emphasis on the h*TAS2R38* genotype of individuals, we tested a single pedigree (n = 86) with *Antidesma bunius* berries and compared their perceived bitter intensities with their modified Harris–Kalmus bitterness recognition thresholds for PTC. We fully replicated the previous results, finding only four subjects who did not fit within the reported categories. We also assessed other taste modalities during ratings of antidesma and found ~70% of PTC bitter tasters rated the fruit berries as sweet; the remaining 30% of PTC bitter tasters found the berries not sweet and somewhat sour. We will assess whether these findings may be explained by haplotypes of the single bitter receptor gene h*TAS2R38*, which accounts for a large portion of PTC taste variability (Kim *et al.*, 2003, *Science*, 299:1221–1225). Thus, by identifying the h*TAS2R38* haplotype for each subject, we hope to determine if variants of this receptor gene account for antidesma's positive sweet and negative bitter correlations with PTC tasting ability.

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Poster: Taste: Human Sensory Performance

Twin-study demonstrates heritability of sensitivity to sour taste from citric acid

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At present, the molecular mechanisms of sour taste in humans are not known. To determine the contribution of genes to sourness perception, a twin study assessed the heritability of sensitivity to sourness from citric acid. Identical (monozygotic, or MZ) twins have ~100% of their genes in common. Fraternal (dizygotic, or DZ) twins, like typical siblings, have only 50% of their genes in common, on average. Assuming MZ and DZ twin-pairs both share similar environments, better agreement between MZ than DZ twins suggests a genetic contribution to sensitivity to sourness. Experimenters tested 77 pairs of MZ twins and 33 pairs of DZ twins at an annual twins festival in Twinsburg, OH. Sensitivity was defined here as the sourness recognition-threshold (modified Harris–Kalmus test) for citric acid. MZ pairs showed significant agreement: intraclass correlation coefficient = 0.60. This agreement approached the limit imposed by test–retest reliability, which a separate study estimated at 0.68. Correction for test–retest variance yielded a correlation among MZ twins of 0.88. Agreement among DZ pairs failed to reach significance: r = 0.17 or 0.24 after adjustment for test–retest variance. Thus, MZ pairs agreed more closely in sensitivity to the sourness of citric acid than did DZ pairs. These results suggest that genes play a predominant role in determining sensitivity to sour taste. Future studies will determine which genes cause variation in sourness perception and whether these genes affect taste receptors or other factors, e.g. buffering capacity of saliva.

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Poster: Taste: Human Sensory Performance

Adaptation versus inhibition: evidence of increased sensitivity to sucrose after adaptation

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Repeated exposure to a taste stimulus often leads to a decrease in magnitude of the perceived intensity. Adaptation to sucrose reduces

the sweetness of subsequent presentations of sucrose as well as other sweeteners. The use of an inhibitor also leads to a decrease in the perceived intensity, the difference being the mechanisms by which the inhibitor interacts with the receptor. The purpose of the present study was to determine the psychophysical functions for sucrose when subjects were previously adapted to either water or sucrose and compare them to the psychophysical functions of sucrose mixed with sweet taste inhibitors. Sweetness intensity ratings were given by a trained panel for sucrose and sucrose mixed with sweetness inhibitors. The concentrations for the sucrose function ranged from 10 to 2000 mM in water. To obtain the adaptation curve, panelists were given seven samples of 400 mM sucrose before they tasted the sucrose concentration series and after each stimulus in the series as a rinse. When subjects were adapted to sucrose, there was a reduction of the sweetness intensity that translated to a right shift of the curve as well as an increase in slope. Thus, solutions of equal or lower concentration than the adapting stimulus were perceived as less intense, while sensitization was evident at concentrations higher than the adapting stimulus. On the other hand, sweetness inhibitors caused a right shift of the concentration-intensity curve without modifying the slope. The differences in the psychophysical functions obtained for adaptation and inhibition suggest that it is possible to differentiate their physiological effects with this approach.

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Poster: Taste: Human Sensory Performance

Genetic and environmental variation in taste mediates vegetable sweetness, bitterness and intake

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Only 1 in 4 adults in the USA consumes sufficient servings of vegetables for optimal health. Since consumers report that taste drives food choice, we predicted tastes from and preference for sampled vegetables as well as vegetable intake from taste genetic (6-*n*-propylthiouracil bitterness) and taste function (chorda tympani nerve/whole mouth ratio for quinine) markers in 68 women and 40 men (aged 18–59 years). With the general Labeled Magnitude Scale, adults reported prototypical tastes from and preference for Brussels sprouts, kale and asparagus; and bitterness from 1.0 mM quinine (tongue tip and whole mouth) and 3.2 mM PROP (whole mouth). Data were analyzed with multiple linear regression (alpha = 0.05), controlling for age and sex effects. Subjects who tasted PROP as less bitter reported less bitterness from sampled vegetables. Those with lower quinine ratios reported less sweetness and less bitterness from vegetables. Vegetable sweetness and bitterness were independent predictors of both vegetable preference and intake. PROP bitterness predicted vegetable intake directly or via vegetable bitterness. In summary, accounting for positive and negative vegetable tastes increased ability to predict intake. Vegetable bitterness was a negative predictor of both preference and intake. Vegetable sweetness was a positive predictor of preference and intake. Those with signs of chorda tympani nerve damage tasted less sweetness from vegetables. By modifying vegetable tastes, dietary quality could be improved.

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Poster: Taste: Human Sensory Performance

Taste and dietary predictors of central adiposity in adult females

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Greater waist circumference, a measure of central adiposity, increases risk of diabetes and heart disease. Here, 75 females (47 ± 7 years) reported bitterness of 6-*n*-propylthiouracil (taste genetic marker) and quinine on the tongue tip (taste function marker) and preference for high-fat foods (sampled and from questionnaire) on the general Labeled Magnitude Scale. Intake of high-fat foods was assessed via validated survey. Waist circumference was >35 inches for 25 females, indicating higher disease risk. Data were analyzed with multiple regression (alpha = 0.05), controlling for age. Although the taste markers were correlated, each contributed uniquely to predicting adiposity. Lower bitterness from PROP and quinine predicted greater adiposity. PROP predicted adiposity only in normal to mildly obese females (n = 66). Quinine predicted adiposity across all females. The PROP–adiposity relationship appears mediated in part by dietary fat behaviors. Females with higher waist circumferences preferred and consumed more high-fat foods. Those who tasted PROP as less bitter reported a greater liking for high-fat foods and consumed 38 of 45 high-fat foods most frequently. Quinine was not a unique predictor of dietary fat behaviors; its influence on adiposity risk may act via other dietary behaviors (eg, vegetable intake—Dinehart *et al*, AChemS 2005). In summary, lower taste function measured by bitter taste markers was associated with greater adiposity and diets that may increase the risk of obesity. Using multiple measures of taste improved the ability to predict adiposity in both normal and obese females.

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Poster: Taste: Human Sensory Performance

Application of structural equation modeling to TAS2R38 genotype, 6-*n*-propylthiouracil bitterness and supertasting

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Drayna and colleagues suggest that between 55 and 85% of the variance in phenylthiocarbamide threshold is explained by TAS2R38 haplotype. However, intense bitterness from 6-*n*-propylthiouracil (PROP), a related compound, is associated with heightened intensity from diverse oral stimuli. Here, Structural Equation Modeling (SEM) using AMOS 5.0 was applied to general Labeled Magnitude Scale-derived data from the prototypical tastants (quinine, sucrose, citric acid, sodium chloride) and PROP. Participants were genotyped and characterized as AVI homozygotes, PAV/AVI heterozygotes or PAV homozygotes; individuals with rare alleles were

excluded from the analysis. Using two-stage SEM, as advocated by Kline, a measurement model with acceptable fit is presented prior to testing any structural models. Structural models tested here include fungiform papillae density and TASR38 genotype as exogenous variables. Reported measures of fit include the chi-square test, Tucker–Lewis Index and Root Mean Square Error of Approximation. Findings suggest that failure to account for fungiform papillae density results in a poor fitting structural model, confirming that TAS2R38 genotype alone cannot explain differences in suprathreshold response across stimuli.

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Poster: Taste: Human Sensory Performance

Inhibition of taste by chlorhexidine in humans

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The antiseptic chlorhexidine (ChX) impairs identification of salty and bitter taste qualities by unknown mechanism(s). A range of stimulus concentrations was used to test if actions of ChX on the tastes of NaCl and quinine were due to competition for receptor sites. Each trained subject (four women, five men, age 21–23 years) tasted test stimuli followed by water rinses in random order. On separate days, two stimulus replicates were presented before, and four replicates 5 min after rinsing with either 0.3 mM or 3.0 mM ChX. Test stimuli were 0.03, 0.1 and 0.3 mM quinine hydrochloride; 0.03, 0.1 and 0.3 M NaCl; and water. Subjects rated taste intensity on a labeled magnitude scale and identified taste quality. On average, 3 mM ChX reduced taste intensity by 75%, greater than the 47% reduction for 0.3 mM ChX ($P < 0.01$). Consistent with a competitive model, ChX treatment reduced the taste intensity of 0.1 M NaCl and 0.1 mM quinine by 75%, which was greater than the 54% reduction for 0.3 M NaCl and 0.3 mM quinine ($P < 0.01$). However, results for 0.03 M NaCl and 0.03 mM quinine were not consistent with competition, nor were results for 3 mM ChX and quinine. Identification of NaCl as 'salty' and quinine as 'bitter' deteriorated with decreases in taste intensity. As the ChX effect on moderate and strong salt tastes appears to be competitive, adding a sodium salt to the mouthrinse may alleviate taste distortions that result in discontinued use of ChX, a detriment to treatment of periodontal disease.

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Poster: Taste: Human Sensory Performance

Adaptation and recovery-from-adaptation to sucrose and sodium chloride

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In a study of adaptation and recovery-from-adaptation that we reported at AChemS last year, we found that taste intensity declined rapidly during adaptation to a solution of 0.5 M sucrose, after which perceived intensity recovered more slowly. Two experiments, using the precise temporal control made possible by pressurized air regulating solution delivery with the automated 'TASTE' system (Ashkenazi *et al.*, 2004), expanded these findings. Sodium chloride, as well as sucrose, and two experimental methods were used to assess the effects of adaptation. In both experiments, we presented subjects with adapting and test solutions of 0.5 M sucrose and 0.5 M NaCl. In the first experiment, subjects rated taste intensity, on an LMS scale, at fixed time intervals during exposure to the adapting stimuli and then afterwards during recovery. In the second experiment, subjects rated taste intensity continuously. As in the study reported last year, the results of the present study indicate that taste intensity adapts relatively quickly, and this adaptation is followed by a slower recovery.

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Poster: Taste: Human Sensory Performance

Experience induced changes in human sweet taste

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An apparent plasticity in glucose sensitivity was first noted while studying human taste variants (Eylam and Kennedy, 1998, *Proc. Natl Acad. Sci. USA*, 172), but the experimental design did not rule out regression to the mean. Since then, a human taste induction hypothesis was supported by experience-induced changes in taste identification of monosodium glutamate (MSG) (Kobayashi and Kennedy, 2002, *Physiol. Behav.*, 60). Here we tested the taste induction hypothesis for sweet taste. Thirty-seven subjects treated their tongues with blue fructose 43 mM, while 30 others treated with blue distilled water, briefly each day for 10 days at home. On day 11 or 12, all tasted paired samples of green glucose (17.5, 27, 42, 65, 100 mM) and water in the laboratory and were asked to identify the sugar of each pair in a forced choice paradigm. The data were analyzed by assigning threshold categories (1–6) based on the lowest concentration and all higher concentrations identified correctly. There was a significant difference in identification abilities (ANOVA, $P = 0.005$). Threshold categories of fructose treated subjects were significantly lower than those of controls ($P = 0.003$). There was no effect of gender ($P = 0.244$) (contrasts). These results support the taste induction hypothesis for sweet taste. They also suggest that taste mechanisms for fructose and glucose are the same at some point in the sensory processing pathway.

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Poster: Taste: Human Sensory Performance

Experience-induced changes in taste identification for monosodium glutamate are reversible

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Taste identification for monosodium glutamate (MSG) varies with experience. Subjects identified MSG at lower concentrations after brief daily exposure for 10 days to crackers with MSG than subjects exposed to candies without MSG (Kobayashi and Kennedy, 2002, *Physiol. Behav.*, 57). Here we evaluated the temporal properties of such change. In phase I, subjects underwent 10 days of brief exposure at home to treatment foods—crackers with MSG (MSG group) or crackers without MSG (control group). On day 11 or 12, all were tested in the laboratory. First, they tasted MSG 5mM and were told 'This is MSG'. Then they tasted pairs of MSG and NaCl solutions (0.63, 0.93, 1.25, 1.85, 2.5 mM) and indicated the MSG of each pair. The MSG group identified MSG at lower concentrations than the control group ($P < 0.05$, *t*-tests). For phase II, two subgroups continued treatment for 10 days (MSG- or control-continued group), while two others discontinued treatment (MSG- or control-stopped group). On day 21 or 22, all were tested again. There was a difference in MSG indentification among all the phase I and II groups ($P < 0.05$, ANOVA). The MSG-stopped group recognized MSG at lower concentrations in phase I than phase II ($P < 0.01$), while the other groups did not differ between phases I and II ($P > 0.1$) (contrasts). These results confirm experience-induced increases in the ability to recognize MSG and show that without continued experience, the effect is reversed.

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Poster: Taste: Human Sensory Performance

The effect of a nutritional preload on gustatory activation: an ER–fMRI study

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The present event-related functional magnetic resonance imaging (er-fMRI) study investigated changes in brain responses to gustatory stimuli between physiological states of hunger and satiety. Each participant fasted for 12 h prior to testing, and participated in two er-fMRI sessions, one with no preload and the other with a preload of Ensure Plus. During the scan participants engaged in two functional runs, each consisting of six gustatory stimuli (caffeine, guanosine 5′-monophosphate, sucrose, NaCl, saccharin, citric acid) randomly presented eight times in each run, for a total of 16 repetitions. Results showed that brain activity was globally stronger in the hungry than in the satiated state. In the satiated condition, activation was found in left insula, frontal and rolandic operculum. In the hungry condition, activation was found in right posterolateral and left anteromedial orbitofrontal cortex, thalamus and hypothalamus, caudate nucleus, ventral and dorsal insula, right frontal and rolandic operculum, superior and middle frontal gyrus, amygdala, parahippocampal gyrus and posterior hippocampus. These findings suggest that activation in the primary gustatory cortex is found independent of the physiological state of the subject and that activation in regions associated with changes in the physiological state/reward value, such as orbitofrontal cortex, amygdala and hypothalamus, is found in the hungry state but decreases as a result of satiation.

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Poster: Taste: Human Sensory Performance

Bitterness of *Ilex paraguariensis* infusions: affected by flavor manipulations or caffeine?

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Ilex paraguariensis (Ip) infusion, a complex sensory stimulus with characteristic flavour and bitter notes, is traditionally consumed in South America as a mild stimulant beverage. Thus, the aims of this study were: (i) to examine the bitter responses to caffeine and Ip infusions; and (ii) to investigate whether there is a systematic change in bitterness due to Ip flavor information. Sixteen habitual Ip users distinguished the bitterness of caffeinated distilled water (2.5 versus 5.0 mM). Discrimination of Ip 2% w/v versus Ip 2% w/v + caffeine 10 mM was determined using the *R*-index values outlined by O'Mahony (1992, *Sensory Stud.*, 7:1–47). Three concentrations of caffeine (3.2, 6.5 and 13.0 mM) and Ip (2.5, 5.0 and 7.5% w/v) were evaluated by magnitude estimation. Both, discrimination and intensity scaling tasks were performed with and without use of nose clips. Neither the ability to discriminate bitterness in Ip infusions nor the bitter concentration–response functions were affected by the flavor manipulation. The psychophysical bitter functions for the Ip infusions were steeper than the function for caffeine. Furthermore, this difference is stimulus dependent, both Ip brands showed comparable bitter functions. These data suggest that the bitter experience was not significantly modulated by the Ip flavor. Results showed also that bitterness of Ip is mediated by caffeine plus other Ip components as caffeoyl derivatives and flavonoids.

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Poster: Taste: Human Sensory Performance

Properties of lactisol

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Differences in the sweet-blocking efficacy of lactisol [2-(4-methoxyphenoxy) propanoic acid] for different sweeteners (sucrose and aspartame) and for various exposure areas of the mouth were found. Twenty participants rated the sweetener solutions mixed with and without lactisol for sweetness, sourness, saltiness, bitterness and umami for anterior tongue, posterior tongue and whole mouth stimulations. For sweetness ratings, suppression was significant for all stimulation areas for both sucrose and aspartame. Posterior tongue yielded significantly higher sweetness ratings than did the anterior tongue in the presence of lactisol for aspartame but not for sucrose. Sourness and bitterness ratings were significantly higher for anterior tongue stimulations than posterior tongue stimulations for aspartame but not for sucrose. There were increases in sourness and bitterness ratings in the presence of lactisol for both sweeteners; likely due to the sour taste lactisol has at the concentration used. Results imply a difference between the front and the back of the tongue in the mechanisms involved in the perception of sweetness. While other preliminary findings indicated lactisol might suppress perceived irritation, additional data did not support this initial

discovery. It does, however, raise questions as to why a combination of lactisol and sucrose did reduce rating of burn from capsaicin.

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Poster: Taste: Human Sensory Performance

Perceived saltiness and the sodium evoked lingual surface potential correlate

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We have demonstrated in humans that Na^+ evokes changes in the lingual surface potential (LSP) using a custom gustometer (2003, *J. Neurophysiol*., 90:2060). To assess whether a relationship exists between the Na^+ evoked changes in the LSP and the perceived salt intensity, we measured the LSP and the perception of saltiness simultaneously in sevensubjects. The test solutions (50, 100, 300 and 1000 mM NaCl) superfused the lingual surface for 5 s; they were given in random order. The modulus was 100 mM NaCl and was assigned a value of 100, while the rinse solution was 10 mM NaCl. The evoked LSPs and the reported intensity scores increased with the NaCl concentration in a curvilinear pattern. The evoked LSPs and intensity scores correlated with one another well (r = 0.996, $P < 0.01$). After transforming the data by converting the intensity scores to a logarithmic scale and by normalizing the evoked LSPs to the LSP evoked by the modulus, the values still correlated well (r = 0.989, $P < 0.02$). An almost identical protocol was repeated in eight subjects and the same statistically significant relationship existed between the evoked LSPs and the perceived salt intensity. The existence of a statistical correlation between the perception of saltiness and the Na^+ evoked changes in the LSP suggests that the LSP is a component of the signal transduction system involved in human salt taste.

Poster: Taste: Human Sensory Performance

Taste blindness to 6-*n*-propylthiouracil and body weight in a genetically isolated population in southern Italy

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Taste blindness to the bitterness of 6-*n*-propylthiouracil (PROP) is a stable genetic trait that may be a marker for increased body weight (Tepper and Ullrich, 2003). This study assessed the stability of the PROP phenotype with age as well as the relationship between PROP phenotype and body weight in a genetically isolated population. Subjects were 321 inhabitants (179 women; 142 men) of the village of Campora in Southern Italy who were >15 years of age at the time of the study. PROP tasting was assessed with the paper disk method (Zhao *et al.*, 2003); height and weight were measured to calculate body mass index (BMI; kg/m^2). The prevalence of the non-taster phenotype was 28.6% in this population and did not vary with age. Among women, mean PROP ratings were highest at 31–50 years of age and declined modestly thereafter ($P < 0.05$). Mean PROP ratings were lower in men than in women, but did not vary with age in men. Until age 50 years, non-taster women had higher BMIs than taster women ($P < 0.01$). However, this difference disappeared in older age groups. Non-taster men had higher BMIs than taster men only after age 70 years ($P < 0.05$). These data suggest that PROP tasting characteristics vary with gender and to a lesser extent with age. Age may moderate the relationship between PROP phenotype and BMI, especially in women.

Poster: Taste Development

Neuronal death in the developing rat geniculate ganglion

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In contrast to most other neuronal systems, the pattern of developmental neuronal degeneration in the rat geniculate ganglion (gg) has not yet been defined. To determine this pattern we have examined sections through gg from embryonic day E13 to postnatal day P3 rats using standard H&E histology, TUNEL, BrdU incorporation and neuronal marker beta-neurotubulin III immunohistochemistry (IHC). Results show a rise in the percentage of neurons degenerating, from 3.6% at E15 to a peak of 9.5% at E17, followed by a fall to 1.7% at E18 and to 0.1–0.2% by E22 and later. TUNEL analysis at E16–18 shows that 95–98% of cells counted as degenerating cells were indeed apoptotic. Beta-neurotubulin III IHC at E13, E14 and E17 indicates that 93–98% of the degenerating cells were neurons. The percentage of degenerating cells showing BrdU incorporation 48 h after BrdU injection was 82.5% at E18 but 48.5% at E19, reflecting a concomitant decline in overall ganglionic labeling. Interestingly, the percentage of neurons degenerating was significantly higher at E13 (6.8%), when the gg is separating from the lateral cranial ganglionic mass, than at E14–16 (~4%). This higher E13 value may well reflect morphogenetic rather than target innervation-associated histogenetic cell death. Finally, the mean number of neurons counted showed a continual rise from E13 through E18 (from ~300 to 760) followed by a slight decline at E19 (to ~630) and then a final leveling off at 800–825, counted over every third section, by E20. Thus, in contrast to most other developing neural systems, gg neurons show only a small decrease rather than a major population crash during inital target contact.

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Poster: Taste Development

Dietary protein restriction produces attenuated salt responses in the chorda tympani nerve to sodium-specific stimuli in rats

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Protein malnourishment is a prevalent global dilemma that affects 25.6% of children worldwide. Under experimental conditions,

perinatal protein deprivation has resulted in multiple effects, including decreased body weight, decreased cortical thickness and malformation of the hypothalamic nuclei. In order to determine if dietary protein restriction affects gustatory nerve taste responses, multi-fiber neurophysiological recordings were made from the chorda tympani nerve in rats fed a protein-restricted or protein-replete diet (6% and 20%, respectively). The dietary regimes commenced on embryonic day 8 and continued until the time whole nerve recordings were performed (P35–50). Responses to concentration series of NaCl, Na acetate and KCl, and to 0.50 M sucrose and 0.03 M quinine–HCl, revealed attenuated responses (30–60%) to sodium-specific stimuli in rats fed the 6% protein diet compared with those fed the 20% protein diet. Responses to all other stimuli were similar between groups. These results are similar to the results previously seen in rats fed a sodium-restricted diet throughout development. These data suggest that prenatal malnutrition through sodium or protein restriction may contribute to the improper development of the gustatory system.

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Poster: Taste Development

Patterns of fungiform taste bud innervation in C57BL/6J and *bax* knockout mice

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In the rat and hamster, the number of geniculate ganglion cells innervating a taste bud is correlated with the size of the taste bud; the larger the taste bud the more geniculate ganglion cells that innervate it. The current experiment tested if the same pattern of innervation occurs in C57BL/6J mice and mice lacking the pro-apoptotic gene *bax*. Since mice lacking a functional *bax* gene have larger taste buds than wild type mice, we hypothesized the increase in taste bud size would result in increased innervation. Fungiform taste buds of C57BL/6J and $bax^{-/-}$ mice were injected with a fluorescent retrograde tracer DiI to identify innervating geniculate ganglion cells. The tracer was allowed to transport for 3 days before mice were sacrificed, the ganglion dissected and the tongue sectioned. Taste bud volumes were obtained and plotted against the number of innervating geniculate ganglion cells. Unlike rats and hamsters, mice did not show a correlation between taste bud size and number of labeled ganglion cells ($r = 0.081$). Furthermore, despite larger taste bud volumes in $bax^{-/-}$ mice, there was no difference between $bax^{-/-}$ and wild type mice in the mean number of ganglion cells innervating each taste bud ($P = 0.918$) or the total number of chorda tympani neurons innervating the anterior tongue ($P = 0.386$). These results suggest there is a fundamental difference in the relationship between chorda tympani innervation and taste bud structure in the mouse compared with other rodents.

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Poster: Taste Development

Developmental susceptibility of fungiform papillae following lingual nerve transection in rats

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The present study examined the developmental effects of lingual nerve transection on fungiform papillae morphology. Sprague–Dawley rats underwent unilateral lingual nerve transection at 10, 25 or 65 days of age. Care was taken to ensure the chorda tympani nerve was not damaged. Following 2, 8, 16 or 50 days post-transection, rats were sacrificed and tongues extracted. After at least 1 week post-fixation in 8% paraformaldehyde, ventral muscle layers were removed and the dorsal epithelium was stained using a 1% methylene blue solution. Fungiform papillae were visualized and counted using a combination of brightfield and phase contrast microscopy. Fungiform papillae were classified as 'Pore', 'No Pore' or filiform-like. Results show no significant reduction in the total number of fungiform papillae across groups following lingual nerve transection. However, a significant increase in the number of No Pore papillae occurred within both the 10 day and 25 day surgical age groups. Additionally, the number of filiform-like papillae increased soon after surgery in the 10 day and 25 day surgical groups. These results suggest that the lingual nerve is important for maintaining the structural integrity of fungiform papillae during development. Recovery of fungiform papillae morphology appears to occur by 50 days post-transection. Interestingly, previous studies from our laboratory (Sollars *et al.*, 2002) found that neonatal chorda tympani transection at 10 or 25 days of age results in a more pronounced and permanent disruption of fungiform papillae morphology.

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Poster: Taste Development

Developmental regulation of the geniculate ganglion by neurotrophins BDNF and NT-4

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Brain derived neurotrophic factor (BDNF) and neurotrophin-4 (NT-4) belong to the neurotrophin family of growth factors. Both of these neuronal peptides play a critical role in the development and maintenance of the gustatory neurons localized in the geniculate ganglion. Mice lacking BDNF ($BDNF^{-/-}$) and NT-4 ($NT\text{-}4^{-/-}$) show a significant loss of 50% of their geniculate ganglion neurons by birth compared with wild type animals. To determine when during development BDNF and NT-4 regulate geniculate neuron loss, we quantified geniculate ganglion cell loss on embryonic day 14.5 (E14.5), the age when geniculate neurons initially innervate fungiform papillae via the chorda tympani nerve. We counted geniculate neurons and total cells in $BDNF^{-/-}$, $NT\text{-}4^{-/-}$ and wild type embryos. Compared with wild type mice, $BDNF^{-/-}$ and $NT\text{-}4^{-/-}$ mice showed a significant loss, 27 and 33% respectively, of geniculate ganglion neurons. An equivalent number of non-neuronal cells were lost by E14.5. Although geniculate cell loss is already significant at E14.5, it has not reached the 50% loss that occurs by birth in $BDNF^{-/-}$ and $NT\text{-}4^{-/-}$ mice. These findings indicate that geniculate neuron loss in both $BDNF^{-/-}$ and $NT\text{-}4^{-/-}$ mice begins prior to target innervation (E14.5), but continues after E14.5 of development. These findings are consistent with the hypothesis that both BDNF and NT-4 function as target-derived neurotrophins. We plan to quantify geniculate ganglion neurons at

E12.5, E16.5 and E18.5 to determine when geniculate cell loss begins and when it is completed in BDNF$^{-/-}$ and NT-4$^{-/-}$ mice.

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Poster: Taste Development

Bone morphogenetic proteins regulate fungiform papilla development in embryonic rat tongue

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Bone morphogenetic proteins (BMPs) have important roles in development of patterned ectodermal specializations including tooth, hair and feather. During development of the rat tongue, BMPs alter from an early diffuse lingual distribution (embryonic day 13, E13) to distinct localization within fungiform placodes and papillae (E14–15). We hypothesized that BMPs play a role in papilla patterning and used an embryonic tongue culture system at E14 to study fungiform papillae development, by adding BMPs to culture medium or via blue gel beads soaked in BMP and placed in the tongue. During 2 days in culture, exogenous BMP 2, 4 or 7 decreased the number of fungiform papillae on the tongue in a concentration-dependent manner compared with standard medium conditions. When the BMP inhibitor noggin was added to culture medium or via beads, there was a 60% increase in fungiform papilla number. Noggin effects were concentration—dependent and confined to the anterior tongue. Based on Ki67 immunoreactions, cell proliferation was increased in lingual epithelium near noggin beads. Fungiform papillae that remained after BMP addition to cultures, or new papillae that formed after noggin addition, all contained the sonic hedgehog (Shh) protein papilla marker. Disruption of Shh signaling with cyclopamine did not prevent the BMP-dependent decrease in papilla number. We propose that BMPs act to inhibit new fungiform papilla development in inter-papilla spaces, contributing to the regulation of papilla pattern, and that inhibitory BMP actions within forming papilla are opposed by noggin.

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Poster: Taste Development

Roles for PI3K, MEK and P38 MAP kinase signaling in regulating the fungiform papilla response to epidermal growth factor during tongue development

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Epidermal growth factor (EGF) regulates patterning and number of fungiform papillae on the embryonic rat tongue. In cultures of embryonic day 14 (E14) rat tongues, there is a concentration-dependent decrease in fungiform papilla number and an increase in inter-papilla spacing with exogenous EGF. To understand intracellular signaling for these papilla effects, we investigated roles for phosphatidylinositol-3 kinase (PI3K) and mitogen-activated protein kinases (MEK and p38 MAPK) in regulating the functional responses to EGF. Specific inhibitors to PI3K (LY294002), MEK1 and 2 (U0126), or p38 MAPK (SB203580), across a range of concentrations (3–30 μM), were added to the medium for E14 rat tongue cultures, in a pre-incubation period and during subsequent exposure of cultures to EGF. Number and distribution of papillae were quantified after 48 h in culture. Addition of inhibitors alone to tongue cultures did not alter fungiform papilla numbers compared with controls. However, inhibition of PI3K activity with LY294002, and of p38 MAPK with SB203580, partially reversed the EGF-dependent decrease in fungiform papilla numbers in tongue cultures. Inhibition of MEK activity with U0126 completely reversed the decrease in numbers, and papillae were distributed as in control cultures. The data provide evidence that fungiform papilla effects in response to EGF during development are mediated by intracellular signaling cascades that include PI3K, p38 MAPK and MEK.

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Poster: Taste Development

Development of gustatory nerve terminal field volumes following neonatal chorda tympani nerve transection

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Taste buds within fungiform papillae are innervated by the chorda tympani nerve. A permanent reduction in taste bud and papillae structure occurs following neonatal chorda tympani transection (NeoCTX) in the rat, differing dramatically from the effects observed following chorda tympani transection in the adult rat. NeoCTX further results in a moderate reduction in total chorda tympani terminal field volume in the nucleus of the solitary tract. This study examined greater superficial petrosal nerve (GSP) and glossopharyngeal nerve (GL) terminal field volumes following NeoCTX. Total field volumes, dorsal, intermediate and ventral volumes, and regions of overlap were analyzed in adult rats that received NeoCTX at either five or ten days of age. The GSP was labeled with biotinylated dextran amine and the GL labeled with tetramethylrhodamine dextran. Dye was allowed to transport for 2 days, animals were perfused and brainstems sectioned. Tissue sections were processed with streptavidin Alexa 488 (Molecular Probes) and visualized using fluorescence. Field volumes were reconstructed using a computerized tracing program (Neurolucida). There is evidence of volume changes in the intermediate zone of the GSP when NeoCTX occurred at five days of age, however there were no significant differences in all other analyzed areas. Despite the profound effects seen in the periphery after NeoCTX at both 5 and 10 days of age, central processes appear to develop normally when NeoCTX occurs at 10 days of age.

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Poster: Taste Development

BMP4 and Sonic hedgehog coordinate the development of taste papillae

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The BMP and SHH signaling pathways interact in many developmental events throughout embryogenesis. In mice, both of these diffusible factors are expressed in early taste papillae. Functionally, inhibition of Shh signaling *in vitro* results in more taste papillae, which are larger and closer together, as judged, in part, by their expanded expression of BMP4 (Hall *et al.*, 2003; Mistretta *et al.*, 2003; Liu *et al*, 2004). As Shh manipulation alters BMP4 expression, we aimed to test the functional link between BMP and Shh in taste papilla patterning. Tongue explants were cultured for 3 days beginning on embryonic day 11.5 (E11.5) just prior to the formation of papillae, and exposed to either the BMP antagonist, Noggin, or to control medium. In noggin-treated tongues from transgenic mice with lacZ under control of the BDNF promoter, BDNF-lacZ expressing papillae were more numerous, larger, and appeared closer together, mirroring the effects reported by others when Shh signaling is blocked. We also examined the expression of Shh mRNA via *in situ* hybridization, and found that papillary expression of Shh in noggin-treated tongues was similarly expanded. As a next step in understanding the interplay between these two powerful signaling systems, we are testing the hypothesis that overexposure of tongue explants to BMP4 or Shh protein either blocks or reduces papillary development.

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Poster: Taste Development

BDNF is required for targeting of gustatory fibers during development

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Brain-derived neurotrophic factor (BDNF) is normally expressed in the fungiform papillae and is important for gustatory neuron survival during embryonic development. We found that ectopic overexpression of BDNF throughout the tongue epithelium misdirects chorda tympani fibers to innervate non-gustatory filiform papillae. This finding suggests that BDNF is an important targeting signal, allowing gustatory neurons to recognize and innervate fungiform papillae during development. To determine if BDNF is required for gustatory fibers to initially innervate fungiform papillae during embryonic development, we labeled chorda tympani innervation in BDNF null mutant mice ($BDNF^{-/-}$) at E14.5 and E16.5 with DiI. Wildtype mice have distinctly ordered arrays of innervated fungiform papillae and branching to non-gustatory papillae does not occur. Although many geniculate neurons are lost by E14.5 in $BDNF^{-/-}$ mice, numerous chorda tympani fibers still innervate the tongue. $BDNF^{-/-}$ mice have severely disrupted patterns of remaining chorda tympani innervation. Most chorda tympani fibers fail to reach fungiform papillae and there is excessive branching through the surface of the tongue in $BDNF^{-/-}$ mice. These chorda tympani fibers innervate many non-gustatory filiform papillae. This disruption of chorda tympani innervation continues to intensify through E16.5 of development. We conclude that BDNF is imperative for initial targeting of chorda tympani axons to fungiform papillae during development.

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Poster: Taste Development

Identification of molecular markers selectively expressed in taste basal cells

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Taste cells within the taste bud constantly regenerate themselves, indicating that progenitor and/or stem cells reside within or around the taste bud. By analogy to how intestinal stem cells and skin epithelial stem cells arise, it is most probable that the basal epithelium is the site for taste stem cell origin. Previous work by ourselves and other groups using the pulse chase technique suggest that mitotically active cells reside in the basal epithelium of the taste buds. This indicates that the basal epithelium may contain taste stem or progenitor cells. Although it is known that taste cells turnover in ~10–14 days, there is currently a paucity of molecular and biochemical information making it difficult to identify taste stem cells or progenitor cells. To gain an understanding of the microenvironment of taste stem cells it is critical to identify markers that are selectively expressed in the basal epithelium of the taste bud. We used Representational Difference Analysis (RDA) and gene chip analysis to compare mRNA from circumvallate (CV) papillae ('taste') versus surrounding non-sensory lingual epithelial tissue ('non-taste'). Most of the genes cloned were confirmed to be differentially expressed in taste versus non-taste tissues. *In situ* hybridization revealed that taste tissue-expressed genes contain not only taste cell specific markers but also molecules expressed in regions proximal to, but not within, the taste buds. We have identified several genes that are selectively expressed in basal cells surrounding taste buds. We are currently investigating the roles of these molecules in taste cell development.

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Poster: Taste Development

Expression of FOXA2 and gustducin in taste buds

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Foxa2 is a winged helix/forkhead transcription factor known to be expressed in a variety of endodermal cells. By comparing mRNA from circumvallate (CV) papillae ('taste') versus surrounding non-sensory lingual epithelial tissue ('non-taste') we identified Foxa2 gene transcripts within CV papillae. By immunohistochemistry we have determined that Foxa2 is coexpressed with PLCbeta2, but not with gustducin, in a particular subset of type II taste cells in CV papillae. Foxa2 was also identified in the gustducin negative cells in fungiform papillae and soft pallate. These results suggest that Foxa2 and gustducin may arise from different lineages during taste cell development. To test this hypothesis, we examined ES cells *in vitro* in cell culture. When ES cells were induced to differentiate into the endoderm lineage we observed increased levels of Foxa2

expression. In contrast, when ES cells differentiated into the ectoderm lineage, we observed gustducin expression along with ectodermal marker expression. We are examining the roles of Foxa2 and other transcription factors in the development of taste cell lineages.

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Poster: Taste Development

SEMA3F expression and repellent effects in the developing gustatory system

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We previously demonstrated that Sema3A influences lingual sensory nerve pathfinding at least until target penetration. Less is known about the role of Sema3F in this pathfinding. We found that Sema3F is expressed in rat dorsal lingual and palatal epithelium from embryonic day 15 (E15) to E18, during target contact and penetration. At E15, Sema3F was not observed in fungiform papilla epithelium, but it was detected in E18 fungiform papillae. Surprisingly, Sema3F mRNA is also robustly expressed in the geniculate and trigeminal ganglia from E15 to E18. To determine if Sema3F repels sensory axons at these and earlier stages, we co-cultured the ganglia and COS7 cells expressing Sema3F or GFP in collagen gels. Sema3F repelled geniculate neurites at least through E16 and trigeminal neurites until E14. The severity of the effects of Sema3F on geniculate axons is influenced by developmental stage and the neurotrophic factor used to stimulate outgrowth. Sema3F robustly repels or eliminates neurites at E12–13, and the repellent influence decreases progressively thereafter. NT-4-stimulated geniculate outgrowth is more dramatically inhibited than BDNF-stimulated outgrowth. Given that Sema3F does not repel late stage geniculate outgrowth and that Sema3F is expressed in the dorsal epithelium and ganglia during target penetration, Sema3F is unlikely to act as a repellent for sensory axons at late stages *in vivo*. Experiments are underway to determine if papillae innervation is altered in Sema3F$^{-/-}$ mice and if exogenous Sema3F perturbs sensory axon pathfinding *in situ*.

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Poster: Taste Development

T1R3 expression across taste fields during postnatal mouse development

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T1R3 is a G-protein coupled receptor involved in the recognition and response to sweeteners and amino acids. In rodents, the anterior, posterior and palatal taste fields respond to sweeteners to different extents. We asked whether the taste receptors that initiate these taste responses are expressed at differing levels across taste fields in the mouse. Using real-time RT-PCR, we examined the expression pattern of T1R3 mRNA in circumvallate, foliate, palate and fungiform taste buds at several developmental stages (2, 4, 6, 10 and 20 weeks of age). The expression level of T1R3 was normalized to keratin 20, a gene expressed by differentiated taste cells. In the adult mouse, the highest level of T1R3 mRNA was detected in fungiform and palatal taste buds, whereas levels in circumvallate and foliate taste buds were 3- to 6-fold lower. Within each taste field, across the postnatal developmental stages tested, we observed that the level of T1R3 mRNA was constant relative to the level of keratin 20 mRNA. That is, T1R3 expression appeared to increase in proportion to the general increase in numbers of differentiated taste receptor cells during development. In summary, these results indicate that the expression levels of T1R3 vary among the taste fields (fungiform, palatal, etc.). Furthermore, the data suggest that T1R3 expression normalized to keratin 20 expression in each field reaches adult levels by 2 weeks of age.

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Poster: Taste Development

Postnatal development of gustatory nerve terminal fields in control rats

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Previous work done by Lasiter examined the development of the facial nerve and the glossopharyngeal (IX) nerve terminal fields in the nucleus of the solitary tract (NTS). These experiments revealed that the facial nerve terminal field (i.e. combined chorda tympani (CT) and greater superficial petrosal (GSP) nerve terminal fields) develops from postnatal day 1 (P1) until ~P25 and that the glossopharyngeal nerve terminal field develops from ~P10 until adulthood (~ P45) in the NTS of the rat. To determine the development of the CT and GSP nerves independently, as well as the interactions among the three primary gustatory afferent nerve terminal fields, we used an anterograde triple fluorescent labeling technique on all three nerves and examined the terminal fields in the NTS in rats aged 25 days, 35 days and >40 days (adults). Preliminary results indicate that chorda tympani terminal field volumes dramatically decreases between P25 and P35, with the overlap between CT terminal field and the other terminal fields being larger at 25 than at 35 days of age. The GSP and IX terminal field volumes of 25-day-old rats look similar to adults, as do all three terminal field volumes in 35-day-old rats. These results indicate that the CT field becomes significantly smaller between P25 and P35 and that the CT field continues to develop after the field is reportedly mature.

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Poster: Taste Development

Eph/ephrin expression in the developing gustatory system

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We are studying the divergence of two nerves that emerge from the geniculate ganglion, the greater superficial petrosal nerve (GSP) and the posterior auricular/chorda tympani nerve (PA/CT).

In Sema3A$^{-/-}$ mice, divergence is only partly perturbed, suggesting that other cues contribute to the divergence. Cell-contact-dependent Eph/ephrin signaling guides axons during pathfinding throughout the nervous system, but it is not known if this signaling guides sensory axons in the gustatory system. To profile Eph/ephrin expression, we are conducting antisense RNA amplification on microdissected geniculate neurons (10–30/nerve) and antibody staining on cryosections and wholemounts from E10–11 mice. We compared mRNA expression frequencies using Fisher's exact test ($P < 0.05$ for significance) and have so far found four differentially expressed Eph receptors: A4 and B3 were expressed in significantly more GSP than PA/CT neurons; the converse for B2 and B4. Two receptors were expressed at nearly equal frequencies in the GSP and PA/CT: EphA8 (~45%/nerve) and EphB6 (5%/nerve). Using antibody staining, we detected EphrinB2 in the cerebral vasculature in E10–11 wholemount labeled embryos, but staining within and near the ganglion was not detected. We have begun characterizing GSP and PA/CT pathfinding in Eph/ephrin mutant mice (provided by Dr M. Henkemeyer). At E10.5, we found no pathfinding defects in EphB2$^{-/-}$ mice or in double mutants (EphB3$^{-/-}$ mice expressing truncated EphB2), perhaps owing to redundancy. We will try to perturb Eph/ephrin signaling *in situ* by implanting beads in cultured intact embryos.

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Poster: Taste Development

A comparative analysis of the ultrastructural morphology of the three gustatory nerve axons in the nucleus of the solitary tract in developmentally sodium-restricted and control rats

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Dietary sodium restriction during a critical period in gestation results in an ~2-fold increase in total volume of both chorda tympani (CT) and glossopharyngeal (IX) nerve terminal fields in the adult rat nucleus of the solitary tract but does not affect the volume of greater superficial petrosal (GSP) terminal field. To examine this plasticity, we previously characterized the ultrastructural morphologies of CT and GSP axons, and now include the analysis of IX axons. In order to determine whether the expansion of area occupied by each input is due to the addition of new arbors or to the reorganization of NTS neuropil, we quantified the volumetric density of labeled synapses and the synapsing frequency of axons using electron microscope morphometry. While there is a >3-fold increase in the density of synapses with CT terminals in sodium-restricted rats compared with controls, there is not a difference in how frequently synapses occur along the axons, suggesting that sodium restriction leads to the addition of new axon arbors bearing synaptic terminals. In contrast, for both GSP and IX terminals there are no diet-related differences in the density of synapses, while a significant 2-fold decrease in synapsing frequency occurs. These findings suggest that the arborization of GSP and IX axons after sodium restriction is secondary to the changes that occur in CT inputs to this region, and that the sodium restriction during the critical period may primarily affect CT input to NTS.

Supported by NIH grants DC00407 and DC06332.

Poster: Taste Development

Characterization of a long-term primary taste cell culture

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While much is known about molecular and biological basis of taste, comparatively less is understood about factors governing proliferation and differentiation of taste receptor cells. Our objective was to develop a protocol to maintain taste cells in culture for >2 weeks and to establish a longer-term culture in which new taste cells would be generated from basal cells in the initial culture. In this study, we report an *in vitro* culture method to maintain and generate rat taste cells in primary culture with good viability, physiological function and expression of taste cell specific markers. This optimized culture system maintains primary taste cells obtained from rat tongue foliate and vallate papilla and supports the *de novo* generation of new taste cells for at least two months. Gustducin and phospholipase Cβ2 (PLCβ2) expression was shown by immunocytochemistry and Western blot. PCR analysis indicated that mRNA for gustducin, PLCβ2 and taste cell receptors (T1R3, T2R5) was present in cultured taste cells. Labeling cultured cells with bromodeoxyuridine to identify cells that divided in culture concurrently with taste cell markers indicated that taste cells both proliferated and differentiated *in vitro*. Functional studies using the ratiometric calcium indicator Fura-2 showed that a subset of cells responded to taste stimuli with increases in intracellular calcium. This system will enable studies of processes involved in proliferation, differentiation and stimulus responses of mammalian taste receptor cells in an *in vitro* preparation.

Poster: Feeding & Drinking

Calcium intake and weight

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Current findings with human subjects in short-term studies (less than 3 weeks) have demonstrated that calcium enriched diets lead to decreases in body weight. However, few studies have followed the effects of calcium over a longer period of time. The following study examines the microstructural intake of calcium enriched diets (1.2%) and weight gain/loss in rats over a 6 week period. A special calcium enriched diet was fed to eight rats and a standard laboratory chow was fed to eight control rats throughout the study. Weights for all rats were recorded each week and the total amount of food consumed each day was also noted. Results reveal that the calcium diet did lead to weight loss over the first 3 weeks compared with the control diet. However, over the next 3 weeks the calcium group gained weight faster than the control rats and by the end of the study all 16 rats were of similar weight. Furthermore, changes in the microstructural patterns of food intake were observed with the calcium diet initially leading to lower total intake, but progressively increasing each week. Thus, the effects of a calcium enriched diet may be due to changes in taste perception and provide only short-term effects on weight.

This research was funded by Millsaps College and the Hearin Foundation.

Poster: Feeding & Drinking

Behavioral effects of caffeinated cola consumption on first graders

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Introduction: use of caffeinated cola by children is ubiquitous in our society. The potential psychological effects of this include DSM-IV caffeine-induced anxiety and sleeping disorders, and withdrawal symptoms. Manufacturers continue to add caffeine to cola and target young children for marketing despite that the effects of caffeinated cola in this age group have not been explored. Methods: in a double blinded fashion, 20 first graders (10 of each gender) were presented with caffeine-free cola and caffeinated cola for *ad libitum* consumption in three h epochs sequentially over 2 weeks. Average consumption of caffeine free cola and of caffeinated cola was 7.55 and 9.45 oz, respectively. After completion of each session, teachers rated each student with a six question modified Connors test. Results: the modified Connors score was an average of 5.45 points higher for caffeine than for caffeine-free cola ($P = 0.0017$). In response to caffeine intake, 60% (12) of the students' scores increased compared with 12% (3) which decreased ($P = 0.0079$). Even after adjusting for number of ounces, there was still a significant increase in the Connor score comparing caffeine to caffeine-free soda ($P = 0.0151$). Conclusion: first graders manifested behavioral problems when presented with caffeinated cola, suggesting that consumption of this should be minimized in this age group.

Poster: Feeding & Drinking

Altered taste sensitivity in obese, prediabetic OLETF rats

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OLETF rats lack the CCK-1 receptor, are hyperphagic, progressively become obese, and develop type-2 diabetes. To assess taste functions in this strain, we used an automated lickometer (Davis rig) with 10 s access to six concentrations of each of 12 sapid stimuli—one stimulus per day. Tests were repeated at 10 and 18 weeks of age (six OLETF, six control LETO), representing non-diabetic and pre-diabetic stages, respectively, verified by oral glucose tolerance tests. The OLETF rats showed higher preference for sucrose compared with controls at both ages and, at 18 weeks, this difference was accentuated for higher concentrations. For prediabetic OLETFs, this exaggerated intake also occurred for saccharin, alanine, and fructose, but not for Polycose. Similarly, OLETFs preferred MSG more at the lower concentrations compared with LETO controls, an effect that age also accentuated. In contrast, a slightly reduced preference for higher concentrations of NaCl in younger OLETFs was noted. There were no statistical strain or age differences in responses to other salts ($MgCl_2$, $CaCl_2$), citric acid and quinine–HCl. Trigeminal stimulation with capsaicin revealed no major impairment in OLETFs. When deprived, OLETFs showed an increased intake of water tested alone or along with normally aversive moieties. They did not differ from the LETOs when water was presented with normally preferred stimuli. These differences in taste sensitivity may contribute to hyperphagia and subsequent development of obesity and type-2 diabetes in this strain.

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Poster: Feeding & Drinking

Motivational substitutions in food and drug addiction

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Although there is strong evidence for common brain mechanisms for all types of cravings, it is unclear how abstinence from cocaine affects responses to natural rewards such as food. We predicted that in abstinent cocaine abusers, food cravings would be stronger and more common than in controls. In Study 1, a food craving questionnaire (see Pelchat, 1997) was administered to 21 male abstinent cocaine addicts and 33 healthy males. Twenty-one out of 22 addicts (95%) reported food cravings in the past year whereas only 22 out of 33 controls (67%) reported cravings (Fisher's exact test, $P = 0.0175$). So, abstinent cocaine addicts expressed more food cravings. In Study 2, an fMRI study of food cravings in abstinent cocaine addicts (for the method, see Pelchat *et al.*, 2004), the pattern of patient activation was similar to that in healthy pilot subjects. As previously, activation accompanying food craving was seen in the fusiform gyrus, the amygdala and parahippocampal gyrus, thalamus, caudate nucleus and middle frontal gyrus. In contrast, patients showed bilateral rather than left hemispheric activation in most regions. Patients also produced activation in several additional regions. Thus, there may be a greater spatial extent of craving-related fMRI activation in patients and this is also consistent with the notion of greater food craving in patients. These results bolster the argument that drug addiction may alter the response natural rewards, although, alternatively, the increased frequency of food cravings in the addicted sample may be the result of pre-existing (perhaps genetically based) individual differences in perception or motivated behavior.

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Poster: Feeding & Drinking

Effect of taste and palatability on lingual pressure during swallowing

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Objectives: because lingual pressure and high citric acid may be associated with swallowing safety in neurogenic dysphagia, the effect

of taste and palatability on lingual pressure was examined in 10 healthy young adults. Methods: participants swallowed 10 ml samples (all % w/v) of sucrose (10, 30), citric acid (0.15, 2.7), sodium chloride (0.5, 2.7) caffeine (0.15, 0.30), barium sulfate (40), barium sulfate-citric acid (40 $BaSO_4$, 2.7 acid) and deionized water. Barium sulfate is commonly used in swallow tests. Lingual pressure was measured using a spineless three bulb lingual array secured in an A–P position along the hard palate. The 9-point hedonic scale measured palatability. A mixed model with Tukey *post hoc* analysis was performed. Results: significant main effects were observed with taste ($P = 0.0002$) and pressure sites ($P = 0.001$). Both barium samples, 10% sucrose, and the higher levels of citric acid and salt evoked significantly higher lingual pressures than water. Pressure was higher in the anterior bulb site compared with the middle ($P = 0.003$) and posterior ($P = 0.046$) sites. There was no significant main effect of palatability on pressure ($P = 0.886$). Conclusions: in this small pilot study, several tastants appear to increase lingual swallowing pressure. Because barium sulfate is more viscous than water and viscosity increases pressure, this result was not surprising. However, the mechanism(s) responsible for the increased pressure in response to moderate sucrose and higher levels of citric acid and salt are unknown. Palatability does not appear to play a role in lingual swallowing pressure.

Poster: Feeding & Drinking

Effects of oral movements on perceived food flavors

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Food that is processed in the mouth is subject to mechanical (by oral movements) and enzymatic (by saliva) degradation to make the food suitable to swallow. During this process, the degrading food elicits texture and flavor sensations which are important for food preference. Flavor release from foods is typically measured instrumentally using in-vitro static head-space methods, although in-vivo dynamical methods are used occasionally (MS-Nose). To verify effects of oral movements on texture and flavor sensations, we defined a set of five specific oral manipulations and investigated their effects on the perception of low and high fat versions of two semi-solid foodstuffs, vanilla custard desserts and mayonnaise's. Behavior modifications ranged from simply placing the stimulus on the tip of the tongue to vigorously moving it around in the mouth. Most attributes showed a similar pattern, with the lowest attribute ratings where the tongue movement was restricted and gradually increasing ratings with increasing complexity of the tongue movements. An individual's normal mastication behavior typically resulted in the most intense sensations of flavor and mouth-feel. Flavor intensities grew by as much as 100%. For low fat custards, these flavors were dominated by off-flavors, presumably stemming from the non-fat ingredients. For high-fat custards, these flavors were dominated by positive flavors stemming from the fat. These results indicate that oral movements need to be considered for realistic instrumental flavor release measurements. The effects of oral movements on mouth-feel sensations were reported elsewhere (de Wijk *et al.*, 2003, *Appetite*, 40:1–7).

Poster: Feeding & Drinking

Induction of kallikreins in rat submandibular saliva by sweet taste inhibitors contained gymnema diet

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Gymnema sylvestre (gymnema), a tropical plant, contains two different compounds, gymnemic acid and gurmarin. that specifically inhibit sweet taste in humans, and neural and behavioral sweet responses in rodents, respectively. Our previous studies demonstrated that when rats fed gymnema-containing diet, rat kallikrein 2 (rK2) and 9 (rK9) increased in the submandibular saliva, and the proteins purified from saliva inhibited immunoreaction between gurmarin and antigurmarin antiserum and reduce its sweet suppressing effect in rats. The kallikrein induction was abolished by denervation of the glossopharyngeal nerve, indicating importance of neural inputs from the oral cavity on the induction. In the present study, we further examined mechanisms of the induction by using rats injected with 5 mg/kg/day phenylephrine (α-adrenergic agonist) for 5 days and rats given 10 μg/ml pure gurmarin or 0.3 mg/ml gymnemic acid solution for 6 days. The results indicated that electrophoretic pattern of the submandibular saliva in rats fed gymnema diet was similar to that in rats undergoing phenylephrine treatment. Ingestion of either gurmarin or gymnemic acid increased rK2 and rK9 in the rat submandibular saliva. These results suggest that chemosensory information for gurmarin and gymnemic acid contained in the gymnema diet activates α-adrenoceptors in the submandibular gland and caused the secretion of rK2 and rK9.

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Poster: Feeding & Drinking

Effects of sham intoxication: impact on mood, pain perception and threshold, level of aggression, and physiology

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Past research has found that perceived intoxication significantly undermines psychomotor skills. The present study was designed to assess the effects of perceived intoxication on pain tolerance, mood, and workload. Thirty-one participants completed two within-factor testing sessions. In the control session, participants completed questionnaires assessing aggression, personality and beverage preferences. In the experimental condition, participants consumed 48 ounces of non-alcoholic beer, while experimenters intermittently recorded their physiological measurements (heart rate, oxygen saturation and blood pressure). In both conditions, participants made ratings of mood and workload, and completed a cold pressor pain task. Two-way ANOVAs revealed that participants' pain ratings in the experimental condition were significantly lower than those in the control condition. Participants in the experimental condition also indicated a greater pain tolerance time. Regarding workload, participants reported significantly less physical demand and significantly greater self-evaluated performance in the

experimental condition. When examining the physiological changes over time with one-way ANOVAs, there was a significant decrease in pulse and a significant increase in oxygen saturation, systolic blood pressure and diastolic blood pressure from pre-intoxication to post-intoxication. Implications of the present study are particularly salient in regards to minimizing pain through professional suggestibility and sham consumption.

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Poster: Feeding & Drinking

Sex differences in adult obesity risk associated with childhood tobacco exposure

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Maternal smoking during pregnancy promotes childhood obesity, but its impact on adult body mass (BMI) remains unclear. Also, potential effects of early tobacco exposure on BMI have largely been overlooked, even though chronic exposure to secondhand smoke confers extensive health risk. Early tobacco exposure promotes childhood ear infections, which alter oral sensation by damaging the chorda tympani; for some supertasters of PROP (6-*n*-propylthiouracil), such changes may encourage fat intake and adult-onset obesity. We have shown previously that adult men raised among 2+ smokers before age 10 have elevated BMIs compared with men raised among fewer smokers. Here, we explore this finding in greater detail. Adult participants in a smoking cessation program ($n = 279$) provided height, weight, and family smoking history. As expected, postnatal exposure to 2+ household smokers was associated with increased BMI in men; further analysis showed this effect to be wholly independent of maternal smoking during pregnancy. Of particular interest, elevated BMIs were observed in adult men and women reporting specific postnatal exposure to smoking by both parents. Thus, both men and women show an association between early tobacco exposure and long-term BMI gain; men seem to be affected by a threshold of household secondhand smoke (i.e. 2+ smokers), while women appear to be sensitive to smoking by specific individuals (i.e. both parents). We believe that postnatal tobacco exposure extends obesity risk into adulthood by supporting pathologic changes in oral sensation.

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Poster: Feeding & Drinking

Ingestion of non-caloric palatable food mash did not induce c-fos expression in the rat PVN and NTS

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Oropharyngeal–esophageal and gastric cues contribute to meal-induced neuronal activation, referred by c-fos expression, in the paraventricular nucleus (PVN) and the nucleus tractus of solitarius (NTS). This study was conducted to determine if ingestion of tasty but non-caloric meal induces c-fos expression in brain regions. Male Sprague–Dawley rats (300–350 g) underwent 48 h food deprivation, and received *ad libitum* access to standard rodent chow or non-caloric palatable food mash (2.5 parts by weight alpha-cellulose, 1.0 part mineral oil, and 10.0 parts of a deionized water solution containing 0.1% sodium-saccharin and 0.2% artificial vanilla extract) for 1 h. Rats were overdosed with pentobarbital, and transcardially perfused with 4% paraformaldehyde. Free fed and 48 h deprived rats were included as control groups. Forty micron brain sections from the rostrocaudal extent of PVN and NTS were processed for c-fos immunohistochemistry. Weights of food consumed during 1 h of refeeding period were 5.38 ± 0.83 g in chow group and 7.33 ± 1.30 g in non-caloric group, respectively. The numbers of c-fos-ir nuclei were decreased by 48 h food deprivation significantly in the intermediate NTS, and without statistical significance in PVN and the caudal NTS. One hour of chow refeeding markedly increased c-fos-ir nuclei in all three regions examined, compared with either free fed or deprived control. The number of c-fos-ir nuclei in the PVN and NTS of non-caloric refeeding group did not differ from fed or deprived controls. These results suggest that ingestion of non-caloric meal may not produce effective cues to induced neuronal activation in the PVN and NTS.

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Poster: Feeding & Drinking

Brain mechanisms of hedonic value of taste and ingestive behavior

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The palatability of taste influences the preference of food and fluids, and the level of consumption. Highly rewarding tastes can lead to considerable overconsumption. To elucidate the brain mechanisms of palatability-induced consumption, the functions of the mesolimbic reward system and orexigenic neuropeptides were studied with different experimental approaches in rats. Lesions of the ventral tegmenal area (VTA), the origin of the mesolimbic dopamine system, selectively reduced the consumption of a normally preferred taste fluid. Single neuron activities of the VTA increased in more than half of the neurons immediately before drinking. Microinjections of bicuculline, a GABA receptor antagonist, into the ventral pallidum increased intake of saccharin but not water and quinine solution. These results suggest that dopaminergic and GABAergic modulations in the reward system play important roles in enhancement of the consumption. Next, we studied the roles of hypothalamic neuropeptides, Orexin, melanin-concentrating hormone (MCH), neuropeptide Y (NPY), Ghrelin and agouti-related protein (AgRP) in palatability-induced ingestion of saccharin. Administrations of Orexin, MCH and NPY among the five peptides increased saccharin intake. When the hypothalamic mRNA levels were measured, drinking of saccharin elevated Orexin and NPY but not MCH mRNA levels. The present study suggests that

overconsumpton of sweet palatable food may be attributed to the activities of the reward system and enhancements of orexigenic neuropeptides such as Orexin and NPY.

Poster: Feeding & Drinking

Mouse strains show differences in intake of alkalines

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Strain differences in intake behavior for sweet- and bitter-tasting stimuli have promoted the discovery of genes that underlie transduction of these stimuli. In the current project, we examined strain differences for stimuli characterized by humans as tasting sour (acids) or alkaline (bases), about which much less is known. We did not measure robust or reproducible variation in sensitivity towards acid stimuli, suggesting a common mechanism may exist for this class of stimulus in these strains. However, dramatic strains differences were seen for $CaOH_2$ and NaOH, in which some strains avoided (SWR/J and C57BL/6J) and some preferred (C3HeB/FeJ and BALB/cByJ) these compounds. The SWR/J versus C3HeB/FeJ intake difference to 3 mM $CaOH_2$ was static over an extended test period, and C3×SW F1 mice displayed an intermediate phenotype to 3 and 10 mM $CaOH_2$. Interestingly, these strains are also disparate for $CaCl_2$, suggesting influence of the cation as well as anion. However, conditioned taste aversions to $CaOH_2$ generalized to NaOH but not $CaCl_2$, indicating that alkalines may share a distinctive and distinguishing taste. Alkaline taste is not typically considered to be a separate primary taste modality; the behavioral strain differences could in fact result from variation in either a taste- or trigeminal-based sensory mechanism.

Poster: Feeding & Drinking

Rats form taste–nausea associations in 8 min: new methods to explore the temporal and qualitative dynamics of conditioned taste aversion processing

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LiCl ingestion markedly affects several measures of licking microstructure and causes a suppression of licking during a 15 min access period (Baird *et al.*, 2004, AChemS). This suppression could be due to the formation of a taste–nausea association or to malaise. To evaluate these possibilities, thirsty rats were offered 0.12M LiCl for 8 min (T1) followed by 8 min access (T2) to equimolar LiCl, NaCl, sucrose, or dH_2O. LiCl uniformly suppressed lick rates in T1. In T2, lick rate curves diverged depending on the tastant offered. Rats drinking NaCl or LiCl greatly reduced lick rates in the first minute, which then rapidly declined in parallel to negligible values within 5 min. In contrast, rats offered water or sucrose in T2 exhibited a reinvigoration of ingestion rate; initial lick rates more than doubled that for T2 LiCl or NaCl and remained significantly different from LiCl and NaCl for the remainder of T2. Analysis of microstructural changes for T2 NaCl after T1 LiCl generally replicated our previous observation that NaCl licking patterns were shifted to resemble the microstructural pattern of drinking for aversive QHCl. These results reveal that associative links between taste and nausea are made very rapidly, within at least 8 min, which to our knowledge is the shortest interval reported to date. This rapid generalization test paradigm can be used in future studies to better isolate the functional underpinnings of pharmacological treatments and brain nuclei that contribute to conditioned taste aversion processing.

Poster: Feeding & Drinking

Selective breeding of mouse lines divergent in sweetener consumption

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Inbred mouse strains differ in their ingestive 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 several other genetic loci are also involved. The goals of this study were to confirm the existence of such loci and to create a model for their genetic and physiological analyses. To achieve this, we began selective breeding of mouse lines divergent in sweetener consumption. To eliminate effect of the *Sac*/*Tas1r3* locus, we crossed B6 inbred mice with 129.B6-*Sac* congenic mice; thus, all mice in this cross had only B6 *Sac*/*Tas1r3* allele. Despite genetic identity at the *Sac*/*Tas1r3* locus, mice from the F2 generation varied widely in consumption of 20 mM saccharin and 30 mM glycine; there was no correlation between these two traits. We therefore began selection of separate lines with high and low saccharin intakes, and high and low glycine preferences. Beginning from the F2 generation, mice with highest and lowest scores were mated with phenotypically similar animals. Response to selection (i.e. significant difference between the High and Low lines) was observed in the first (S1) or second (S2) generations of selection for saccharin and glycine respectively. The strains continued to gradually diverge with each generation of selective breeding, up to current S4. These results demonstrate that genes other than *Sac*/*Tas1r3* affect consumption of these two sweet-tasting compounds. We plan to genotype these mice to detect regions of genome differentially retained in the divergent lines, and to conduct experiments elucidating mechanisms that underlie the line differences.

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Poster: Trigeminal: Cellular

Solitary chemosensory cell turnover in the nasal epithelium

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Nasal trigeminal chemosensitivity in mice and rats is mediated in part by solitary chemoreceptor cells (SCCs) in the nasal epithelium (Finger *et al.*, 2003, *Proc. Natl Acad. Sci. USA*). SCCs express the

G-protein α-gustducin as well as other elements of the bitter taste signaling cascade such as PLCβ2 and T2R bitter taste receptors. While some populations of sensory cells are known to be replaced throughout life (taste and olfaction), others are not (hair cells in the mammalian ear). These experiments were designed to test the hypothesis that the population of nasal SCCs does undergo turnover. Adult wild type C57/B6 mice were injected with the thymidine analog 5-bromo-2′-deoxyuridine (BrdU) to label dividing cells. At various times after injection (1–12 days), the mice were perfused with 4% paraformaldehyde. Immunocytochemistry was used to identify dividing cells with a biotinylated mouse anti-BrdU antibody, SCCs with rabbit anti-gustducin, and all cell nuclei were labeled with propidium iodide. Double labeling with gustducin and BrdU was seen as early as 3 days post-BrdU and remained for as long as 12 days post-injection indicating that SCCs do undergo turnover like the rest of the nasal epithelium.

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Poster: Trigeminal: Cellular

Investigation of chemosensory properties of trigeminal neurons using adenovirus based expression of a PIP2 signalling marker

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Facial sensory innervation is provided by the trigeminal (V cranial) nerve, which comprises neurons that transduce mechanical, thermal and chemical stimuli probably including odorant molecules. The signal transduction mechanism in sensory nerve endings remains unclear. We were interested in the role of phosphoinositol (PI)-signalling in trigeminal chemosensation, and focused on phospholipase C (PLC) and PI_3 kinase pathways. We used a pleckstrin homology domain of phosphplipase C(delta)1 fused to GFP (referred to as PH-GFP) as a PIP2 signalling marker and tested its properties in transiently transfected HEK cells expressing various chemosensitive TRP ion channels. In a second approach, an adenoviral vectorsystem is used to deliver PH-GFP to primary cultured trigeminal neurons. Live confocal laser scanning imaging of transfected HEK cells and infected neurons is performed to observe the subcellular distribution and stimulus induced translocation of the fluorescent PIP2 binding marker. The application of TRP receptor agonists lead to the stimulation of neurons and results in a decrease of the PH-GFP signal at the plasma membrane indicating a decrease in membrane PIP2 and translocation of PH-GFP to the cytoplasm. Further experiments will investigate the involvement of phospholipase C and PI3-kinase in this second messenger pathway. Our data indicate that chemical substances known to activate chemosensory trigeminal neurons (trigeminal odorants) could use and modulate PI-signalling pathways in chemosensory signal transduction.

Poster: Trigeminal: Cellular

Calcium-dependence of anktm1 function

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ANKTM1 is a member of the transient receptor potential (TRP) ion channel family and was initially reported to sense noxious cold temperatures. It is found in a subset of nociceptive sensory neurons of the dorsal root and trigeminal ganglia. We studied the Ca^{2+}-dependent modulation of ANKTM1 transiently expressed in HEK cells. Application of mustard oil (Allyl isothiocyanate, 25 μM) activated transient currents in presence and absence of extracellular Ca^{2+}. Mustard oil-induced currents showed rapid desensitization in presence of extracellular Ca^{2+}, but hardly desensitized in Ca^{2+}-free solution. During repeated application of agonist the current amplitudes declined in Ca^{2+}-containing solution. However, in the absence of extracellular Ca^{2+}, the inducible currents increased until maximal amplitude was reached and then declined again. Interestingly, we found that human ANKTM1 could be activated by intra- and extracellular Ca^{2+}. Increasing the intracellular Ca^{2+} concentration to 5 mM $CaCl_2$ via the patch pipette leads to a fast but diminished activation of ANKTM1 shortly after obtaining the whole-cell configuration. In addition, extracellular Ca^{2+} induced robust ANKTM1-mediated currents as well: $CaCl_2$ in concentrations as low as 2 mM were sufficient to activate ANKTM1. The elicited currents were blocked by the unspecific TRP channel inhibitor Ruthenium Red. Increasing the extracellular Ca^{2+} concentration up to 10 mM leads to a faster activation of the channel. This activation is delayed upon chelating the intracellular Ca^{2+} with 5 mM BAPTA. Our findings show that ANKTM1 function is strongly dependent on intra- and extracellular Ca^{2+} concentration. Experiments will be conducted to clarify the mechanism underlying the Ca^{2+} dependency of the ANKTM1 channel.

Poster: Trigeminal: Cellular

Chemosensory properties of trigeminal neurons innervating the nasal cavity of mice

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Trigeminal chemosensation of odorants is mediated by trigeminal neurons (TGs) that innervate the mucouse membranes of the nasal cavity. Several types of receptor proteins and their composition define the chemosensory spectrum of single neurons, thus providing a basis for perception and discrimination of trigeminal stimuli. Tracing with marker protein expressing variants of pseudorabies virus was performed to identify nasal TGs of mice in a cell culture system. Calcium imaging measurements and patch-clamp recordings were done in combination with trigeminal agonists to analyse the receptor profile of traced nasal cells. Their sensitivity for capsaicine, menthol, GABA and ATP was compared with TGs that were traced from the orbital facial skin. This double tracing approach revealed two neuronal populations varying in cell sizes and distribution of purinergic receptors. Cutaneous tracing identified large neurons that predominantly carried P2X3 receptors whereas P2X2 currents dominated nasal neurons that were of smaller size and furthermore were negative for IB4. In contrast GABA and capsaicine sensitivity was the same for both groups. However, both populations exhibited mean cell sizes that were

significantly larger than the average size of TGs pointing to a role beyond pain sensation. In conclusion we identified a population of trigeminal neurons that might account for a certain role of nasal somatosensation and suggests relevance for purinergic receptors in trigeminal chemoperception.

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Poster: Trigeminal: Cellular

Electrophysiological characterization of nasal trigeminal neurons identified by viral tracing

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The trigeminal nerve is the major mediator of sensations from the mammalian head and comprises neurons that transduce mechanical, thermal and chemical stimuli. Thereby single neurons mediate sensory input from selective areas of the head. Physiological features of peripheral neurons depending on their function and area of innervation remain largely unclear. Viral tracing using a neurotropic alpha-herpesvirus, a recombinant Bartha strain of the pseudorabies virus (PrV), was performed to identify trigeminal neurons mediating information from the murine nasal cavity. Twenty-four hours after intranasal application of GFP expressing PrV, green fluorescent ganglion neurons could be identified in cryosections of the gasserian ganglion. Simultanous subcutaneous injection of a RFP-expressing PrV variant allowed identification of red fluorescent cells innervating the facial skin and revealed an exclusive separation of both subpopulations within the ganglion. Both populations could be identified after dissociation and plating and allowed activity measurements of identified neurons in primary cell culture. Relevant cells were characterized electrophysiologically and compared with *in vitro* infected and not infected control neurons. Electrophysiological properties of traced neurons were not altered by PrV infection compared with control neurons: resting membrane potential, amplitudes and kinetics of voltage-activated sodium-, as well as Ih-currents were not affected. These data indicate the benefit of PrV (Bartha) for life-cell tracing studies. Intrinsic properties of identified subpopulations of cells concerning specific receptor settings and the distribution of TTX-R and TTX-S sodium currents will be investigated in future research.

Poster: Trigeminal Neurophysiology

Trigeminal thresholds correlate to the width of the nasal cavity

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Aim: the investigation of correlation between width of the nasal cavity and trigeminal function. Material and methods: a total of 33 normosmic subjects participated, all of whom were between 18 and 35 years of age (18 female, 15 male). Subjects were healthy and had a normal nasal endoscopy. All of them received an MRI of the nasal cavity (CISS sequence) which allowed for volumetric analyses. Using the 'Sniffin' Sticks' test kit olfactory function was measured in a lateralized fashion for phenyl ethyl alcohol thresholds, and odor discrimination; odor identification was obtained bilaterally. CO_2 detection thresholds were obtained using air-dilution olfactometry (stimulus duration 250 ms, total flow 8 l/min). All subjects additionally underwent rhinomanometry, and provided ratings of their nasal patency and olfactory abilities. For correlations between functional measures and width of the nasal cavity MR scans of each nasal cavity were divided into several slices the area of which was obtained. Results: There were significant correlations between CO_2 thresholds and intranasal areas, indicating that subjects with smaller nasal cavities were more sensitive to CO_2-induced irritation ($r > 0.5$, $P < 0.01$). In contrast, such correlations were not found for all other measures. Conclusions: these data suggest that, at threshold level, nasal anatomy plays a role for interindividual differences in the sensitivity to trigeminal stimuli.

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Poster: Trigeminal Neurophysiology

Excitation of cold-sensitive trigeminal caudalis neurons by mustard oil and other irritants

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The transient receptor potential (TRP) channel TRPA1 expressed in trigeminal ganglion cells is activated by both intense cooling and irritant chemicals including mustard oil and cinnamaldehyde. We investigated if nociceptive neurons in trigeminal subnucleus caudalis (Vc) are excited by cooling and irritant stimulation of the oral mucosa in a manner consistent with input from primary afferents expressing TRPA1. In halothane-anesthetized rats, single-unit recordings were made in superficial laminae of dorsomedial Vc from units identified by their responses to tongue cooling (3°C water). Nearly all cold-responsive units also responded to noxious heat as well as application of the chemoirritants pentanoic acid (200 mM), mustard oil (10%), cinnamaldehyde (1%) and capsaicin (0.1%). Vc responses to repeated cooling were stable at 0.5–2 min intervals, but exhibited a significant decrease across trials at 15 s intervals. Cold-evoked responses were significantly attenuated following application of mustard oil or cinnamaldehyde. Vc responses to repeated application of mustard oil or cinnamaldehyde exhibited significant desensitization (tachyphylaxis). Vc units responded to capsaicin, which cross-desensitized responses to subsequent mustard oil. The results indicate that a population of Vc neurons is excited by intense cold, noxious heat, and chemical irritants via input from sensory afferents coexpressing TRPA1 and the capsaicin receptor TRPV1.

Poster: Trigeminal Neurophysiology

Ethanol affects the mechanical response in trigeminal nerve endings on the tongue of rhesus monkeys

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We have previously shown that ethanol administered orally elicits a powerful response in lingual non-gustatory nerve endings in rhesus monkey. Here we focus on the interaction between ethanol and mechanical stimulation. First, we characterized a LN mechanosensitive fiber with adequate stimuli. The mechanical threshold and receptive field area were measured with a set of von Frey hairs producing forces ranging from 0.005 to 80 mN. Then the receptive field was repeatedly stimulated using constant force with a strain gauge for 1 s with a 4 s interval, while ethanol (1–12 M) flowed over the receptive field for 52 s with a 2 min rinse interval. Responses of 42 LN fibers were recorded. We found that 86% the fibers responded to both ethanol and mechanical stimulation when applied together and ethanol modulated the response to mechanical stimulation. First, the response to simultaneous stimulation with ethanol and the strain gauge was not equal to sum of the responses. Even though some fibers did not respond to ethanol alone, when mechanical stimulus was applied to these same fibers together with ethanol, an interaction of ethanol and mechanical stimulation was revealed by a potentiation of the mechanical responses. Second, the pattern of activity in response to mechanical stimulation changed during the time when ethanol was applied. In 55% of the recorded fibers, high concentrations of ethanol inhibited the response to mechanical stimulation.

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Poster: Trigeminal Neurophysiology

Cholinergic receptor cells in nasal respiratory epithelium

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Recently, we reported that, in addition to free nerve endings, an extensive population of solitary chemosensory cells exists within the nasal respiratory epithelium (Finger *et al.*, 2003, *Proc. Natl Acad. Sci. USA*). In this study, since animals are sensitive to inhaled cholinergic agents, we examined whether cholinergic chemoreceptor cells exist and whether the respiratory epithelial cells are responsive to acetylcholine (ACh). Immunohistochemistry reveals some solitary chemoreceptor-like cells reacted with antibodies against vesicular acetylcholine transporter (VAChT), a key element of ACh-containing synaptic vesicle. The results suggest that the solitary chemoreceptor cells in nasal respiratory epithelium could release ACh. In a Ca^{2+}-imaging study, many epithelial cells that were enzymatically isolated or in intact nasal respiratory epithelium increased intracellular Ca^{2+} levels in response to ACh. The responses were blocked by a muscarinic ACh receptor antagonist atropine, but not significantly suppressed by a nicotinic ACh receptor antagonist hexamethonium. The data indicate that the responses were mediated by muscarinic ACh receptors. Some of the ACh-responsive cells also increased intracellular Ca^{2+} levels in response to irritant stimuli. These results suggest that a cholinergic system is present in nasal respiratory epithelial cells possibly including solitary chemoreceptor cells, and may function in cell-to-cell and/or cell-to-nerve communication.

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Poster: Trigeminal Neurophysiology

Activation of the human secondary somatosensory cortex following moderately painful trigeminal stimulation of the nasal mucosa: fMRI study

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Objectives: the application of carbon dioxide (CO_2) stimuli to the nasal mucosa is a well-established model of acute experimental trigeminal pain. We studied the brain activation induced by moderately painful trigeminal stimulation of the bilateral nasal mucosa without concomittant tactile or thermal stimulation. With reference to the results of a previous MEG study we were especially interested in the role of the primary and secondary somatosensory cortices (S1, S2). Methods: functional images following CO_2-stimulation were obtained from 30 healthy volunteers using a 1.5T MRI scanner (T2*-weighted EPI sequence, block-design). Images were analyzed using SPM2. Results and conclusions: we found activation of brain areas known to be involved following chemical stimulation of the nasal mucosa (orbitofrontal cortex), as well as association cortex (inferior, middle and superior frontal gyri, superior parietal lobule) and areas specific to the processing of painful and aversive stimuli (ventroposterolateral thalamus, S2, amygdala). Interestingly, moderately painful stimulation of the nasal mucosa activated S2, but not S1 cortex. Our data indicate that the experimental pain model of CO_2-stimulation of the nasal mucosa specifically activates the nociceptive but not the primary somatosensory cortex.

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Poster: Trigeminal Neurophysiology

Peripherial trigeminal nerve responses to a homologous series of carboxylic acids

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The trigeminal nerve responds to a variety of irritants in the environment and serves a protective role. Among the most effective trigeminal stimuli are acids. Several receptors on the trigeminal nerve respond to acids and low pH: ASICS (acid sensing ion channel), TRPV1 (vanilloid receptor 1) and P2X (purinergic receptor). The present study examined peripheral trigeminal nerve responses to a homologous series of carboxylic acids. Analysis of these responses can help determine characteristics that make an effective trigeminal stimulus. Neural recordings were obtained from the ethmoid nerves

of adult rats in response to acids delivered in solution to the nasal cavity. The ethmoid nerve response to formic, acetic, propionic, butyric, valeric and hexanoic acid were recorded. As the size of the acid increased, the threshold decreased up to hexanoic. Responses to the six acids at 10 mM also increased up to hexanoic. This suggests that as the acid becomes more hydrophobic it becomes a more effective stimulus. The data also suggest that there is a 'cut off' after valeric acid where there is a trade off between the acid's efficacy due to its hydrophobicity and its possible inability to bind to a receptor site.

Poster: Trigeminal Neurophysiology

Trigeminal nerve responses to pungent spices

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Trigeminal chemoreception is a well established and important component of flavor in foods and drinks. In mammals, there is vast trigeminal innervation of both the nasal and oral cavities, and a considerable amount of our everyday flavor experience comes from activation of trigeminal afferents in the nose and mouth by stimuli such as carbon dioxide and pungent herbs and spices. In the present preliminary study, we tested the effects of six commonly used spices on the trigeminal nerve response of adult Sprague–Dawley rats. Multi-unit neural activity was recorded from the ethmoid branch of the trigeminal nerve in response to irrigation of the nasal cavity by various spice solutions. Black pepper, cinnamon, cloves, ginger, mustard and red pepper were purchased from a local supermarket and stock solutions were made by 'steeping' the ground form of each spice in heated rat Ringer's for 30 min. Serial dilutions were then prepared from filtered stock solutions and delivered at room temperature. Application of all six spices to the nasal cavity resulted in activation of the trigeminal nerve. At the concentrations tested, ginger and red pepper appeared to elicit the most vigorous responses. These results lay the groundwork for future experiments which will examine the mechanisms by which the pungent ingredients of each specific spice elicit trigeminal responses.

Poster: Olfactory CNS

A cortical high-pass filter contributes to olfactory figure–ground separation

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Detection and discrimination of odors generally, if not always, occurs against background odors. On any given inhalation, olfactory receptor neurons will be activated by both features of the target odor and features of the background stimuli. In order to identify a target odor against a background, therefore, the olfactory system must be capable of grouping a subset of features into an odor object distinct from the background. Our previous work has suggested that rapid homosynaptic depression of cortical afferents and intracortical association fiber plasticity within the anterior piriform cortex (aPCX) allows for experience-dependent odor object formation and enhances cortical odor discrimination. We hypothesize here that this process would also allow for figure-ground separation of a target odor from background stimulation. Single-unit recordings were made from both mitral/tufted cells and aPCX neurons in urethane-anesthetized rats. Single-unit responses to 2 s odorant stimuli and their binary mixtures were determined. One of the odorants was randomly selected as the background and presented for 50 s. At 40 s after the onset of the background stimulus, the second odor was presented for 2 s, producing a binary mixture. The results suggest that mitral/tufted cells continue to respond to the background odor and when the target odor is presented, responded similar to the binary mixture. In contrast, aPCX neurons are capable of filtering out the background stimulus while maintaining responses to the target stimulus—providing a potential mechanism for olfactory figure–ground separation.

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Poster: Olfactory CNS

Odotopic map in the forebrain of channel catfish

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Functional information on odor representation central to the olfactory bulb (OB) is rudimentary in mammals and nonexistent in teleosts. Odor-responsive neurons were located in the olfactory tract terminal zones in the forebrain (FB) of channel catfish (Finger, 1975; Bass, 1981). We previously reported the existence of a FB odotopic map for amino acids and bile salts (Nikonov and Caprio, 2004, *AChemS*), and now include similar information for a third class of biologically relevant odorants, i.e. nucleotides. Tested were ATP, ITP and IMP along with bile salts (Na^+ salts of taurocholic, taurolithocholic and lithocholic acids) and amino acids (L-met and L-arginine HCl)—all effective odor stimuli in catfish (Nikonov and Caprio, 2001). A total of 74 FB neurons were excited by odor stimulation: (i) 17 units located in posterior/central olfactory terminal field (otf) were selectively excited by 1μM nucleotides; (ii) 25 units located in the medial otf were selectively excited by 1μM bile salts; and (iii) 24 neurons located ventrolaterally (lateral otf) were selectively excited by amino acids. Both the medial–lateral distinction between excitatory responses to bile salts and amino acids and the rostro-caudal distincition between amino acids and nucleotides, respectively, reflect a similar topographical organization within the OB. Emergent cell types not previously observed within the OB include: (i) eight FB units that were excited by both amino acids and nucleotides; and (ii) seven amino acid FB units that were excited by both neutral (L-Met) and basic (L-Arg) amino acids.

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Poster: Olfactory CNS

The cloning and characterization of the distribution of an octopamine receptor and two serotonin receptors in the olfactory system of the tobacco hawkmoth *Manduca sexta*

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Biogenic amines have been demonstrated to be important modulators of olfactory based neural processing and behavior in many species of both vertebrates and invertebrates. However, few olfactory models have been used to examine the effects of biogenic amines at the molecular, anatomical, electrophysiological and behavioral levels. The tobacco hawkmoth, *Manduca sexta*, is an ideal animal in which to study the modulatory roles of biogenic amines in olfactory processing as it can be studied using many different levels of analysis. The neuroanatomy of both serotonin and octopamine-immunoreactive neurons has been documented in *M. sexta* and it has been shown that serotonin serves as a modulator of olfactory sensitivity within the antennal lobe of *M. sexta*. The behavioral and physiological effects of octopamine in *M. sexta* remain unknown. We also have little information about the specific molecular mechanisms underlying aminergic modulation in this system. In preparation for a detailed pharmacological analysis of aminergic receptors in the olfactory system, we obtained the full length sequences of an octopamine receptor (MsOAR) and two serotonin receptors (Ms5HT1a and Ms5HT1b), and examined their localization within the olfactory system at the peripheral, first and second levels of olfactory processing using *in situ* hybridization.

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Poster: Olfactory CNS

Olfactory working-memory in primary olfactory cortex

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Although many studies with humans have contributed to our understanding of olfactory memory in general, few have contributed to elucidating the specific role of primary olfactory cortex (POC) in this process. Recently, imaging techniques have shed some light on the topic. PET studies have found specific POC activation during odor recognition that exceeded that during odor discrimination. Additionally, fMRI studies have found that POC was activated during the successful retrieval of old as compared with new objects. Aside from the aforementioned results, most studies conducted on olfactory memory have focused on regions receiving secondary and tertiary olfactory projections. In light of this, we set out to further elucidate the specific role of POC in olfactory memory using fMRI. We have scanned one subject to date using the following protocol. Each trial of an event-related design began with an auditory primer instructing the subject to prepare to sniff at the tone. At the tone, the olfactometer administered an odorant of a particular concentration to the subject. After each trial, the subject was asked to make an odor intensity estimate. In three conditions, the subject was cued to key the estimate either (i) immediately after the sniff, (ii) 10 s later or (iii) 15 s later. This design forced the subject to keep the intensity estimate in mind for the various durations. The entire experiment included 24 trials of each condition randomly ordered across five functional scans. Activity in POC was maintained at a constant high level until the intensity estimation was reported. This result suggests working memory in primary olfactory cortex. Data will be presented from 16 additional subjects.

Poster: Olfactory CNS

CNS processing of pulse duration in the crayfish, *Procambarus clarkii*

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Two major issues facing sensory ecologists are how olfactory information is coded in the nervous system and how that information correlates with behavior. Previous results indicate that crayfish orientation behavior is sensitive to temporal components of an odor signal. Due to this, we expect to find neural correlates within the olfactory system that underlie the organism's behavior. In this study, we investigated how the temporal aspects of an odor signal are processed in the central nervous system of the crayfish. Neural ensemble recordings were made on an isolated head preparation perfused with oxygenated crayfish saline. Silicon multichannel electrode arrays were inserted into the deuterocerebrum of the crayfish brain. The medial antennule was placed into an olfactometer and stimulated with three types of stimuli: glutamine, glycine and shrimp extract. The stimuli were presented at a specific molar concentration (10^{-5} M) and inter-pulse interval (5000 ms), varying only the duration of the odor pulses presented to the antennule. Our results suggest that cells are able to encode duration of stimulus presentation. In addition to the ability of the CNS to encode pulse duration we are also investigating how the CNS is habituating or adapting to the various pulse durations presented. These results are consistent with our behavioral data demonstrating that crayfish orientation is sensitive to the temporal dynamics of odor plumes.

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Poster: Olfactory CNS

Differential habituation of unit activity, beta activity and gamma activity in olfactory cortex

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Odor processing in olfactory cortex was examined using single unit and local field potential recordings in freely breathing male Long-Evans rats anesthetized with urethane (1.4 g/kg, i.p.). Brief (2 s) odorant presentation increased beta activity (8–35 Hz) and gamma activity (35–90 Hz) in both the olfactory bulb (OB) and the piriform cortex (PC). The response of PC units to an odorant was heterogeneous, with some increasing and others deceasing their firing rate. Long (50 s) odorant presentation caused rapid habituation of the initial beta response in both the OB and PC; in marked contrast, the gamma response remained elevated in both sites throughout

the presentation. Habituation of PC unit activity depended upon the nature of the initial response: units exhibiting an initial increase in firing habituated rapidly, but units that were initially inhibited exhibited a progressive increase in the strength of that inhibition over the course of the 50 s presentation. Previous research (Neville and Haberly, 2003, *J. Neurophysiol.*, 90:3921–3930) has demonstrated that beta activity requires an intact LOT but gamma activity does not. Combined with the present results, this suggests that gamma activity originates in the OB and may be related to coding of the physical characteristics of an odorant (thus remaining elevated as long as an odorant is present), while beta activity requires participation of the PC and may be related to the motivational significance of an odorant (thus habituating rapidly in the presence of a non-reinforced odorant).

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Poster: Olfactory CNS

Real-time gas concentration monitoring method for chemosensory event-related potential measurement

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A new technique is described for the accurate, reliable measurement of gaseous molecule concentrations. Precision gaseous stimulus monitoring with a high time accuracy is indispensable to measure event-related potentials and event-related magnetic fields for an olfactory stimulus, as Evans *et al.* proposed in 1993. In our method, a continuous ultrasonic wave generated from the transmitter was modulated by the average molecular weight in a chamber. Through the process of amplification of the received signal, smoothing, a half-wave rectification and peak-hold, we could detect the temporal change of average molecular weight (gas concentration) within the chamber. This device could in principle measure the change of the gas concentration over a 100 kHz sampling rate (10 μs temporal resolution). We investigated the potential of this high-speed gas concentration sensor using the olfactometer developed by Kobal. We used 100% nitrogen for the odorant (molecular weight 28) and air (containing 78.03% N_2, average molecular weight 28.996) for constant flow, and observed the gas exchange, i.e. the stimulus with 1 kHz sampling time. The signal/noise ratio was >42 dB, and no artifacts to potentials or magnetic fields were found. Using the current method to observe the contrast between nitrogen and air, it is highly probable that this sensor will detect small molecular gas changes, such as air and air with odorant.

Poster: Olfactory CNS

Synaptic correlates of olfactory recognition memory

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We have investigated the synaptic basis of a specialized form of olfactory learning in mice, in which a female forms a memory to the pheromonal signal of the male that mates with her. This memory is of vital importance in avoiding the abortion of the mating male's own offspring. The neural changes underlying the memory require the association of the pheromonal signal and mating signal, which is conveyed by noradrenergic projections, in the accessory olfactory bulb (AOB) and involve changes at the reciprocal synapses between mitral and granule cells. In support of this, we show in the AOB slice that pairing of 10 Hz stimulation of mitral cell axons with noradrenaline, acting at presynaptic α_2-adrenoceptors, induces NMDA receptor-dependent long-term potentiation (LTP) at the mitral to granule cell synapses. We have previously shown that the activation of mGluR2, a metabotropic glutamate receptor, in the AOB permits the formation of a pheromone-specific memory. To further investigate the role of mGluR2 in memory formation, we have used genetically engineered mice lacking mGluR2. Pairing of 10 Hz stimulation of mitral cell axons with DCG-IV, an mGluR2 agonist, induces NMDA receptor-independent LTP at the mitral to granule cell synapses in wild type mice but not in mutant mice. These results indicate that there are at least two independent signalling pathways leading to the induction of LTP at the mitral to granule cell synapse.

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Poster: Olfactory CNS

Eye closure in darkness animates olfactory cortical areas

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Objectives: open-eyed and closed-eyed conditions in darkness are frequently used as rest conditions in brain imaging studies but could have non-trivial effects on brain activity. In this study we tested whether the olfactory cortex is animated by eye closure without any external stimulation. Methods: 12 healthy volunteers lying in complete darkness in the MRI scanner alternately opened and closed their eyes in response to an acoustic signal during fMRI measurements (1.5 T). Images were analyzed using SPM99. Additional region of interest (ROI) analysis was performed using MarsBaR. Results: for the contrast eyes-closed versus eyes-open, we identified activation clusters bilaterally in visual, somatosensory, vestibular and auditory cortical areas. ROI analysis revealed that both the specific olfactory regions in the piriform cortex and anterior insula, which had been activated in previous experiments following olfactory stimulation, were significantly activated by eye closure in darkness without any external stimulation. The contrast eyes-open versus eyes-closed showed activation clusters in cortical and subcortical ocular motor structures. Conclusions: the state chosen as rest condition may have a considerable impact on the interpretation of brain activation studies. Our data confirm that sensory areas of the visual, somatosensory, vestibular and auditory system are activated by eye closure in darkness without external stimulation. Moreover, we were able to demonstrate that this holds true for olfactory sensory areas as well.

Poster: Olfactory CNS

Evidence of olfactory cross-adaptation of an unpleasant mercaptan odorant by a pleasant mercaptan as revealed by chemosensory event-related potentials

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Olfactory cross-adaptation has been observed for structurally similar yet perceptually different odorants (Pierce *et al.*, 1998). The study reported here extends this work by recording chemosensory event-related potentials (CSERP) for an unpleasant mercaptan odorant following an adaptation phase. Fifteen participants took part in the experiment and were pre-screened for olfactory acuity using 'Sniffin' Sticks'. Chemosensory stimuli were applied using a birhinal olfactometer. This involved either presentation of air or an unpleasant mercaptan, a structurally related pleasant fruity mercaptan or a non-related pleasant ethyl-ester in the adaptation phase. Using these stimuli, four adaptation conditions were presented. All experimental conditions took the following form: first, a 15 s adaptation phase consisting of one of the above mentioned stimuli; second, a short random delay of 4–8 s (air); finally, the mercaptan target (300 ms) was presented. Two seconds after the target, behavioural data was acquired using a visual analog scale. There was an ITI of 20 s. Dependent measures were the CSERP's and individual magnitude estimates of the stimulus intensity for the target mercaptan. All odorant and air conditions were presented twenty times in four separate counterbalanced blocks. EEG results for P2 demonstrated evidence of adaptation (reduced amplitudes) following the unpleasant mercaptan (self-adaptation) and the pleasant mercaptan (cross-adaptation) compared with the pleasant ethyl-ester and the air controls (maximum at CPz). Behavioural results, the intensity magnitude estimates, revealed a similar pattern of results.

Poster: Olfactory CNS

Variability in olfactory fMRI study results

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Small sample size and diverse acquisition hardware complicate comparability in olfactory functional imaging (fMRI) studies. To evaluate reproducibility of cortical activation in a passive smelling paradigm with phenylethyl alcohol (PEA) we analysed data from two functional imaging studies (14 subjects each). Stimulus delivery and intensity were kept identical. In the first study image acquisition differed in regard to the receiving head coil (one channel versus eight channels). To evaluate the influence of the head coil used we acquired a subset of the data with both coils (eight subjects). This allowed for a within-subject analysis (different coils) and a between-study comparison. Subjects were examined in a 1.5 T MRI scanner (Siemens Sonata). The paradigm was a block design (30 s odorant, 30 s air, 1 s stimulus duration, 3 s ISI). Stimuli were given by an olfactometer and a tube in the right nostril. In a region-of-interest approach we assessed brain regions commonly activated in olfactory fMRI studies (Gottfried *et al.*, 2002, *J. Neurosci.*, 22:10819–10828). On the second level paired *t*-tests and two sample *t*-tests were employed. Inter- and intrasubject variability was considerable and differed for individual brain areas. Cingulate cortex and insula were most reliably activated. In conclusion, the same paradigm assessed in different subgroups and differences in technical equipment leads to divergent results. Similarly, large within- and between-subject variability was found in an earlier study assessing temporal aspects of the hemodynamic response (Neumann *et al.*, 2003, *Neuroimage*, 19:784–796). Thus, the within-subject variability seen here may not be attributed to different technical equipment alone.

Poster: Olfactory CNS

Single odorants versus binary odor mixtures: a PET investigation

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Although a great deal of literature on the functional neuroanatomy associated with perception of single odorous compounds exists, no study has investigated the neural correlates of perception of odor mixtures. The aim of this study was to compare brain activations obtained by passive smelling of binary odor mixtures versus single odorants. Twelve healthy volunteers participated in the positron emission tomography study. During the acquisition scans, subjects were presented one of two individual compounds (citral, pyridine) or one of five mixtures composed of varying proportions of the two odorants. All individual odorants and mixtures were selected to be isointense. During the baseline condition subjects were presented an odorless stimulus (water). A direct comparison of binary mixtures versus single odorants showed increased activation in the left medial cingulate cortex and right lateral orbitofrontal cortex during perception of mixtures. The opposite contrast did not reveal any increased activation for perception of single odorants. These preliminary results show that the perception of odor mixtures recruits more neural activity in traditional olfactory regions than does perception of single odorants.

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Poster: Olfactory CNS

Age-dependent impairment in ltp in anterior piriform cortex of mice lacking the fragile X mental retardation protein

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Mutations in the gene encoding the fragile X mental retardation protein (FMRP) cause the Fragile X syndrome, the most common inherited form of mental retardation. Knockout (KO) mice lacking

FMRP show learning deficits, including impairments in olfactory discrimination learning. The present study tested for alterations in long-term potentiation (LTP) in primary olfactory cortex of FMRP-deficient mice. Slices of anterior piriform cortex were prepared from adult (3–18 months old) KO and wild type (WT) mice (C57BL/6J strain) and maintained *in vitro* using conventional methods. Synaptic responses evoked by associational fiber stimulation were recorded extracellularly in layer Ib. Input–output curves were generated by varying stimulus intensity from 2.5 to 160 μA, paired-pulse stimulation used inter-pulse intervals of 50–1600 ms and LTP was induced by theta burst stimulation (TBS) in the presence of 10 μM picrotoxin. LTP was monitored for at least 60 min after TBS. Input-output curves were slightly, but significantly, depressed in slices from KO mice aged 3–6 months and 12–18 months, compared with slices from age-matched WT mice. Short-term synaptic plasticity (paired-pulse depression) did not differ in KO and WT slices at any ages. LTP induced by TBS was normal in FMRP-deficient mice aged 3–6 months but was substantially reduced in KO mice aged 6–12 and 12–18 months. These results indicate that absence of FMRP is associated with an age-dependent impairment in long-term synaptic potentiation in primary olfactory cortex. It will be of interest to determine if behavioral impairments in FMRP KO mice are also sensitive to age.

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Poster: Olfactory CNS

Context and chemistry as factors for innate odor responses

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2,4,5-Trimethylthiazoline (TMT, a component of fox feces) has been used as a predator odor to elicit fear responses in rodents. More recently, the use of TMT as such has been questioned. In this study, we test the hypothesis that odors can produce innate electrophysiological and behavioral responses. Rat olfactory bulb local field potential responses are recorded and filtered into the theta (3–15 Hz), beta (15–40 Hz), low gamma (35–60 Hz) and high gamma (60–115 Hz) frequency bands. In the first experiment odors are delivered to both awake and anesthetized rats on cotton swabs for 12 presentations of 10 s. We find that certain chemical characteristics of odors are highly correlated with responses, indicating that odorant chemistry may be responsible for immediate response variance in this context. We next examine the role that context plays in determining odor responses by habituating context. Odors are delivered to awake, implanted rats in the air of an enclosed chamber for five 2 min presentations. In this situation, TMT does not produce behavioral or electrophysiological signatures distinct from other odors. This supports the hypothesis (McGregor *et al.*, 2002) that TMT may not be perceived by rats as a predator odor. We consider these results as compared with those from shock fear conditioning and suggest that TMT may become part of an associative threat context when combined with other features.

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Poster: Olfactory Receptor Neurons

Detection of MHC class I peptides by the mammalian main olfactory system

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Genes of the major histocompatibility complex (MHC), which play a critical role in immune recognition, influence mating preference and other social behaviors in fish, mice and humans but the cellular and molecular mechanisms by which this occurs remain unclear. We have proposed that small peptide ligands of MHC molecules provide a functional link between the immune system and the sense of smell, converting an immunological genotype into a chemosensory quality. Here, we use a combination of dye tracing, electrophysiological, and behavioral approaches to show that MHC peptides are not only detected by the specialized vomeronasal organ (VNO) but also by neuronal populations in the mouse main olfactory epithelium (MOE). MHC peptides activate olfactory sensory neurons at picomolar concentrations *in vitro* and affect social preference of mice *in vivo*. Both effects depend on the cyclic nucleotide-gated channel gene *CNGA2*. Thus, two distinct mechanisms have evolved in the mammalian main and accessory olfactory systems for the detection of MHC peptides. Our findings suggest a more general chemosignaling function of MHC peptides even in those vertebrates that do not possess a functional accessory olfactory system. They also indicate that the strict division of chemosensory systems based on the detection of volatile versus nonvolatile stimuli needs to be reconsidered.

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Poster: Olfactory Receptor Neurons

Lipocalins in the olfactory epithelium of the newt, *Cynopus pyrrohogaster*

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We constructed a cDNA Library with messenger RNA obtained from the olfactory epithelium of the newt, *Cynopus pyrrhogaster*. By a preliminary exhaustive analysis of the library, we found three types of clones that are abundantly expressed in the olfactory epithelium of the newt. Analysis of the nucleotide sequence of the clones revealed that each of them showed high sequence similarity to frog olfactory specific protein (OSP), lipocalin and rat potential ligand-binding protein (RY2G5; PLBP), respectively. The molecular phylogenetic tree constructed by a neighbor joining method showed that Cy-OSP and Cy-lipocalin are highly similar to each other and that they belong to the lipocalin superfamily. The transcripts of the three clones were highly expressed in the newt olfactory epithelium but not in brain, liver or intestine. Based on *in situ* hybridization analysis using serial sections of the olfactory organ of

the newt, they all expressed in cells composing the Bowman's grand, but the distribution of the cells expressing each transcript showed different patterns on the olfactory organ. These results suggest that the different types of possible odorant-binding proteins are expressed in the different parts and form a heterogeneous mucous environment of the olfactory organ of the newt.

Poster: Olfactory Receptor Neurons

Nonselective cation channels in rat olfactory receptor neurons

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A background nonselective cation channel was identified in inside-out membrane patches taken from acutely dissociated rat olfactory receptor neurons. The channel was active at –60 mV and was insensitive to cAMP (1–200 μM) (n = 22). The channel differed from the cyclic nucleotide-gated (CNG) channel previously reported in these cells. Such channel activity occurred in 22 of 66 patches tested. Exposing a patch containing background nonselective cation channel activity to cAMP (1–200 μM) triggered CNG channel openings in 7 of 22 patches tested, and failed to do so in the remaining 15 patches. Sixteen patches showed only the CNG channel activity. The I–V relationship of the channel was linear between –100 and +100 mV. The single channel conductance was 18.7 ± 0.01 pS (n = 3) and the open probability was strongly dependent of membrane potential (Popen = 0.005 ± 0.001 at –60 mV; Popen = 0.58 ± 0.05 at +60 mV; n = 3–6). The channel was impermeable to Cl^- and was relatively nonselective between Na^+ and Cs^+ based on ion substitution.

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Poster: Olfactory Receptor Neurons

Plasma membrane calcium pumps of mouse olfactory neurons

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Plasma membrane calcium pumps play significant roles in calcium clearance in sensory cells, such as hair cells and retinal cells. Previously we have shown that the transient increase in intracellular calcium arising from depolarizing stimuli or the application of IBMX-forskolin to dissociated olfactory sensory neurons still resolves when mitochondrial calcium uptake or Na/Ca transporter inhibitors are applied to the cells. Therefore, we are examining the roles of plasma membrane calcium pumps in calcium removal in olfactory sensory neurons. The calcium pump proteins are expressed from 4 genes, and we have shown that isoforms 1 and 2 are the most highly represented isoforms in Western blots and sections of the olfactory epithelium and VNO. Confocal and deconvolution microscopy of dissociated olfactory sensory neurons show isoforms 1, 2 and 4 distributed from the cilia to the cell body, with a preponderance of the fluorescence from the pumps on the dendritic knob and where the dendrite enters the cell body. Dissociated cells from homozygous PMCA2 knockout mice (a kind gift of G. Shull) show no staining for PMCA2, but normal staining with antibodies for PMCA1, 4 and pan-PMCA. The homozygous wild type show typical staining for PMCAs 1, 2, and 4. We are in the process of examining the differences between the cells from wild type and knock out mice for calcium clearance after stimulation.

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Poster: Olfactory Receptor Neurons

Unconventional neurons in the nasal cavity of humans

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The nasal cavity of humans can roughly be divided into a sensory and a so-called non-sensory area: the main olfactory epithelium (MOE) housing ciliated ORNs, and the respiratory epithelium (RE) containing free nerve endings. However, the morphology and function of both the MOE and the RE are more complex, as indicated by studies on rodents and humans. Previous investigations of the human MOE revealed cell types other than the ciliated ORNs. Contrary to the traditional concept that the RE contains only free nerve endings, we (Finger *et al.*, 2003, *Proc. Natl Acad. Sci. USA*) discovered a chemosensory cell in the RE that strikingly resembles solitary chemosensory cell (SCC) that detect irritants. Since humans show aversion to inhaled noxious chemicals, it is likely that the human RE, too, contains receptor cells. The aim of this study was to obtain a thorough knowledge of the cell types and their structural features involved in the nasal chemosensory system. This knowledge is critical for understanding disorders and diseases of the olfactory system. Standard techniques of immunocytochemistry, and both SEM and TEM electron microscopy, were used to characterize unconventional neurons in the human MOE and RE. Our studies reveal a possible receptor cell in the human MOE that strikingly resembles the crypt ORN hitherto described only in fish. Also, electron microscopic studies of the RE indicate several potential chemosensory cell types. Immunocytochemical experiments show cell types positive for gustducin, calbindin and/or VAchT that closely resemble the rodent SCCs.

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Poster: Olfactory Receptor Neurons

Identification of crypt-type ORN in the olfactory epithelium of the European sea bass *Dicentrarchus labrax*

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Crypt-type ORNs were identified for the first time in the olfactory epithelium of the European sea bass, *Dicentrarchus labrax*, using immunohistochemistry for the Na^+/K^+-ATPase, a staining method originally used to localize chloride cells in fish. The ultrastructure of the olfactory epithelium was then re-examined by scanning and transmission electron microscopy for a specific description of the crypt-type ORN. The sea bass crypt-type ORNs are scattered within the sensory epithelium and relatively less abundant than ciliated and microvillous ORNs. As described in other fish species, these ORNs are characterized by the presence of ciliae and microvilli. The immunostaining for the Na^+/K^+-ATPase was observed in specific supporting cells closely associated with the crypt-type ORNs but not in the receptor neuron itself. The immunostaining procedure also revealed the presence of numerous chloride cells located in the non-sensory epithelium, at the base and the edge of the olfactory lamellae. The use of the Na^+/K^+-ATPase immunohistochemistry provide a new tool to study the distribution of crypt-type ORNs in marine fish. The abundance of the Na^+/K^+-ATPase in the specialized associated supporting cells remain to be explained and deserves more investigation.

Poster: Olfactory Receptor Neurons

Signal transduction proteins in the squid, *Lolliguncula brevis*

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In the squid, *Lolliguncula brevis*, paired olfactory organs are located anterior to the mantle cavity, where they are ideally positioned to sample water flow. The pseudostratified olfactory epithelium is composed of five morphological neuron types and one or two support cell types. To see whether G-protein mediated signal transduction pathways are present in *L. brevis* olfactory receptor neurons, we tested antibodies against $G\alpha_{s/olf}$, $G\alpha_q$ and PLCβ2 (Santa Cruz Biotechnology, CA). Squid olfactory organs were dissected and immersion fixed in 4% paraformaldehyde, cryoprotected and cut into 10 μm thick sections. We found positive immunoreactivity (IR) with all three antibodies. $G\alpha_q$-like IR was present in the cilia pockets of 2–3 neuron types. $G\alpha_{s/olf}$-like IR was present in cilia pockets of 4–5 neuron types. PLCβ2-like IR outlined the cells and was present in the cilia of type 2 neurons. Pre-absorption of the primary antibodies with peptide antigens showed that staining was specific. Zero primary and secondary controls also verified that staining was specific to the protein, and not an artifact for all antibodies tested. We conclude that $G\alpha_{s/olf}$, $G\alpha_q$ and PLCβ2-like IR is present in squid olfactory neurons and is located in cellular regions that are predicted to contain the signal transduction machinery responsible for odor transduction.

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Poster: Olfactory Receptor Neurons

Molecular identification of a TRPC protein homolog from lobster olfactory receptor neurons

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Lobster olfactory receptor neurons signal in part through a phosphoinositide pathway that targets a well-characterized Na^+-gated non-selective cation (SGC) channel (Zhainazarov and Ache, 1995, 1998). The physiological and pharmacological properties of the channel are consistent with the channel being a member of the transient receptor potential (TRP) superfamily (Bobkov and Ache, 2004). To address more directly the possibility that the SGC channel is a TRP channel, we attempted to clone one or more TRP channel proteins from a lobster olfactory organ library. We were able to isolate a cDNA sequence homologous to TRPC in mammals, TRP-1 in *Caenorhabditis elegans* and TRPA in *Limulus polyphemus*. The sequence subsequently could be identified from ORN RNA through RT-PCR. Once the full-length sequence is verified we will attempt to functionally express the protein to determine if the physiological and pharmacological properties of the cloned molecule match those of the native channel. If so, the lobster SGC channel will be among the best characterized native TRP channels in chemosensory cells.

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Poster: Olfactory Receptor Neurons

Characterization of a functionally different subpopulation of lobster olfactory receptor neurons

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The olfactory organ in the lobster appears to possess functionally heterogeneous subpopulations of olfactory receptor neurons (ORNs) that differ in both their spontaneous discharge and evoked responses to odorants. Using extracellular and whole-cell recordings from individual ORNs, we characterized the latency, rate of rise, patterning, gain coefficient and dynamic range of the cells. The predominant subpopulation, comprising an estimated 70% of the population, is the one usually reported. This cell type is tonically active between 0.1 and 8.2 Hz and responds to odors by increasing the rate of discharge in a concentration dependent manner. However, another subpopulation can be identified that exhibits spontaneous rhythmic bursts of action potentials (0.03–0.9 burst/s). In these cells burst frequency is voltage-dependent. Bursting behavior is presumably intrinsic since bursting persisted in acutely dissociated ORNs. Excitatory odor input triggers a burst in a phase-dependent manner. Such odor-evoked bursting could serve to amplify the input to the cell in a strong nonlinear manner. Rhythmic excitatory odor input, as might be generated by flicking (sniffing), within limits can entrain the intrinsic bursting rhythm of the cell. We are in the process of characterizing potential differences in the ion channel(s) targeted by phosphoinositide signaling in these two subpopulations of lobster ORNs.

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Poster: Olfactory Receptor Neurons

Characterization of a *Drosophila melanogaster* chemosensory specific SNMP

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SNMP (sensory neuron membrane protein; Rogers *et al.*, 1997, *J. Biol. Chem.*, 272:14792; 2001a, *Cell Tissue Res.*, 303:433; 2001b, *J. Neurobiol.*, 49:47) is an antenna-specific two transmembrane domain protein abundantly present in the receptive membrane of olfactory neurons in moths. SNMP is expressed late in adult development and in adult life, well after morphogenic events have occurred. These temporal and spatial expression patterns suggest SNMP is functionally involved in odor detection, either in odor recognition or clearance. *Drosophila melanogaster* contains 13 SNMP homologues; one of these shares significant similarity with the moth SNMPs. We have constructed a transgenic fly containing the promoter for this gene; this promoter drives expression of cd8::GFP, labeling cells ostensibly that express the *Drosophila* SNMP homologue. We have also conducted *in situ* hybridization experiments confirming the validity of the cd8::GFP expression pattern.

Poster: Olfactory Receptor Neurons

Functional characterization of two human olfactory receptors expressed in the baculovirus SF9 insect cell system

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Olfactory receptors (ORs) are the largest member of G-protein coupled receptors which mediate early olfactory perception in discriminating among thousands of odorant molecules. Assigning odorous ligands to ORs is a prerequisite to get understanding of the mechanisms of odorant recognition. The functional expression of ORs represents a critical step to address this issue. Due to limitations in heterologous expression, very few mammal ORs have been characterized and so far only one is from human origin. Consequently, OR function still remains poorly understood especially in human whose genome encode a restricted chemosensory repertoire compared with most mammal species. In this study, we have designed cassette baculovirus vectors to coexpress human OR 17-209 or OR 17-210 with either $G_{\alpha olf}$ or $G_{\alpha 16}$ proteins in Sf9 cells. Each OR was found to be expressed at the cell surface and co-localized with both G_{α} proteins. Using Ca^{2+} imaging, we showed that OR 17-209 and OR 17-210 proteins are activated by esters and ketones respectively. Odorant-induced calcium response was increased when ORs were coexpressed with G $G_{\alpha 16}$ protein whereas coexpression with $G_{\alpha olf}$ abolished calcium signaling. This strategy has been found to overcome most of the limitations encountered when expressing an OR protein and has permitted odorant screening of functional ORs. Our approach could thus be of interest for further expression and ligand assignment of other orphan receptor proteins.

Poster: Olfactory Receptor Neurons

Response profiles of olfactory receptor neurons in *Xenopus laevis* tadpoles: a theoretical analysis

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We have recently shown that the sensitivity spectra of olfactory receptor neurons (ORNs) of *Xenopus laevis* tadpoles to amino acids become more selective over ontogenetic stages. In the present work we performed a theoretical analysis of the above mentioned data and determined the correlational relationships among odorant responses represented as binary response vectors. We first show that, on the one hand, the number of 204 ORN classes (out of 283 recorded ORNs) cannot be explained by a random expression pattern of olfactory receptors (ORs). On the other hand, this number does not appear to be reconcilable with the idea that individual ORNs expresses one type of OR each. The covariance matrix of stimulus responses shows that the responses to some stimuli are correlated to those of others. Furthermore, the response vectors show positive as well as negative correlations among each other. While the positive correlations can partly be explained by the differing response frequencies to the odorants used, the negative ones cannot. Finally, we analyse the similarity among responses using the Hamming distance as a distance measure, the result being that most response vectors differ from others by small Hamming distances. Such vectors are shown to form pattern cascades. In conclusion our data analysis suggests a decreasing number of ORs being expressed over ontogenetic stages.

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Poster: Olfactory Receptor Neurons

Investigations on presence and function of nitric oxide in the olfactory system of mice

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The gaseous signaling molecule nitric oxide (NO) is involved in many biological events including vascular smooth muscle relaxation, inhibition of platelet aggregation, immune regulation and neurotransmission, as well as neurogenesis and cell differentiation. In the peripheral olfactory system NO seems to have functions in the embryonal development of the olfactory neuroepithelium (OE) and its regeneration after injuries. Nevertheless, existence and function of NO in the intact OE of adult rodents are still controversially discussed. In the present study we have begun to investigate the existence and function of NO as well as the expression of its synthesizing enzyme, NOS, in the OE of adult and neonatal mice. Using antibodies against different isoforms of NOS we were able to show the presence of the endothelial isoform (eNOS) in a subpopulation of olfactory sensory (OSN) neurons in adult mice. eNOS could be detected only in the somata, in the dendrites and knobs,

but never in the axons of the OSNs. The neuronal isoform of NOS, on the other hand, has been shown to reside in the axons of OSN in embryonal and neonatal mice. Experiments on the functionality of eNOS in the olfactory epithelium showed that NO is produced by this enzyme upon stimulation of the olfactory receptor neurons with odorants or other activators of the olfactory signal transduction cascade. Taken together, our results favour the idea of a function ofor eNOS in the olfactory signal transduction, in contrast to the function of nNOS in development and regeneration.

Poster: Olfactory Receptor Neurons

Determining the location of CNG channels in olfactory cilia

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Ion channels often have spatial arrangements within compartments of sensory neurons that dictate their timing and degree of activation. Olfactory neurons have narrow extensions called cilia, which contain a high density of ion channels. These ion channels, the cyclic-nucleotide-gated (CNG) channel and the Ca^{2+}-activated Cl^- channel, are activated sequentially during an odor response. Ca^{2+} from the CNG channel activates the Cl^- channel. The distribution of the two channel types in the ciliary membrane may dictate their relative timing of activation. It may also influence the amplitude of the response for the neuron, as a signal from the distal region of the cilium may diminish before it reaches the dendrite. We have used a mathematical model, which interprets patch-clamp experiments from isolated cilia, in order to determine the location of the CNG channels. The model consists of two nonlinear differential equations and a constrained Fredholm integral equation of the first kind. The results from the model suggest that CNG channels in olfactory cilia are located in clusters.

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Poster: Olfactory Receptor Neurons

Gonadotropin releasing hormone (GNRH) modulation of olfactory responses in coho salmon

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The coordination of maturation and reproductive behaviors depends upon the timing of neuromodulatory activity and circulating hormone levels. The synchronization of these events relies upon general environmental cues as well as the exchange of visual and pheromonal cues between the sexes. One of the critical coordinators of maturation and sexual behaviors in vertebrates appears to be gonadotropin-releasing hormone (GnRH). GnRH is present not only in inputs to the portal vasculature of the pituitary gland, where it stimulates the secretion of gonadotropins, but also within brain circuits projecting to sensory organs including the retina and olfactory epithelium. Here we present immunohistochemical and real-time PCR data demonstrating the presence of GnRH receptors within the olfactory epithelia of coho salmon (*Oncorynchus kisutch*). We also present electrophysiological evidence that GnRH inputs to the olfactory system may serve as neuromodulators, altering the electrical responses of olfactory neurons to environmental odors during sexual maturation and thereby altering olfaction-dependent behaviors.

Poster: Olfactory Receptor Neurons

Cyclic-AMP dynamics and adenylyl cyclase activities within the olfactory cilia

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It is now fairly well accepted that the vertebrate olfactory transduction is mediated by AC-cAMP system. However, little is known about quantitative kinetics of the molecular elements underlying this signal transduction. Generally, the activity and temporal dynamics of the enzymes and cytoplasmic cAMP are indispensable information to understand the molecular manner mediating cellular systems. However, sensory cilia express a fine structure (200 nm), which has actually limited experimental manipulations for long period of time. Here, we present a novel concept to estimate cytoplasmic cAMP dynamics in real time within such a fine structure. The activities of cAMP-gated membrane channels were monitored, while cytoplasmic cAMP concentration was freely manipulated through the UV-photolysis of intracellulary loaded caged cAMP. Since in this condition production of cAMP is proportional to the intensity of light, we could obtain the relation between the cytoplasmic cAMP activity and membrane current. Therefore, cytoplasmic cAMP concentration could be estimated inversely from the appeared current size, regardless of the type of stimuli (either UV or odor). Using such logics and techniques, we monitored ligand (odorant)-activated adenylyl cyclase activity and its temporal dynamics within fine cilia. The present study will show that G-protein-mediated enzymatic activity increases monotonically with time after the GPCR stimulation, as has been estimated from the previous theoretical work based on the thermodynamic modeling of the rod photoreceptor cell. In addition, we calculate the actual number for the cAMP production rate in living olfactory cilia at the response.

Poster: Olfactory Regeneration

The role of transporter proteins in release of the neuromodulator atp in the mouse olfactory epithelium

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Our previous work has shown that (i) ATP is a neuromodulator, reducing olfactory sensory neuron sensitivity to odorants, and (ii) activation of purinergic receptors initiates a stress signaling cascade that results in expression of heat shock protein (HSP) 25. Ischemic, stressed and injured cells release ATP in millimolar amounts; moreover, intracellular ATP significantly decreases when the olfactory epithelium (OE) is damaged by noxious fumes (Kilgour *et al.*, 2000, *Toxicology*, 145:39–49). We also have evidence that ATP is tonically released at nanomolar levels in the OE (Hegg *et al.*, 2003, *J. Neurosci.*, 23:8291–8301) and thus we hypothesize that ATP release is regulated in the OE. Recent evidence suggests that the connexons (gap junction hemi-channels) and the ATP binding cassette (ABC) transporters have an important role in extracellular paracrine signaling in many epithelial tissues. In the current study, we examined the role of connexons and ABC transporters in the release of the neuromodulator ATP into the extracellular milieu of mouse OE. ATP release was evoked by low concentrations of 3-methylindole, a potent olfactotoxicant. Concomitant lactate dehydrogenase release was measured to insure that ATP release was not solely due to cell lysis. We found that inhibitors of ABC transporters altered ATP release while connexon inhibitors did not have any effect on toxicant-evoked ATP release. ATP released by injured cells could act as a signal of cell damage, evoking HSP expression and initiating regeneration due to the mitogenic and growth-promoting effects of purinergic receptor activation.

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Poster: Olfactory Regeneration

Molecular events during regeneration of olfactory sensory neurons

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Regeneration of olfactory sensory neurons (OSNs) involves at least four distinct cellular stages. The sheer scale of these events is expected to require massive molecular changes during olfactory regeneration, some of which can be predicted. Using expression profiling and bioinformatics, with validation by quantitative RT-PCR and *in situ* hybridization of key gene products, we tested these predictions in an olfactory bulbectomy (OBX) model. The 1205 affected probe sets represented several biological processes, including apoptosis, immune response, cell cycle activation, proliferation, differentiation and mature neuron markers (which decreased). Many of the several hundred probe sets that decreased after OBX were not previously known to be expressed by mature OSNs. The immune response was temporally complex, consisting of chemokine recruitment of monocytes, suppression of their migration and then dendritic cell maturation. Activators of all phases of the cell cycle and enzymes necessary for DNA replication were increased. Several dozen cell signaling mRNAs and at least 16 transcription factors known to be involved in proliferation, development and differentiation in other tissues were increased. A cohort of mRNAs involved in the elaboration of neurites and cytoskeletal biogenesis also increased. These results indicate that the major processes involved in olfactory regeneration are known, but we found that the identity of many of the individual gene products in these groups is often surprising.

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Poster: Olfactory Regeneration

Delayed olfactory nerve regeneration in apoE-deficient mice

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Apolipoprotein E (apoE), a lipid transporting protein, is extensively expressed in the primary olfactory pathway, but its function is unknown. We previously reported increased apoE levels in the olfactory bulb (OB) following olfactory epithelium (OE) lesion in mice, and hypothesized that apoE may play a vital role in olfactory nerve (ON) regeneration. To directly test this hypothesis we examined the rate of ON regeneration following OE lesion in apoE deficient/knockout (KO) and wild type (WT) mice. OE was lesioned in 2- to 3-month-old mice by intranasal irrigation with Triton X-100 (TX). OB were collected at 0, 3, 7, 21, 42 and 56 days post-lesion. OB recovery was measured by both immunoblotting and immunohistochemical analysis of growth cone associated protein (GAP) 43 and olfactory marker protein (OMP). The results revealed that (i) OMP recovery in the OB was significantly slower in apoE KO compared with WT mice; (ii) recovery of glomerular area was similarly slower; and (iii) GAP43 increases and return to pre-lesion levels in the OB were slower in KO mice. Together, these results show that olfactory nerve regeneration is significantly slower in KO mice as compared with WT mice, suggesting apoE facilitates olfactory nerve regeneration.

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Poster: Olfactory Regeneration

Recovery after olfactory axotomy in mice: pharmacologic and genetic effects

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Apoptotic death of olfactory sensory neurons (OSNs) has been implicated in most cases of peripheral smell loss. The standard experimental model of OSN apoptosis is surgical axotomy resulting in the rapid death of most neurons within 72 h; recovery occurs by the regeneration of new OSNs over the next several weeks. Genetic deletion of the Bax protein has been shown to result in resistance to many pro-apoptotic stimuli. Similar resistance has been documented with the drug minocycline, which is believed to induce Bcl-2 protein a Bax antagonist. Histologic studies from our laboratory have demonstrated inhibition of post-axotomy OSN apoptosis in both bax KO mice and mice treated with minocycline but it is unclear if these OSNs remain viable and capable of participating in the recovery process. In the current study, the time course of electrical recovery post-axotomy will be tracked in mice wherein apoptosis has been inhibited. Methods: unilateral olfactory axotomy

was performed in three groups of mice: controls, bax KO and mice treated with minocycline prior to axotomy and daily for 4 days post-op. Electrical olfactory responses (EOR) and histology were assessed post-injury. Results: anatomic and electrical recovery in bax KO and minocycline-treated mice occurred significantly earlier when compared with controls. Conclusion: recovery following olfactory axotomy is faster in those mice wherein apoptosis is inhibited. These findings support the following hypothesis: OSNs that survive axotomy are able to recover, re-sprout axons and synapse with the bulb. Minocycline may be useful in the management of a wide range of olfactory disorders.

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Poster: Olfactory Regeneration

EASE analysis of gene expression profiles from murine olfactory mucosa following target ablation

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Degeneration of olfactory sensory neurons (OSNs) following target ablation (OBX) induces altered gene expression profiles in the olfactory epithelium (OE). The specific aim was to identify over-represented categories of significantly regulated genes at 48 h following OBX-induced OSN apoptosis in C57BL/6 male mice. Genes that were significantly up- or down-regulated with $P < 0.05$ when compared with sham controls were uploaded into EASE (Expression Analysis Systematic Explorer) and run separately against the entire Affymetrix MG-U74Av2 GeneChip population with GO Biological Process as the classification parameter. Categories with an EASE score of <0.01 were considered over-represented. Significantly down-regulated genes had five over-represented categories, with a minimum EASE score of 1.13E–3. In contrast, significantly up-regulated genes had 14 over-represented categories, with a minimum EASE score of 8.68E–9. Eleven of the 14 categories (79%) fell under the inclusive 'immune response' designation. These results identify the up-regulation of immune response genes as a key genomic trend at 48 h post-OBX to facilitate the degeneration/regeneration cycle of the OE following target ablation.

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Poster: Olfactory Regeneration

Remodeling of the olfactory bulb and epithelium in response to NMDA-mediated excitotoxicity

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We have used an NMDA lesion of the olfactory bulb in mice to excitotoxically kill ORN target neurons and examine the ORN response to target loss. More than 90% of mitral and tufted cells were lost by 4 days after lesion. Despite the widespread destruction of multiple bulb neurons and disruption of olfactory bulb cytoarchitecture, some OMP-expressing axons persisted in glomeruli. From 4 to 30 days after lesion, the proportion of GAP 43+ nerve fibre layer and glomerular axons steadily increased, suggesting either a regeneration or neurogenesis response within the OE. At 4 days after NMDA, we detected a slight increase (20–38%) in TUNEL+ ORNs only in patches of OE (compared with contralateral OE). By 8 days, the OE decreased its mature OMP+ ORN population by 20% and increase its complement of PCNA-positive basal cells. This neurogenetic response and increase in GAP43+ ORNs indicated that only 20–30% of ORNs died after target neuron loss. In the olfactory bulb, the radial migratory path of doublecortin+ neuroblasts from the rostral miratory stream was lost by 4 days following lesion. Despite being cut off from the rostral migratory stream, TH+ periglomerular neurons persisted, and appeared to maintain glomeruli during the regeneration response. From 4–30 days, Doublecortin+ neuroblasts accumulated in the internal plexiform layer. This suggests mitral cells are essential for neuroblast migration in the olfactory bulb, and that both OE and bulb mount an attempt to rebuild the olfactory neuraxis following this lesion.

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Poster: Olfactory Regeneration

Altered chemokine expression profiles following target ablation in the olfactory epithelium of scavenger receptor A-deficient mice

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Olfactory bulbectomy (OBX) results in apoptosis of olfactory sensory neurons (OSNs). Resident macrophages infiltrate the olfactory epithelium (OE) and phagocytose dying OSNs; this triggers chemokine release, causing additional infiltration of phagocytic macrophages that stimulate neurogenesis and tissue remodeling. Scavenger receptor A (SR-A) is a macrophage adhesion receptor that participates in the uptake of apoptotic cells. We investigated cellular and gene expression changes in wild type C57BL/6 (wt) and C57BL/6 SR-A-deficient ($MSR^{-/-}$) mice at 0 h (sham OBX) and at short time intervals (2, 8, 16 and 48 h) after OBX. The occurrence of reduced numbers of macrophages and fewer proliferative OSN progenitor cells in the OE of $MSR^{-/-}$ compared with wt mice was demonstrated with immunohistochemistry. Using Affymetrix MG-U74Av2 GeneChips (three per time point, 30 in total), we identified chemokines whose expression was altered in $MSR^{-/-}$ mice. A 2×5 ANOVA and 1% false discovery rate calculation demonstrated significant differences in the direction and/or magnitude of gene expression for 4 CC chemokines, 3 CXC chemokines and 1 chemokine receptor in the $MSR^{-/-}$ mice. These results identify possible mechanisms associated with impaired infiltration of macrophages and neurogenesis in $MSR^{-/-}$ mice.

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Poster: Olfactory Regeneration

Proteomic and transcriptional analysis of aging in murine olfactory epithelium

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Decline of olfactory function has been associated with aging. The aim of our study was to gain insight into the proteomic and transcriptional regulation occurring in the olfactory epithelium (OE) of aging mice. The OE was harvested from C57BL/6 male mice, 6 weeks old (young, $n = 5$) and 80 weeks old (old, $n = 5$); protein was extracted for proteomic analysis, and RNA was extracted for transcriptional analysis. The proteins were separated using 2-D gels, first by p*I* with isoelectric focusing (pH 3–10) and then by size with electrophoresis. The gels were stained with Sypro Ruby. Nine significantly different ($P < 0.05$) spots were excised and subjected to trypsin digestion and analysis by MALDI-TOF mass spectrometry. The spectra were matched to the MASCOT database and six proteins were identified (Mowse > 61). The proteins were classified using the Swiss-Prot database under the functional categories of metabolism, stress and transport. Based on their known association with aging, several proteins were selected for transcriptional validation of corresponding genes using real-time RT-PCR. The direction of regulation at the transcriptional level was concordant with that at the proteomic level for 66% of the genes assayed. These initial results characterize altered molecular profiles, both at the proteomic and transcriptional levels, in the aging murine OE.

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Poster: Olfactory Regeneration

Expression of Pax6 in olfactory epithelium

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The paired homeodomain transcription factor Pax6 plays an important role in multiple systems and stages during development. The nasal cavity does not form in homozygous Pax6 mutants, suggesting that Pax6 may be crucial in olfactory development. Taking advantage of the complete reconstitution of olfactory epithelium (OE) after direct damage by inhalation of methyl bromide (MeBr), we examined the expression pattern of Pax6 in normal and MeBr-lesioned rats using immunohisochemistry. In normal OE, Pax6 is expressed strongly in horizontal basal cells and sustentacular (Sus) cells, but is also present in duct/gland cells and some globose basal cells (GBCs). Of the Pax6 (+) GBCs the majority also express Ki67 and are proliferating. However, other Pax6 (+) GBCs are not labeled with anti-Ki67, but do express p27Kip1 and are presumably quiescent. Still other Ki67 (+) GBCs are not labeled with anti-Pax6. Therefore, the expression pattern of Pax6 not only varies across the epithelial cell types, but also defines subsets of GBCs in normal OE. At early stages in OE regeneration, Pax6 is expressed by most remaining cells and their progeny, including basal cells; at later stages the normal pattern re-emerges as Pax6(–) neurons come to reside between the differentiating Sus cells and the basal cells. The results in normal and regenerating OE suggest that Pax6 is expressed by multipotent GBCs and is consistent with the anri-Pax6 labeling of radial glia in the developing cerebral cortex, which also function as multipotent neural progenitors.

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Poster: Olfactory Regeneration

Analysis of the spatial organization of the retinaldehyde dehydrogenases in the adult olfactory mucosa

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In many biological settings, position within a tissue field correlates with gradations in the concentration of RA, which in turn derive from differential expression of the retinoic acid synthetic enzymes known as the retinaldehyde dehydrogenases (RALDH-1, RALDH-2 and RALDH-3). This may also be true in the olfactory mucosa (OM) based on published work suggesting that the enzymes and the cellular response to RA in the embryo are more concentrated in the ventrolateral regions of the OM as compared with dorsal, as is RALDH2 expression in adults. We have used *in situ* hybridization and immunohistochemistry to demonstrate that the pattern of RALDH expression in the embryo is maintained in the adult OM. All three enzymes are expressed at high levels in ventrolateral OM, and the shift from high to low expression of the RALDHs matches the boundary defined by differential expression of OCAM/mamFasII. In addition, we have shown that each of the three RALDH enzymes is expressed by a specific subset of cells in the mucosa. Both RALDH-2 and -3 are expressed in the lamina propria, but in different cell types. In contrast, RALDH-1 is expressed by sustentacular cells. Following recovery from lesion of the OE by methyl bromide the expression patterns of the RALDHs are re-established. These results suggest a potential role for RA both in maintaining the spatial organization of the OE in the normal state and in re-establishing it during regeneration after lesion.

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Poster: Multimodal Neurophysiology & Behavior

Electrical activity of local olfactory interneurons in the crayfish brain is modified by hydrodynamic input

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Lateral antennular filaments of the crayfish *Procambarus clarkii* possess four types of cuticular sensilla. The most conspicuous of these are aesthetasc sensilla (AE), blunt hair-like structures roughly 100 μm long and ~10 μm wide, which house the distal

dendrites of olfactory receptor neurons (ORNs), the only sensory neurons known to send axons to the olfactory lobes (OL) of the midbrain. The three other sensillum classes, homologous with those described on another crayfish, *Cherax destructor*, are short (50–100 μm long) companion hairs, long (200 μm) guard hairs and non-innervated procumbent feather hairs. Axons of the innervated, non-AE sensilla probably terminate within the lateral antennular neuropil (LAN); local interneurons receiving input in the LAN can also be excited by input to the OL. Here I report that sharp electrode record responses to hydrodynamic as well as to odorant stimuli stimuli from interneurons within the OL itself or within cell cluster 11. Among the most commonly encountered neurons within the OL are those responding to the onset of water flowing past the antennules with a brief EPSP, a prominent IPSP and a post-inhibitory spike discharge; some of these cells also respond to odorant stimuli, and the odorant response was inhibited when superimposed upon the response to hydrodynamic input. Other cells that responded with excitation to both stimulus modalities exhibited reinforcement; their summed response was stronger than the response to either modality by itself. These findings indicate that mechanoreceptor inputs from the lateral filament access interneurons within the OL and can modulate or reinforce olfactory input from the AE.

Poster: Multimodal Neurophysiology & Behavior

Interactions between the posterior piriform cortex, forelimb motor cortex and red nucleus during an olfactory guided, skilled reaching task

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Olfactory cortex is thought to be the most ancient vertebrate cortex, and its connections with motor cortex and midbrain motor nuclei constitute an ancient sensorimotor interface (Lacalli, 2001; Aboitiz *et al.*, 2003). Using simultaneous recordings of multi-single units and local field potentials, we tested the flow of information between the output layers of the posterior piriform cortex, the forelimb motor cortex and a midbrain motor site, the magnocellular red nucleus, as rats performed a well-learned, olfactory driven reaching task. We applied partial directed coherence to the local field potential data to determine whether activity in any area preceded or lagged behind activity in any other area during the task. Strikingly, throughout all analyzed trials, activity in the piriform and motor cortices preceded that in the red nucleus at all frequencies between 0 and 100 Hz. In contrast, on 40% of analyzed trials, activity in the motor cortex preceded that in the piriform cortex at the end of target sniffing, followed within 30 ms by classical coherence between the two areas in the low gamma (30–50 Hz) range. During target sniffing, the firing rate of a large subpopulation of neurons in each area was transiently inhibited, and the presence or absence of this inhibition strongly predicted task outcome. We are currently testing the hypothesis that the gamma oscillations resulting from odorant sniffing, and deriving, at least in some vertebrate olfactory systems, from the activity of networks of inhibitory interneurons (Friedrich *et al.*, 2004), cause transient unit inhibition starting in the motor cortex and speading to the posterior piriform cortex.

Poster: Multimodal Neurophysiology & Behavior

Chemosensory event-related potentials: different from other senses?

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The event-related potential (ERP) technique is an excellent method of investigating rapid perceptual processes. However, there is yet limited understanding for how variation in ERP parameters compares between the senses. The present study investigated how individual variation in ERP parameters was correlated between sensory modalities. It introduces a multi-sensory ERP protocol, in which a simple auditory, visual and chemosensory (15% pyridine) stimulus is presented in one randomized sequence. Thus, by keeping properties of task (button-press), stimulus presentation (duration, ISI) and state of the individual (attention, arousal) constant across sensory modalities, direct cross-modal comparisons are enabled. Eighteen healthy young adults were subjected to testing. The results show that auditory, chemosensory and visual stimuli evoked similar ERP responses, including N1, P2 and P3 components. Cross-modal correlations occurred mainly between P3 amplitudes of the ERP at the parietal recording site (Pz). These findings suggest that individual, modality-unspecific factors affect ERP parameters, and that the chemosensory P3 resembles the visual and auditory P3 in this aspect. However, the central processing of chemosensory information differs from that of visual and auditory senses in two aspects: the chemosensory P3 was smaller than the P3 of the other senses, even though the P2 was equivalent between senses; and furthermore, chemosensory processing was slower than auditory and visual processing, as measured by the latency difference between components. The findings are relevant for the discussion on the nature of the late positive complex within the chemosensory ERP.

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Poster: Multimodal Neurophysiology & Behavior

Ear and nose: similar networks in familiarity processing

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Odor and music share multiple features, including their ability to evoke strong memories and emotions and the difficulty to be described and identified. To examine common neural substrates of olfactory and auditory events, our study focused on recognition memory and more particularly the feeling of familiarity. Thirteen right-handed male students underwent two fMRI scans (3 T). Each scan was divided in 12 periods of 90 s, six for odors and six for musical excerpts. Each period included six familiar and six unfamiliar items selected on the basis of behavioral pretests. Subjects pressed one of two buttons to judge item familiarity. We performed whole brain random-effects analysis (SPM2) in a short-block design and region of interest analysis (ROI, MarsBaR). We compared neural networks involved in familiarity judgements in olfaction and audition, and differentiated responses pattern of familiar and unfamiliar items

(i.e. based on individual responses). Compared with resting state, familiarity judgements involved the following neural network common to the two modalities: left cingulate gyrus, amygdala and medial insula, and right cerebellum. Compared with unfamiliar items, familiar items activated additional regions previously observed for semantic and memory processing: left inferior frontal gyrus, angular gyrus, anterior cingulate gyrus and precuneus. The left hippocampal ROI was also activated. The unfamiliar items activated no additional regions. This outcome suggests a multimodal network of familiarity, and that the feeling of familiarity needs the activation of an additional neural network in comparison to that of unfamiliarity.

Poster: Multimodal Neurophysiology & Behavior

The amygdala/piriform encodes incentive salience of odors predicting an appetitive drink

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In everyday life, the sight and smell of food often signify the availability and subsequent ingestion of that food, and thus can trigger conditioned responses (e.g. salivation). This indicates that these sensory cues have incentive salience; that is, experiencing them causes a 'wanting' of the reward that is distinct from the pleasure associated with receipt of the food itself (Berridge *et al.*, 1996). Previous studies have investigated the neural response to visual stimuli predicting food odors (Gottfried *et al.*, 2003) or tastes (O'Doherty *et al.*, 2002) but none have looked at olfactory cues predicting taste rewards. In the present study we used fMRI to assess the neural response in 15 subjects to the smell of a pleasant drink (CS+) generally followed by receipt of that drink and to the smell of a pleasant drink (CS–) followed by a tasteless solution. After modeling out the effects of receiving the taste reward or tasteless solution, comparison of CS+ versus CS– showed that CS+ odors preferentially activated the right posterior OFC extending through frontal and temporal piriform cortex, lateral amygdala and anterior hippocampus. Receipt of the pleasant drink versus receipt of the tasteless solution did not activate this region. These results are in accordance with previous findings indicating a role for the amygdala/piriform cortex in encoding incentive salience. Furthermore, we show that the response is specific to incentive salience and does not generalize to the pleasure associated with receipt of reward.

Poster: Multimodal Neurophysiology & Behavior

Simultaneous gustatory stimuli modulate ortho- and retronasal olfaction

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The aim of this study was to examine whether brain activity differs during ortho- and retronasal odorant perception when sensed simultaneous with gustatory stimuli. Both olfactory event-related potentials (OERP) and functional MRI (fMRI) were used. Thirty-two healthy subjects took part in three test sessions. OERP (sessions 1 and 2) were recorded in response to phenylethyl alcohol or vanillin. Each session consisted of four randomized blocks which were applied either orthonasally or retronasally. Simultaneously we applied sweet or sour gustatory stimuli; 16 subjects participated in the fMRI experiment (session 3), which was performed on a 1.5 T Siemens Sonata system. Olfactory stimuli (vanillin) were applied ortho- or retronasally; in addition, small volumes of intensity matched gustatory stimuli (sucrose, citric acid) or water were administered. OERP latencies P2 were significantly shorter during the 'sweet condition' for retronasal stimulation than for orthonasal stimulation, indicating that taste facilitates the processing of olfactory information more during retro- versus ortho-presentation. In the fMRI session (using sweet gustatory stimulation), stronger activation was found in the right-sided posterior temporal lobe when olfactory stimuli were administered retronasally compared with orthonasal stimulation. In contrast, orthonasal compared with retronasal stimulation produced bilateral insular activation. In conclusion, application of a sweet taste significantly enhanced the processing of olfactory information when odorous stimuli were presented through the retronasal route. Moreover, fMRI data revealed the differential influences of gustatory stimulation on ortho- and retronasal odorous stimulation.

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Poster: Multimodal Neurophysiology & Behavior

Ethanol–taste interaction: the role of smell

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The effects of ethanol and ethanol smell on perithreshold tastes were studied. Seventeen subjects (seven male) participated. Aqueous solutions of (mM) sucrose (7.31–61.40), citric acid (0.39–1.29), caffeine (0.25–2.00), NaCl (4.27–43.18) and their ethanol mixtures (final ethanol %: 6, 12) were used. Detection threshold measures were ascending limits with forced choice. Results were expressed as 'detection probability' ('positively detected to total cases' ratio) for each concentration. Data were submitted to MANOVA followed by individual ANOVAs with LSD according to taste quality (SPSS 10.0^{TM}). Ethanol affected sweetness and bitterness: $F_{Sw}(1,64) = 4.02$, $P < 0.05$; $F_{Bitt}(1,64) = 7.54$, $P < 0.008$. Gender dependence for ethanol effects was relevant only for bitterness [ethanol × gender interaction: $F_{Bitt}(1,64) = 4.19$ $P < 0.04$]. Response to ethanol was modified by closing nostrils (no smell) [$F_{nostrils}(1,128) = 4.305$, $P < 0.04$]. Ethanol at 6% did not affect sweetness except for interaction at the lowest sucrose concentration in male [ethanol grade × concentration × gender interaction: $F(1,64) = 5.06$ $P < 0.03$], while 12% ethanol affected sweetness to a lesser extent than observed with nostrils open [$F(1,64) = 6.17$ $P < 0.02$]. Male were more sensitive than women to the ethanol–sweetness interaction. Present results suggest that chemosensory factors other than taste may likely be involved in the ethanol–taste interaction. Also, genetic taste variation may underlie neurophysiological sensitivity to the ethanol–taste interaction.

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Poster: Multimodal Neurophysiology & Behavior

Olfactomotor coupling during skilled reaching in rats

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Despite the existence of electrophysiological techniques for recording from multiple brain areas simultaneously in awake animals, relatively little is known about the nature of the transient coordination between olfactory and motor processing areas during distinct phases of olfactomotor tasks. The aim of our study was to identify the mechanism(s) of coordination among neurons in the output layers of posterior piriform cortex, the forelimb motor cortex and the midbrain red nucleus during performance of an olfactory-guided, GO/NO-GO reaching task. Recording multi-single units and local field potentials in all three areas, we found highly significant, transient, temporally overlapping (i) inhibition of spike firing rate and (ii) membrane hyperpolarization during odor sampling on GO trials with the real food pellet that was not observed on NO-GO trials with chemically inert, visually identical non-food pellets. Moreover, by examining time–frequency-decomposed local field potentials, we found that during odor sampling on GO trials, a transient increase in high-gamma energy occurred, whereas low-gamma energy was present during odor sampling on both the GO and NO-GO trials. All three of the above observed phenomena occurred within half a theta cycle. In concert with studies demonstrating the major role played by the dynamics of inhibitory networks in sculpting neural activity, these findings suggest that the co-occurrence of transient spike rate inhibition, membrane hyperpolarization and high-gamma oscillations are part of the mechanism coupling disparate sensory, motivational and motor circuits prior to task execution.

Poster: Multimodal Neurophysiology & Behavior

Surprising tongue: effects of electrotactile sensory substitution

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Purpose: to use an anterior part of the dorsal tongue surface as an electrotactile brain–machine interface for visual and vestibular sensory substitution. Methods: the tongue electrode array is made of 144 gold-plated 1.5 mm diameter circular electrodes. All electrodes are separated by 2.34 mm, and are gold plated for biocompatibility. The electrotactile stimulus is produced by a custom 144-channel waveform generator in bursts of three 40 μs pulses delivered at a rate of 50 Hz with a 200 Hz pulse rate within a burst. Sensation thresholds was measured under each electrode for 11 subjects, and 2-D intensity maps were plotted. For 22 vestibular subjects with bilateral vestibular loss the signal from a 2-D head-mounted accelerometer was delivered through the tongue. For six blind subjects, the output from a small digital video camera was presented to the individual electrodes on the array. Results: first, we noticed a short learning period that continue for 10–20 min in vestibular and for 3–5 h in blind subjects. Second, we observed very strong effects of sensory substitution: vestibular patients were capable of recovering stationary and dynamic stability almost completely. Blind patients were capable of navigating by themselves in a room, of dynamically correcting their hand position to catch a rolling ball and recognizing simple shapes. Conclusions: the dorsal surface of the human tongue can be used as an efficient brain–machine interface for electrotactile sensory substitution in vestibular and visually impaired subjects.

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Poster: Multimodal Neurophysiology & Behavior

The pH of chemical defenses in sea hare *Aplysia californica* modulates behavior and chemosensory response in the spiny lobster *Panulirus interruptus*

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Ink and opaline secretions are released by sea hares (*Aplysia californica*) upon attack by predatory spiny lobsters (*Panulirus interruptus*), and these secretions increase survival of sea hares. The secretions contain extremely high concentrations of chemicals such as amino acids that are highly excitatory to the olfactory and gustatory pathways of lobsters. This sticky secretion protects sea hares through mechanisms that include phagomimicry (stimulating the chemosensory neurons in the feeding pathway, leading to manipulation of the secretions rather than sustaining the attack on the sea hare) and sensory disruption (strong and sustained stimulation of the chemosensory neurons, leading to grooming and escape behaviors rather than attack). Since ink and opaline are acidic (pH of 4.9 and 5.8 respectively), we chose to examine the role of pH in this chemical defense. We tested a single concentration of ink and opaline at three pHs—4.9, 6.3 and 7.7—on spiking responses of lobster chemosensory neurons in the mouthparts and antennules and on behavioral responses of lobsters (moving toward or grabbing the secretion). The chemosensory neurons responded to ink and opaline more strongly at lower pH. In addition, behavioral responses of lobsters to the secretions were stronger at lower pH. This correlation is consistent with the idea that the low pH of these sea hare defensive secretions improves their defensive properties against lobsters by enhancing the secretions' excitatory properties on chemosensory neurons. We are currently examining these effects at different concentrations of secretions.

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Poster: Multimodal Neurophysiology & Behavior

Verbal context influences flavor perception

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Verbal context has been repeatedly shown to influence the psychophysical attributes of odorants. The effect of verbal context on 45 participants' judgements of the flavor of beverage samples was examined. Participants were randomly allocated to one of three groups and were shown three samples of liquids (2.5, 7.5 and 90% soy milk diluted with water). Depending upon group

membership, participants were either given no description or told one of two cover stories about the origins of the samples: that the samples were an attempt to alter the fat content of milk by adding a natural ingredient (Milk group) or that the samples were obtained from tap water in the city of Syracuse where chalk deposits were plentiful (Chalk group). Participants were then asked to taste each of the three samples and to rate them for pleasantness, intensity, creaminess and chalkiness using unstructured line scales. Separate repeated measures ANOVAs were conducted for each rating. Main effects of dilution level were observed. Results indicated main effects of instruction group only for the ratings of strength [$F(2,42) = 5.7$, $P = 0.006$)] and chalkiness [$F(2,42) = 4.7$, $P = 0.014$], with the Chalk group giving the highest ratings for both cases. These results are similar to those found with olfactory stimuli in that participants seem more susceptible to negative manipulations (Herz, 2003), and underscore the importance of verbal information in providing a context for chemosensory perception.

Poster: Taste: Animal Behavior

Amiloride-adulterated NaCl tastes like KCl and na gluconate tastes like NaCl to the Na-depleted rat

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Following Breslin *et al.* (1993, *Am. J. Physiol.*, 264:R312), I used a brief-access licking test to compare the relative acceptability of several salt solutions for the sodium-depleted rat across a wide range of concentrations (0.028–0.89 M, quarter-log steps). Breslin *et al.* showed that furosemide-injected rats and non-deprived rats treat NaCl similarly (licking maximally at concentrations near isotonic), but that behavior was potentiated at all concentrations in the deprived rat. This inverted-U shaped 'fingerprint', I argue, can be used to estimate both qualitative and intensive similarity to NaCl: leftward or rightward shifts would indicate qualitatively similar stimuli that are either more or less intense at isomolar concentrations; downward shifts or changes in the shape of the concentration-response function would indicate qualitative dissimilarity. Rats treated NaCl and Na gluconate nearly identically; KCl was unmotivating except at a single concentration (0.158 M). Amiloride (10, 30, 100 and 300 μM) paramatrically shifted the shape of the function from NaCl-like to KCl-like. Coupled with previous work using the NaCl transduction blocker cetylpyridinium chloride (St. John and Hallagan, in press), these results have implications for the role of amiloride-sensitive and -insensitive pathways in salt taste quality coding.

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Poster: Taste: Animal Behavior

Lick responsiveness of eight mouse strains to sweeteners: effects of allelic variation in the *Tas1r3* gene

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Recent studies have established that the T1R3 receptor plays a central role in the taste-mediated response to sweeteners by mice. First, transgenic mice lacking the gene for T1R3, *Tas1r3*, show dramatically reduced lick responsiveness to low, mid-range and high concentrations of most sweeteners. Second, strains with the taster allele of *Tas1r3* (T strains) are more sensitive to low sweetener concentrations than strains with the nontaster allele (NT strains). We asked how allelic variation in *Tas1r3* influences taste-mediated ingestive responses to a range of concentrations of sweeteners (sucrose and SC45647). To this end, we compared lick responsiveness of four T strains (FVB/NJ, SWR/J, SM/J, C57BL/6J) with that of four NT strains (BALB/cJ, 129P3/J, DBA/2J, C3H/HeJ). We measured lick responsiveness with two short-term intake tests: a brief-access taste test and a 1 min preference test. Because of the short duration of these tests, the contribution of non-gustatory factors (e.g. negative and positive postingestive feedback) to the licking response was minimized. The T strains exhibited significantly higher lick responsiveness at the low sweetener concentrations, but not at the mid-range or high sweetener concentrations. In fact, at the mid-range and high sweetener concentrations, strain differences in lick responsiveness appeared to vary independently of *Tas1r3* allele status. These findings indicate (i) that structural differences in the T1R3 receptor alone cannot reliably predict a mouse's immediate licking response to mid-range and high concentrations of sweeteners; and (ii) that additional genes must contribute significantly to individual variation in sweetener intake.

Poster: Taste: Animal Behavior

Oleic and linoleic acid alters licking responses to sweet, sour, salt and bitter tastants in rats

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Previously our laboratory demonstrated that the addition of 88 μM linoleic acid to sweet, sour, salty and bitter tastants produced changes in the licking response of rats suggesting a perceived increase in the tastant intensity. This experiment extends that research to 88 μM oleic acid and a mixture of linoleic (66%) and oleic (33%) acid approximating the proportions of each free fatty acid found in corn oil. The licking behavior of 12 male Sprague–Dawley rats to concentrations of sucrose (15–250 mM), glucose (15–500 mM), NaCl (30–1000 mM), citric acid (1.5–60 mM) and quinine–HCl (0.003–1 mM) presented as 20 s trials in a Davis Rig apparatus was examined with and without the addition of either 88 μM oleic acid or a mixture of linoleic and oleic acid approximating an 88 μM concentration of the combined free fatty acids. The addition of either oleic acid or the mixture of linoleic and oleic acid significantly increased the licking responses to glucose in water-replete rats. Also in water-replete rats, the addition of oleic acid produced a slight but insignificant increase in the licking response to sucrose; however, the free fatty acid mixture did significantly increase the licking response to sucrose. The addition of either oleic acid or the mixture of linoleic and oleic acid significantly decreased the licking responses to NaCl, citric acid and quinine–HCl in water-restricted rats. These data further support the theory that free fatty acids may prolong the depolarization of taste receptor cells in response to taste stimuli through inhibition of K^+ channels, thus acting to increase the perceived intensity of tastants.

Poster: Taste: Animal Behavior

The role of the chorda tympani nerve in the detection of free fatty acids in rats

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Previous research has shown that rat taste receptor cells can transduce free fatty acid chemical components of dietary fat. This study characterizes the ability of Sprague–Dawley rats with bilateral chorda tympani transections to detect and avoid short-duration (8 s) stimulus presentations of both linoleic acid (LA) and oleic acid (OA) following a conditioned taste aversion pairing with one of the free fatty acids. Following the single pairing of a LiCl injection with consumption of 88 μM LA, rats with an intact chorda tympani nerve (n = 10) significantly avoided future consumption of LA at concentrations ≥88 μM; however, rats with bilateral chorda tympani transections (n = 16) did not avoid LA at any concentration (44, 88, 176 μM), consuming amounts similar to control animals receiving a saline injection. Previously, our laboratory has demonstrated both taste aversion formation to OA as well as stimulus generalizations between LA and OA using 1 h two-bottle preference tests. Interestingly in this protocol, neither chorda tympani intact nor bilaterally transected animals avoided OA when conditioned to avoid either 88 μM LA or OA. Our data suggest that the ability of rats to detect and avoid LA is likely due to neural signals transduced by the fungiform papillae and transmitted through the chorda tympani nerve. The lack of evidence for a CTA to oleic acid may be due to an inability to form a CTA to OA, an unlikely result given our previous research, or the 8 s stimulus duration may not have allowed adequate stimulation of the circumvallate papillae to permit a behavioral demonstration of a CTA to OA. Future studies will lengthen the stimulus duration during assessment of the CTA formation to OA in order to clarify the detection parameters.

Poster: Taste: Animal Behavior

The role of the chorda tympani nerve in linoleic acid detection by male and female rats

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Previous research from our laboratory shows that female rats increase licking rates to lower concentrations of linoleic acid (LA), a main component of dietary fat, than do male rats in 10 s trials. Although these results suggest that there are different detection thresholds for LA by male and female rats, the mechanisms underlying these differences remain unexamined. The goal of the present studies was to determine what role, if any, the gustatory chorda tympani nerve (CT) plays in LA detection. Bilateral transections of the CT drastically impaired the ability of both male and female rats to generalize a conditioned taste aversion to low concentrations of LA, suggesting that the CT is necessary for LA detection. Accordingly, we also recorded whole nerve, electrophysiological activity from the CT in response to lingual application of a range of LA concentrations (11–88 μM) in male and female rats. Contrary to expectations, the CT was unresponsive to all LA concentrations. These conflicting results suggest that LA detection requires an intact CT; however, detection may be mediated via a small subset of CT fibers whose responses are obscured in whole nerve recordings. Alternatively, LA may produce a subtle inhibition of CT activity that typical recording techniques lack the sensitivity to detect. Finally, LA detection may depend upon a combination of tastants that requires the interaction of multiple gustatory sensory nerves. Any of these mechanisms may underlie sex differences in LA detection thresholds.

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Poster: Taste: Animal Behavior

Microstructural analysis of NaCl consumption after brief dietary Na^+ deprivation

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Previous studies have shown that 8–10 days of dietary sodium deprivation are necessary to increase 24 h ingestion of a concentrated NaCl solution by rats. However, recent work from our laboratory showed that, in short term tests, licking responses to NaCl solutions increased following only 2 days of Na^+-deficient diet, suggesting a rapidly occurring change in NaCl taste responses. This study used microstructural analysis to examine patterns of NaCl consumption after 2 days of Na^+-deficient diet. Following 2 days of adaptation to 0.5 M NaCl, male rats (n = 20) received 48 h of either Na^+-deficient or regular (control) chow, and licking patterns and intakes of 0.5 M NaCl and H_2O then were examined for the next 24 h. As expected, 24 h intakes did not differ; however, patterns of ingestion were altered by the 2 days of Na^+ deprivation. Na^+-deficient rats licked significantly more during the first NaCl intake bout compared with control rats (843.9 ± 161.7 licks versus 113.7 ± 41.4 licks; $P < 0.01$), but the two groups had comparable numbers of licks for the remaining NaCl bouts. Na^+-deficient rats also had a significantly longer first NaCl bout than did controls (11.7 ± 2.1 min versus 2.9 ± 1.2 min; $P < 0.01$), but bouts were of comparable durations thereafter. These results show that 2 days of Na^+ deprivation are sufficient to alter patterns of ingestion of concentrated NaCl and provide further support for early changes in gustatory responses after brief dietary Na^+ deprivation.

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Poster: Taste: Animal Behavior

Relationships between insulin release and taste

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It is known that the food related sensory stimuli induces cephalic phase hormonal release. Thus, tasting sweet food elicits insulin release prior to increasing plasma glucose levels, it is called cephalic phase insulin release (CPIR). The characteristic of the CPIR is that the plasma insulin secrets within 2 min after oral sensory

stimulation, peak at 4 min and return to baseline in the 8–10 min post-stimulus time period. The functional role of CPIR is not known clearly. In this experiment, we examined any tastes which was placed on the tongue induced CPIR or not. We used female Wistar rats and five basic taste stimuli: sucrose (sweet), sodium chloride (salty), HCl (sour), quinine (bitter) or monosodium glutamate (umami). Rats reliably exhibit CPIR to sucrose. Sodium chloride, HCl, quinine or monosodium glutamate does not elicit CPIR. Sucrose has two typical characters, 'sweet 'and 'nutritive'. Then, we tested whether 'sweet 'or 'nutritive' elicits CPIR. As the results, the non-nutritive sweetener saccharine does elicit CPIR. However, the non-sweetener nutrition starch does not elicit CPIR. In addition, we studied whether the CPIR related with the taste receptor cell activity. We carried out the experiment that bilaterality cut off the chorda tympani nerve which is one of the gustatory nerve. Then the CPIR could not be recognized for the sweet stimulation. From these results, it was proven that CPIR was elicited by the conducted taste nerve sweetness information. It is considered that these results must inform the important comprehensible information for CPIR.

Poster: Taste: Animal Behavior

Sodium chloride preference in *Trpv1* knockout mice

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Sodium chloride (NaCl) transduction involves both amiloride-sensitive and amiloride-insensitive pathways. In rodents, the amiloride-sensitive pathway involves the epithelial sodium channel (ENaC), while the amiloride-insensitive pathway is thought to rely on the *Trpv1* channel (DeSimone *et al.*, 2004, *J. Physiol.*, 558:147–149). Studies involving chorda tympani recordings in *Trpv1* knockout (KO) mice demonstrate no functional amiloride-insensitive salt taste. We performed two-bottle preference tests with *Trpv1* KO mice compared with wild type C57/BL/6J mice for NaCl preference with and without amiloride. Surprisingly, *Trpv1* KO mice preferred NaCl (100–200 mM) to H_2O, with preference ratios in excess of 80% when amiloride was present. In contrast, wild type mice showed little or no preference for NaCl at these concentrations unless amiloride was present. These data suggest that additional NaCl detection mechanisms are present. Further studies are in progress to determine if the increased preference for NaCl in the *Trpv1* knockouts results from an increase in the detection threshold for NaCl.

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Poster: Taste: Animal Behavior

Orosensory detection of dietary lipids: role for the fatty acid transporter (FAT/CD36)

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Laboratory animals exhibit a spontaneous attraction for lipids. This observation raises the question of mechanism responsible for the oral detection of lipids. In the rat, the receptor-like fatty acid transporter FAT/CD36 appears to be a plausible candidate for this function since it has been found in lingual circumvallate papillae. To explore this hypothesis, experiments were conducted in wild type and FAT/CD36 null mice. The lipid-binding proteins screening throughout the lingual epithelium reveals that FAT/CD36 expression is restricted to papillae in contrast to the fatty acid transporter-4 (FATP-4). Immunostaining experiments demonstrate that FAT/CD36 is located in the apical side of receptor cells of taste buds in which it is sometimes coexpressed with α-gustducin. FAT/CD36 gene invalidation fully abolishes the preference for linoleic acid-enriched solution observed in wild type mice by the two bottle preference test. This behavior is lipid-specific since FAT/CD36 null mice exhibit the same preference and aversion than intact mice for sucrose and kinin solutions, respectively. Moreover, the rapid and sustained rise in the flux and proteins levels of pancreato-biliary secretions triggered by an oral lipid load in FAT/CD36$^{+/+}$ mice with a ligatured oesophagus is fully suppressed in FAT/CD36$^{-/-}$ mice. These new findings demonstrate that FAT/CD36 plays a crucial role in the orosensory detection of dietary lipids in the mouse.

Poster: Taste: Animal Behavior

Comparison of the tastes of MSG and glycine

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Previous studies have suggested that L-amino acids and 'umami' tastants activate the T1R1/T1R3 receptor (Nelson *et al.*, 2002; Zhao *et al.*, 2003). These findings indicate that L-amino acids and umami tastants have analogous taste qualities. Conditioned taste aversion (CTA) studies indicate that L-serine, L-alanine and L-proline have behaviorally comparable taste qualities to MSG (Duran *et al.*, 2005; Mitzelfelt *et al.*, 2004; Taylor-Burds *et al.*, 2004). However, discrimination methods have shown that L-serine and L-alanine are easily discriminated from MSG. Thus, while these amino acids share taste qualities with MSG, there are also qualitative differences between these substances. To further explore the role of the T1R1/T1R3 receptor in amino acid transduction, this study compared the tastes of glycine with MSG using CTA and discrimination methods in rats. CTA experiments were used to determine if glycine and MSG have similar taste qualities. Rats that were conditioned to avoid glycine or MSG (both with amiloride) and then tested for generalization to the opposite amino acids, showed that glycine and MSG have similar taste qualities ($P < 0.05$). Discrimination experiments were then conducted to assess qualitative differences between the two substances. The results show that rats can easily discriminate between the two substances, but have much more difficulty when amiloride or amiloride + NaCl were added to glycine to control for the Na^+ taste of MSG. Collectively these results suggest that the two tastants are detected by the same receptor but another receptor may also be involved.

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Poster: Taste: Animal Behavior

Generalization of conditioned taste aversion (CTA) to monosodium glutamate (MSG) and inosine monophosphate (IMP) in 129P3/J and C57BL/6BYJ mice

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In the two-bottle tests, mice from the C57BL/6ByJ (B6) strain drink more MSG and IMP than mice from the 129P3/J (129) strain, suggesting that they may differ in perception of MSG and IMP taste. To assess perception of taste quality of these compounds, we developed LiCl-induced CTA to 100 mM MSG or 10 mM IMP and examined its generalization to 25 taste stimuli. In both strains, mice trained to avoid 100 mM MSG avoided 100 mM and higher MSG concentrations, but not 10 mM and lower MSG concentrations or IMP. In both strains, mice trained to avoid 10 mM IMP avoided 3–10 mM IMP and 100–1000 mM MSG. Thus, cross-generalization between MSG and IMP was incomplete. Patterns of CTA generalization to other taste stimuli were not identical for 129 and B6 mice, suggesting that they differ in perception of MSG and IMP taste.

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Poster: Taste: Animal Behavior

Preliminary assessment of sucrose and MSG taste capabilities of T1R3 knockout mice

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Heterologously expressed T1R3 receptors in heterodimeric combination with other T1R family members detect sweet, umami and L-amino acid taste stimuli. Molecular expression studies (Nelson *et al.*, 2002) reported that T1R1/T1R3, a broadly tuned amino acid receptor, responds to monosodium glutamate (MSG) but not to sweet stimuli. In contrast, T1R2/T1R3 receptors respond to a wide variety of natural sugars and artificial sweeteners but not to umami substances. Based on results with knockout (KO) mice, T1R3 has been implicated in sweet and umami perception, although the magnitudes of the effects varied in two published studies. One study reported that T1R3 KO mice had a reduction in umami taste responses (Damak *et al.*, 2003), whereas another study with an independently developed T1R3 KO line reported the complete elimination of umami taste responses (Zhao *et al.*, 2003). We have conducted initial threshold experiments with T1R3 KO mice obtained from Damak *et al.* and found that these mice can detect sucrose and MSG nearly as well as wild type mice of the same genetic background. In a shock-avoidance/water-reinforcement discrimination paradigm, T1R3 KO mice can discriminate between the tastes of citric acid and MSG. They can also discriminate between sucrose and MSG (both with 100 μM amiloride) but only with difficulty. These findings suggest that other receptor mechanisms may also be involved in the detection of these substances.

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Poster: Taste: Animal Behavior

Generalization of CTA between monosodium glutamate and L-proline in rats

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Previous studies have shown that monosodium glutamate (MSG) and L-amino acids activate the same taste receptor T1R1/T1R3 (Zhao *et al.*, 2003). This suggests that MSG and other L-amino acids should elicit similar taste sensations. Conditioned taste aversion (CTA) methods can be used to identify taste substances that have similar taste qualities. Recent CTA studies reported that the amino acids L-alanine, L-serine and L-aspartate have taste qualities that mimic the qualities of MSG in the presence of amiloride (Delay *et al.*, 2004; Mitzelfelt *et al.*, 2004; Taylor-Burds *et al.*, 2004). The results of these studies suggest that a common receptor is detecting these amino acids. The current study used CTA methods to determine if the amino acid L-proline and MSG also have similar taste qualities. Water-deprived rats were conditioned to avoid either MSG or L-proline mixed with amiloride. They were then tested for generalization of the learned aversion to the opposite amino acid using brief access testing procedures. Amiloride was added to all solutions during testing. Analysis of the lick rates showed a cross generalization of CTA between L-proline and MSG, suggesting that L-proline and MSG have similar taste qualities that may be detected by the same taste receptor.

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Poster: Taste: Animal Behavior

P2X2/P2X3 double knockout mice do not respond to most taste stimuli

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Both P2X2 and P2X3 ionotropic receptors for ATP are present on the nerve fibers innervating taste buds (Bo *et al.*, 1999). Since ATP can serve as a fast, excitatory neurotransmitter, we utilized P2X2/P2X3 double KO mice to test whether these receptors were necessary for transmission of taste information. Mice knocked out for either P2X2 or P2X3, or both, were tested using two-bottle taste preference and lickometer tests. Double knockout mice did not respond to denatonium, sucrose, MSGor the artificial sweetener SC45647 at concentrations at which wild type siblings do. However, the P2X2/3 double KO mice do respond to caffeine, quinine and citric acid similarly to wild types. P2X2 and P2X3 single knockouts respond to SC45647 like wild types; P2X3 knockouts also respond to denatonium like wild types (P2X2 KO mice not yet tested). Histological examination of taste buds in P2X2/3 double KO mice

shows essentially normal morphology with a normal expression of gustducin, T1R3, SNAP-25 and other markers of the three principal taste cell types. *in situ* hybridization experiments indicate that expression of T1R and T2R taste receptors in P2X2/3 double KO mice is grossly normal. Our results strongly suggest that both P2X2 and P2X3 receptors on the nerve fibers are crucial for transmission of taste information. The residual chemical sensitivity in P2X2/3 double knockout mice might be mediated laryngeal solitary chemoreceptors (Sbarbati *et al.*, 2004) utilizing different synaptic mechanisms.

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Poster: Taste: Animal Behavior

Removal of BDNF from adult taste buds causes subtle behavioral and anatomical changes in mice

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Brain-derived neurotrophic factor (BDNF) is critical for proper development of the taste system, but BDNF expression persists into adulthood. To test the role of taste bud-derived BDNF in the adult mouse taste system, we have employed the cre-lox system to specifically remove BDNF from adult taste buds. We used a K14-CreERtam mouse (E. Fuchs, Rockefeller University; Vasioukhin *et al.*, 1999, *Proc. Natl Acad. Sci. USA*, 96:8551) in which cre recombinase is expressed in basal epithelial cells and administration of tamoxifen activates cre in these cells. We mated K14-CreERtam mice with BDNFlox mice. X-Gal staining of tongues from tamoxifen-treated K14-CreERtam/BDNFlox mice showed widespread reaction product indicating removal of BDNF from fungiform taste buds, with an incomplete removal from circumvallate taste buds. Taste preference studies in these K14-CreERtam /BDNFlox mice reveal no altered preference for sweet, umami or bitter compounds compared with tamoxifen-treated wild type control mice. However, the K14-CreERtam /BDNFlox mice show a slightly enhanced sensitivity to sour (citric acid). Initial anatomical studies indicate no gross alterations in fungiform or circumvallate taste buds. More quantitative investigation of the BDNF-deficient animals may reveal more subtle alterations in taste bud morphology.

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Poster: Taste: Animal Behavior

Taste effectiveness and preference of sugar alcohols in C57BL/6 mice

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Some sugar alcohols are widely used as anti-cariogenic sweeteners. The preference behavior and the receptor mechanisms for these sweeteners are not well understood. Therefore, in the present study, we conducted electrophysiological and behavioral experiments in C57BL/6 mice. In the electrophysiological study, to elucidate the effectiveness of these sweeteners for the sweet taste receptor, chorda tympani nerve responses to 0.3 M sucrose and four sugar alcohols—mannitol, xylitol, sorbitol and palatinit—were compared, before and after tongue treatment with 2% pronase E, an anti-sweet enzyme. Responses to sucrose and three sugar alcohols were suppressed by this treatment ($P < 0.01$; *t*-test), and the suppression ratios were sucrose > sorbitol > palatinit > xylitol. On the other hand, this treatment did not suppress the response to 0.3 M mannitol ($P > 0.05$; *t*-test). In the behavioral experiment, a 48 h two-bottle preference test, one of the above sweeteners versus distilled water was carried out to investigate the preference for sugar alcohols in C57BL/6 mice. The mice preferred 0.3 M sugar alcohols, except mannitol, rather than distilled water: the preference percents for xylitol, palatinit and sorbitol were >50%. On the other hand, the preference percent for mannitol was <35%. These results suggest that the taste receptor mechanisms as well as preference for sugar alcohols are different from each other.

Poster: Taste: Molecular Studies

Group IIA phospholipase A_2 is predominantly expressed in mature taste receptor cells of rat circumvallate papillae

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Taste buds consist of heterogeneous cells with diverse properties and different stages of maturity which are closely related to each other. It is important to define this relationship for understanding the molecular events involved in intracellular taste signaling. We found, by *in situ* hybridization analysis, that group IIA phospholipase A_2 (PLA_2-IIA) was expressed in a subset of taste bud cells. Immunohistochemical studies showed that PLA_2-IIA was expressed particularly in a subset of cells expressing phospholipase Cβ2 which is an essential molecule for taste signaling in the taste receptor cells, and that some of the PLA_2-IIA-positive cells also expressed gustducin (Ggust), one of bitter taste signaling molecules. Although PLA_2-IIA and Ggust were each expressed with similar frequencies in taste buds, bromodeoxyuridine (BrdU) chasing experiments indicated that Ggust began to express two days after BrdU injection, while the expression of PLA_2-IIA started 4 days after BrdU injection. These results indicate that PLA_2-IIA exists in mature taste receptor cells independent by their taste modalities.

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Poster: Taste: Molecular Studies

Do monkeys taste like humans?

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T2R (Tas2R) receptors represent the largest family of G-protein coupled taste receptors and are linked to bitter taste. Differences between individual T2Rs result in altered taste perception either in specificity or in sensitivity. All 33 human T2Rs are characterized by significant sequence homology. However, with a total of eight pseudogenes and >83 coding region single nucleotide polymorphisms (SNPs), the family displays broad diversity. The underlying variability of individual T2Rs might be the source for personalized taste perception. We have compared all human T2R genes with those of the closely related primate species Pan paniscus (bonobo) and Pan troglodytes (chimpanzee). Regions that change between closely related species or individuals within a species are likely to be involved in fine tuning the T2Rs to their ligands perhaps allowing a role for T2Rs in dietary adaptation and personalized food uptake (Parry *et al.*, 2004, *Proc. Natl Acad. Sci. USA*).

Poster: Taste: Molecular Studies

Regulating calcium in taste cells: expression of calcium ATPases

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Taste stimuli have been shown to activate multiple signaling pathways. Some stimuli bind directly to ligand gated receptors (sour and salty) while other taste stimuli (bitter, sweet and umami) bind to membrane bound G-protein coupled receptors that activate the PLC signaling cascade. Activation of the PLC signaling cascade results in calcium release from intracellular stores followed by an influx of calcium through a store-operated channel. Other taste cells increase intracellular calcium by activating voltage gated calcium channels. Evidence suggests this calcium influx may also have a role in taste transduction. Since calcium is important not only in the signal transduction of taste qualities but in many other cellular processes as well, its intracellular concentration must be tightly regulated. However, the role of calcium buffering mechanisms in taste cells is poorly understood. A few studies using immunocytochemical and biochemical techniques have indicated the presence of calcium ATPases in taste cells and physiological studies have clearly demonstrated the presence of at least one calcium ATPase on the endoplasmic reticulum. To date, these ATPases have not been characterized using molecular techniques to determine which isoforms are expressed. In my laboratory, initial studies on mice indicate the expression of multiple calcium ATPase isoforms on the endoplasmic reticulum and the plasma membrane in taste cells, suggesting that differential expression of these enzymes may contribute to the formation of taste signals.

This work is supported by DC00635801.

Poster: Taste: Molecular Studies

Taste-cell specific overexpression of BDNF in mice leads to the supertaster morphological phenotype

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BDNF is a member of the neurotrophin family and has been shown to play important roles in target innervation, development and synaptogenesis in the peripheral taste system. Here, we used an alpha-gustducin promoter to overexpress BDNF in taste buds. Gust-BDNF mice survive and reproduce. We show that BDNF is overexpressed in taste buds of Gust-BDNF mice. Gustatory papillae are larger in Gust-BDNF mice compared with wild type controls. The morphology of circumvallate papillae trench system is altered such that it resembles that in rats and humans. There is also a larger number of taste buds in the vallate papillae, a 20% increase. Gust-BDNF fungiform papillae are much larger than in wild type controls and have multiple taste pores. The gustatory papillae of Gust-BDNF mice are hyperinnervated compared with wild type controls. These findings suggest that Gust-BDNF mice might provide a useful mouse model to mimic human supertasting. Behavioral and electrophysiological studies will be used to test this hypothesis.

Poster: Taste: Molecular Studies

Functional characterization of a rat mGluR1 variant from vallate papillae

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The purpose of this study is to investigate the functional characteristics of a newly isolated metabotropic glutamate receptor 1 (mGluR1) variant cloned from rat vallate tissue. Recently several G protein coupled receptors (GPCR) were hypothesized to act as umami receptors (Chaudhari *et al.*, 2000; Li *et al.*, 2002; Nelson *et al.*, 2002; Toyono *et al.*, 2003). However, the receptor mechanism for umami taste perception is still in doubt (Damak *et al.*, 2003; Zhao *et al.*, 2003). And data from gustatory nerve recording, receptor distribution and taste cell electrophysiological function cannot all be reconciled solely by reference to already known receptors (Hoon *et al.*, 1999; Ninomiya *et al.*, 2000; Kim *et al.*, 2003). We propose that the sensory input for umami perception presumably involves other receptors still not accounted for. Previously cloned mGluR1 variant displayed a uniquely short 5′ end sequence that contains a stop codon in-frame with the long open reading frame that would ultimately produce a truncated receptor. In electrophysiological and biochemical studies performed in *Xenopus* oocytes expressing the newly isolated mGluR1 variant L-glutamate applied at concentrations that elicit umami taste generated intracellular Ca^{2+} mobilization. These data indicates that *in vitro* mGluR1 variant from taste tissue functions as a receptor for umami substances and has a lower affinity for L-glutamate compared with the receptor expressed in brain tissue.

Poster: Taste: Molecular Studies

Dietary NaCl-induced changes in the aldosterone-regulated salt taste transduction pathway

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Evidence suggests that sodium salt taste transduction pathways involving the epithelial sodium channel (ENaC) are responsive to aldosterone (ALDO) via late genomic effects (12–48 h). Our previous quantitative PCR (qPCR) results indicate that the taste system expresses a number of the early (<30 min) genomic response intermediates activated by ALDO that ultimately alter ENaC expression and/or function. Using qPCR, we examined diet-induced changes in the ALDO-regulated salt taste pathway in rat taste buds. Adult male Sprague–Dawley rats were fed a low (0.26%), intermediate (1.0%) or high (6.0%) NaCl diet for a period of 1, 5 or 14 days. RNA was isolated from taste buds from fungiform and circumvallate papillae as well as from kidney cortex and medulla at each of these time points. Changes in the expression of mRNA of a number of ion channels, transporters and ALDO-regulated intermediates including neural precursor cell expressed, developmentally down-regulated gene 4 type 2, serum- and glucocorticoid-regulated kinase type 1, corticosteroid hormone induced factor, and the GTPase Kirsten-ras were measured to explore how the peripheral taste system responds to long-term dietary and hormonal changes. Preliminary analysis revealed that the gamma subunit of ENaC in fungiform taste buds appears to be the most responsive to changes in dietary NaCl. Currently, we are looking directly at the short term effects (minutes to hours) of ALDO *in vitro* on expression of ENaC as well as the intermediates in rat taste buds.

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Poster: Taste: Molecular Studies

Taste responses in mice lacking AQP5

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Aquaporins (AQP) have been implicated in water movement during gustatory responses to hypoosmotic solutions. Previously, we have examined behavioral and cellular responses to changes in osmolarity in C57/6ByJ and 129X/SvJ inbred mouse strains, which express AQP5 in their taste receptor cells. Using real-time quantitative PCR we have found strain differences in AQP5 expression which may contribute to differences in behavioral responses observed. To examine the role of AQP5 in water taste directly, we are characterizing behavioral responses to taste stimuli in transgenic mice lacking AQP5. Using 24 h three-bottle preference tests, we measured preferences for mannitol, NaCl, sucrose, saccharin, quinine and citric acid in $AQP5^{-/-}$ and $AQP5^{+/+}$ littermates. Preferences for mannitol and NaCl decreased with increasing concentration, however, $AQP5^{+/+}$ mice show significant avoidance of mannitol and NaCl at lower concentrations than $AQP5^{-/-}$ mice. Sucrose preference increased with increasing concentration. Wild type mice show a significant sucrose preference at a lower concentration than $AQP5^{-/-}$ mice. We also examined taste preferences for mannitol, NaCl and sucrose following overnight water deprivation in a 15 m three-bottle preference test; however, no significant genotype differences were found. Therefore, differences in response to taste stimuli are present in $AQP5^{-/-}$ mice, but appear to be affected by hydration state. Currently, we are testing these mice using brief (5 s) access tests.

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Poster: Taste: Molecular Studies

Transsynaptic transport of wheat germ agglutinin expressed in a subset of type II taste cells of transgenic mice

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Anatomical tracing of neural circuits originating from specific subsets of taste receptor cells may shed light on the pattern of taste coding, i.e. labeled line versus across fiber pattern. Genetic tracing by transgenic expression of wheatgerm agglutinin (WGA) or barley lectin has been used to label olfactory and visual neural circuits, but not gustatory circuits. It was not clear if this approach would work for the taste system because the type II taste receptor cells that express signal transduction proteins do not have well elaborated synapses or express classic synaptic markers. To determine if WGA produced in T1r3-expressing type II taste receptor cells can be transported into first- and second-order neurons, transgenic mice expressing WGA-IRES-GFP under the control of the T1r3 promoter were generated. Immunohistochemistry showed co-localization of WGA, GFP and endogenous T1r3 in the taste buds of transgenic mice. Immunoreactivity for WGA, but not for GFP, was found in the geniculate and petrosal ganglia of transgenic mice indicating that WGA was transported across synapses. WGA immunoreactivity was also found in a few cells of the trigeminal ganglion, suggesting that T1r3-expressing cells make synapses with trigeminal neurons. In the medulla, WGA was detected in the nucleus of the solitary tract, in the nucleus ambiguous, the vestibular nucleus, the trigeminal nucleus and in the gigantocellular reticular nucleus. These results show the usefulness of transgenically expressed WGA as a tracer for neural circuits originating from a defined subset of taste receptor cells and argue in favor of the presence of functional synapses in the T1r3-expressing cells.

Poster: Taste: Molecular Studies

Whither the sweet taste in cats?

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Sweet taste perception is widely thought to have evolved to identify sugars found in foods. Species like the domestic cats, which rarely ingest sugars, might have lost the ability to perceive sweet taste due to lack of selective pressure to maintain a functional sweet receptor gene. Other than blindness to sweet, cats maintain an otherwise functional sense of taste. These observations suggest that the sweet receptor dimer (T1R2/T1R3) became non-functional during evolution of carnivory. By screening a feline genomic BAC library and by conducting PCR with degenerate primers, we demonstrated that cat *Tas1r3* gene is functional and expressed in cat taste buds. *Tas1r2*, on the other hand, is an unexpressed pseudogene, making the sweet

receptor dimer non-functional. A similar *Tas1r2* pseudogene is found in tigers and cheetahs. In contrast, the dog possesses a normal *Tas1r2* gene. This alteration in cat *Tas1r2* may have coincided with their food choices favoring protein and fat, making it an important event in the evolution of carnivorous behavior.

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Poster: Taste: Molecular Studies

Identification of voltage-gated pH-sensitive chloride channels in taste bud cells

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Taste bud cells are excitable. Two types of action potentials have been recorded from these cells: fast action potentials with shorter duration and larger currents; and slow action potentials with longer duration and smaller currents. Physiological studies have indicated that various voltage-dependent sodium, potassium, calcium and chloride channels are expressed in different types of taste bud cells. To further investigate the possible roles of receptor potentials and action potentials in taste signal transduction and peripheral coding in the end organs of taste, we molecularly identified and functionally characterized some of these physiologically described voltage-gated ion channels. Using single cell PCR and differential screening methods, we isolated from taste bud cells the cDNAs for a voltage-gated, pH-sensitive chloride channel, ClC-4, and its novel splice variant, ClC-4A. *In situ* hybridization and immunohistochemistry localized these two channels' transcripts and proteins to a subset of taste bud cells. Electrophysiological recordings of the heterologously expressed channels in Xenopus oocytes showed that ClC-4 and ClC-4A had the opposite sensitivity to pH, and distinct ion selectivity. Chloride channel blockers NFA and NPPB had slight or no inhibitory effect on the conductance of ClC-4, but both blockers inhibited ClC-4A.

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Poster: Taste: Molecular Studies

Molecular studies of the gustducin–phosphodiesterase 1A interaction

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Taste receptor cell (TRC) responses to sweet, bitter and umami compounds are mediated by receptors, G-proteins and effector enzymes. Activated taste receptors stimulate their coupled heterotrimeric G proteins, which in turn regulate effector enzymes to alter levels of TRC second messengers (e.g. cyclic nucleotides and inositides). The TRC-expressed G protein gustducin is critical for TRC responses to bitter, sweet and umami compounds. Gustducin's striking similarity to transducin (80% identity), and the presence in TRCs of phosphodiesterase 1A (PDE1A) and transducin suggests that G-protein regulation of phosphodiesterase activity may be important to taste transduction. The alpha subunits of gustducin and transducin, but not of $G_{\alpha i}$, activated PDE1A *in vitro* when coexpressed in HEK 293T cells. To probe the specificity of the gustducin–phosphodiesterse interaction we characterized the properties of a series of chimeras of α-gustducin and $G_{\alpha i}$, as well as several α-gustducin mutants. A specific region of α-gustducin required for the interaction with PDE1A was identified. Replacement of specific amino acids within this region diminished or abolished the ability of the G protein to activate PDE1A.

Poster: Taste: Molecular Studies

The role of calcium-sensitive adenylyl cyclase in taste buds

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Taste buds produce two second messengers, cAMP and IP_3, in response to taste stimuli. Whether these pathways interact is currently unknown. Previously, we showed adenylyl cyclases AC4, AC5/6 and AC8 are present in taste cells. Because AC8 is stimulated by Ca^{2+}, we hypothesized that it could serve to integrate the Ca^{2+} and cAMP second messenger pathways. To confirm the functional presence of a Ca^{2+}-sensitive AC in taste cells, we measured cAMP levels in taste buds using treatments that alter intracellular Ca^{2+} levels. Application of 5 mM thapsigargin (causing a transient increase in intracellular Ca^{2+}) led to a significant increase (162 ± 17%) in cAMP levels. This increase in cAMP required extracellular Ca^{2+} (i.e. may be caused by a capacitative influx of Ca^{2+}). A23187(3 μM), a Ca^{2+} ionophore, similarly stimulated AC activity. Taste stimuli are known to cause both release of intracellular calcium (via PLCβ2 mediated signaling) and subsequent capacitative entry. Immunofluorescent microscopy showed that the majority of AC8-positive cells also express PLCβ2, suggesting that they respond to tastants. Thus, we considered that the cAMP increase previously shown for sucrose stimulation may be a result of enhanced AC8 activity following Ca^{2+} signaling. We measured cAMP levels in taste buds stimulated with sucrose in the presence or absence of extracellular Ca^{2+}. The increase in cAMP in response to sucrose (and decrease in response to MSG) persists in the absence of extracellular Ca^{2+}. Our results suggest that the cAMP signal is not a secondary consequence of Ca^{2+} modulation. Instead, cAMP and Ca^{2+} may represent independent signals downstream of taste receptors.

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Poster: Taste: Molecular Studies

Diet-induced changes in expression of fatty acid-sensitive potassium channels in rat taste buds

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One mechanism for the transduction of fat involves the inhibition of delayed rectifying K^+ (DRK) channels by free fatty acids. We have previously identified and quantified expression of the nine DRK channels of the KCNA, KCNB and KCNC gene families, which represent both fatty acid-sensitive and -insensitive DRK subtypes, in obesity-prone and -resistant rat strains and have suggested that variability in DRK expression underlies the differences in their chemosensory responses to fat. In the present study we have used real-time quantitative PCR (qPCR) to measure the changes in DRK expression in obesity-prone and -resistant rats placed on one of three diets [chow, high fat (HF) and high carbohydrate (HC)] for 60 days. Since early exposure to HF diets is known to predispose individuals to develop obesity, we hypothesized that diets differing in macronutrient content may alter expression of fatty acid-sensitive DRK channels. In fat-preferring, obesity-prone rats (Osborne–Mendel), there are significant differences in the expression of Kv1.5 (KCNA5), the major fatty acid sensitive channel expressed in taste cells, when placed on a HF or HC diet. Interestingly, expression of Kv1.5 in fungiform taste buds is ~50 times greater for rats placed on a HF diet than those on a HC diet, while other DRK channels show little or no change in expression. Similar experiments are underway in a fat avoiding, obesity-resistant rat strain (S5B/Pl). These results suggest that taste cells may alter expression of fat-sensitive channels in response to specific macronutrients.

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Poster: Taste: Molecular Studies

Identification and characterization of human sweet receptor binding sites for agonists and antagonists

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The detection of sweet tasting compounds is mediated in large part by a heterodimeric receptor comprised of T1R2 + T1R3. Lactisole, a broad-acting sweet antagonist, suppresses the sweet taste of sugars, protein sweeteners and artificial sweeteners. Lactisole's inhibitory effect is specific to humans and other primates: lactisole does not affect responses of rodents to sweet compounds. The artificial sweetener cyclamate, a sulfamic acid, activates the human sweet receptor, but not the mouse receptor. By heterologously expressing interspecies (mouse + human) combinations of T1R2 + T1R3 we have determined that the target for both lactisole and cyclamate is human T1R3. From studies with mouse/human chimeras of T1R3 we determined that the molecular basis for species-specific sensitivity to lactisole and cyclamate depends on only a few residues within the transmembrane helices of T1R3. Alanine substitution of residues in the transmembrane region of human T1R3 revealed additional residues required for sensitivity to lactisole and cyclamate. Based on the crystal structure of rhodopsin, we have modeled the transmembrane domain of T1R3 and predicted the receptor's binding sites for lactisole and cyclamate. Our results indicate that lactisole and cyclamate occupy overlapping binding pockets within the seven transmembrane helix bundle of human T1R3. Lactisole stabilizes the receptor's inactive state, while cyclamate stabilizes the receptor's active state.

Poster: Taste: Molecular Studies

X-Ray crystallographic studies of the single chain monellin MNEI: implications for interactions with T1R taste receptors

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A small number of proteins, including monellin and thaumatin, are perceived as intensely sweet by humans and some old world primates. Although such sweet proteins have been extensively studied, little is known about their mechanisms of interaction with the sweet taste receptor T1R2:T1R3. To better understand the interactions of this class of sweet ligands with T1R receptors, we have determined the X-ray crystal structures of a wild type single-chain monellin, MNEI, and a less sweet mutant, G16A-MNEI, at 1.1 and 2.2 Å resolution, respectively. The proteins crystallize in different space groups, $P2_1$ for wild type and $P4_12_12$ for the mutant, but have globally similar structures. Significantly, the wild type crystal structure does not contain the monellin dimer interface seen in previous monellin crystal structures, supporting evidence from solution studies that the monomeric form of single chain monellins is the functional unit. Differences between the wild type and G16A structures, and between wild type MNEI and previously solved monellin structures will be discussed, with particular emphasis on the significance for interactions with the sweet taste receptor T1R2-T1R3.

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Poster: Taste: Molecular Studies

Coding sequence variation in human sweet and umami taste receptor genes

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Sweet and umami (the taste of glutamate) tastes play a major role on the perception of carlorically rich and essential nutrients. In humans, three members of the T1R class of taste-specific G protein-coupled receptors (T1R1, T1R2 and T1R3), which residue on chromosome 1, are known to function in combination as heterodimeric receptors for sweet and umami tastes. We hypothesized single nucleotide polymorphisms (SNPs) or variant haplotypes of the T1R genes in humans may underlie individual differences in the detection and recognition threshold for sweeteners and amino acids. To enable study of genotpe/phenotypic correlation for these two tastes, we identified coding sequence variation by sequencing these genes in a cohort of unrelated individuals. To achieve maximum genetic diversity in our sample, we included an outbred African population of 20 individuals from Cameroon, together with individuals from Asian, Amerindian and European populatons. We found 48 SNPs in these three genes. Of these, 29 cause an amino acid substitution in the encoded receptor

protein, and one SNP, in the T1R1 gene, introduces an in-frame stop codon. Although the size of these three genes is much larger than those of the T2R genes that encode bitter taste receptors, the number of cSNPs in the T1R genes is small compared with the number of cSNPs in T2R genes. This suggests that the sequences of the T1R receptor proteins are more conserved than those of T2R receptors, and may explain why individuals show less variation in these taste modalities.

Poster: Taste: Molecular Studies

Measuring ligand binding to the mouse T1R2 N-terminal domain

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T1Rs are class C G protein-coupled receptors (GPCRs) important for sweet and umami taste. The native sweet taste receptor contains two subunits, T1R2 and T1R3, both of which are required for native stimulus sensitivity and selectivity. The N-terminal domains (NTDs) of T1R taste receptors are thought to contain one or more ligand-binding motifs for sweet ligands. However, little is known about how individual T1Rs interact with chemically distinct sweet ligands (e.g. sugars, sweeteners and amino acids) or contribute to their transduction. To understand how T1Rs control their stimulus specificity and sensitivity, it is important to decouple the ligand-binding event from downstream receptor functions. Using a novel *in vitro* assay system developed in our laboratories, we have measured the binding of sugars to the NTD of the T1R subunit specific to sweet taste, T1R2. We expressed and purified mouse T1R2 NTD protein from a bacterial expression system. Circular dichroism (CD) spectroscopy shows the proteins to be folded. Titration of sweet ligands (e.g. glucose and sucrose) into the T1R2 NTD induces a consistent shift in CD spectra of the protein, indicating a ligand-induced change in secondary and/or tertiary structure. Sweet ligands also change the intrinsic fluorescence intensity of the T1R2 NTD (EC_{50} of glucose = 12 mM). These results show the T1R2 NTD is competent to bind small molecular weight natural ligands, and suggests that it plays an active role in this process *in vivo*.

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Poster: Taste: Molecular Studies

T1R3 binds sweet ligands at physiological concentrations

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T1Rs are class C G protein-coupled receptors (GPCRs) important for sweet and umami taste. The native sweet taste receptor contains two subunits, T1R2 and T1R3, both of which are required for normal stimulus sensitivity and selectivity. It has been suggested that T1R3 plays no direct role in ligand binding, in part because it also pairs with T1R1 to form an umami taste receptor. To test whether T1R3 can bind taste stimuli at physiological concentrations, we have developed an *in vitro* system for measuring ligand binding to the N-terminal domain of T1R3. We expressed and purified mouse T1R3 NTD protein from bacterial expression systems. Circular dichroism (CD) spectroscopy showed the proteins to be folded, with a secondary structure content consistent with that predicted *in silico*. Fluorescence spectroscopy of T1R3 NTD showed that sweet ligands (e.g. glucose, sucrose, glycine and sucralose) changed the intrinsic fluorescence intensity of the protein, with EC_{50}s from 3 to 10 mM; cyclamate, which is not preferred by mice, had no effect. Ligands also induce a consistent shift in CD spectra of T1R3 NTD, indicating a change in receptor structure upon ligand binding. Our results show that T1R3 NTD is competent to bind sweet stimuli at physiological concentrations, and suggest that, in contrast to other heteromeric class C GPCRs (e.g. $GABA_B$ receptors), both T1R subunits may play a role in ligand interactions *in vivo*.

Supported by NIDCD, NSLS/BNL (S.D.M.) and The Wellcome Trust, CCLRC (G.L.C.).

Poster: Taste: Molecular Studies

Functional characterization of the human T2R7, T2R14, T2R43 and T2R47 bitter receptors

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In the present study, we address the signaling properties of human T2Rs using an *in vitro* reconstitution system in which both the ligands and G-proteins being assayed can be manipulated independently and quantitatively assessed. We demonstrate that the hT2R14 and hT2R43 receptors respond selectively to micromolar concentrations of aristolochic acid and that the hT2R47 receptor responds selectively to micromolar concentrations of denatonium. In contrast, hT2R7 is more broadly tuned responding to strychnine, quinacrine, chloroquine and papaverine. Using these defined ligand/receptor interactions, we also demonstrate that the T2Rs display different affinities for G-proteins including both Gα and Gβγ subunits. To further define the signaling pathways for the T2Rs, we assayed the ability of the ligand-activated T2Rs to catalyze GTP binding on divergent members of the Gαi subfamily including transducin, Gαi1, and GαoA. With the exception of hT2R47, all of the T2Rs tested coupled with each of the three Gαi members. Furthermore, we observed different G-protein selectivities among the T2Rs, suggesting that *in vivo* T2Rs may signal using alternative Gα subunits.

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Poster: Taste: Clinical

Trigeminal nerve response in humans for 3 M NaCl solutions, studied by MEG

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Patients with severe taste disorder cannot perceive high concentration (3.42 M) NaCl solutions using a taste examination kit (taste disk) on the tip of the tongue, and does not report any irritation or heat perception either, while both the chorda tympani and trigeminal nerve were reported to be stimulated by 0.4 M NaCl solutions in rat. There is a clear discrepancy between physiological data of rats and clinical data, according to the activity of trigeminal nerve, in the presentation of high concentration NaCl solutions. It is impossible to record solely the response of the trigeminal nerve separate from that of chorda tympani in normal participants. We therefore chose participants whose chorda tympani nerves on both sides had been cut by bilateral cholesteoma. We could investigate only the response of trigeminal nerve by stimulating the tip of the tongue in these participants. We also confirmed the funtionality of the trigeminal nerve of participants by subjective tactile examination (SW test) and evoked magnetic fields. We first administered subjective examination for taste and touch sensation. The participants did not report any perception of a small filter paper dipped in 3.42 M NaCl, but could perceive the lightest touch stimulation at the tip of the tongue. We also measured the magnetic fields evoked by 3 M NaCl solutions presented to the tip of tongue, and also by the tactile stimulation on the same place. We found a remarkable response for tactile stimulation, but no remarkable response for taste under the same experimental conditions. Further experiments are needed to make clear the influence of high concentration NaCl on the trigeminal nerve.

Poster: Taste: Clinical

Objective assessment of terbinafine-induced taste loss

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Terbinafine (Lamisil®), a widely prescribed oral antifungal agent, is associated with subjective reports of taste disturbance. However, the sole study that has empirically evaluated terbinafine-related taste dysfunction found no deficits, and many so-called taste problems reflect olfactory system damage. We employed state-of-the-art taste and smell tests to evaluate six patients reporting terbinafine-related taste disturbances. In all six cases, olfactory function was normal and taste perception was depressed in both anterior (CN VII) and posterior (CN IX) lingual regions, with the greatest deficits occurring for sour and bitter tasting agents. For NaCl, the major decrements were at the rear of the tongue. This study is the first to empirically verify taste dysfunction in patients taking terbinafine. Given that self-report markedly underestimates most chemosensory deficits, quantitative taste testing may well reveal a much higher prevalence of terbinafine-induced taste loss than currently believed.

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Poster: Taste: Clinical

Substance and tongue-region specific loss in basic taste-quality identification in elderly adults

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Past findings of successful health-related effects in elderly by additional flavouring of food for compensation of chemosensory loss motivated the present study of age-related loss in identification of basic taste qualities presented at different tongue regions. Impregnated 'taste strips' with four concentrations of each of the tastants sucrose, NaCl, quinine hydrochloride and citric acid were applied on the tip, midlateral and posteromedial tongue regions to be identified as either sweet, salty, bitter or sour by 30 young and 26 elderly adults. The results showed more pronounced age-related loss in identification for citric acid (eta-square = 0.45 and 0.44) and quinine hydrochloride (0.22 and 0.12) than for sucrose (0.07 and ns) and NaCl (0.08 and ns) at both the tip and midlateral regions, respectively, but not at the posteromedial region, where both age groups performed close to chance level. These findings may have implications for enhancing flavour among elderly populations by adding bitter and sour tastants to food.

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Poster: Taste: Clinical

Confocal microscopy of the peripheral gustatory system: comparison between healthy subjects and patients suffering from taste disorders during radiochemotherapy

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A laser-scanning microscope, a combination of the Heidelberg Retina Tomograph and the Rostock Cornea Module, was used to compare taste buds and epithelia of fungiform papillae of healthy human subjects with those of patients with taste disorders during radiochemotherapy (RCT). The aim of the study was to evaluate whether laser-scanning microscopy (LSM) is applicable for *in vivo* diagnostics of taste disorders. Data from 12 healthy subjects were compared with those from 12 patients with head and neck cancer suffering from taste disorders during RCT. Four parameters were chosen for analysis: (i) measurement of the distance between the pore of the taste bud of fungiform papilla and the crest of the papillary vessels; (ii) quantification of epithelial cells of each taste bud at 34 μm; (iii), calculation of cell density (cells/mm^2); and (iv) calculation of the area of the taste pore at 4 μm. These data were correlated to measures of gustatory sensitivity obtained with 'taste

strips' (filter papers impregnated with tastants) and electrogustometry (EGM). Patients complaining from taste disorders during RCT exhibited a significant decrease of taste function obtained with both taste strips and EGM. In these patients we found significantly thicker epithelia and a smaller area of the taste pore compared with healthy subjects. In 30% of those there were no taste pores, while in deeper sections normal taste buds were apparent. In conclusion, LSM suggests changes of epithelia of fungiform papilla but no changes of the taste bud structure in patients with taste disorders during RCT. Accordingly, LSM appears to be a useful tool in the investigation of the peripheral gustatory system.

Poster: Taste: Clinical

Glossopharyngeal sensory neuropathy: implication in dysgeusia and glossodynia

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There is a large patient population with complaints of dysgeusia and glossodynia for which no local, systemic, or psychological cause can be identified. At the WFU Smell and Taste Center we have evaluated (in a subset of patients) the relationship between dysgeusia or glossodynia and glossopharyngeal neuropathy, the causes of dysgeusia or glossodynia, and the efficacy of treatment for dysgeusia or glossodynia through pathogenesis-based treatment for glossopharyngeal sensory neuropathy. It was determined that in treating dysgeusia or glossodynia, it is important to give high priority in diagnosing and treating glossopharyngeal sensory neuropathy. The clinical efficacy of treatment for dysgeusia and glossodynia will depend on the presence or absence of glossopharyngeal sensory neuropathy and its early diagnosis and course of treatment.

Poster: Taste: Clinical

Taste sense is abnormal in idiopathic Parkinson's disease

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Objective: to determine the prevalence of dysgeusia in idiopathic Parkinson's disease (IPD). Background: patients with IPD rarely complain of difficulty with taste sensation but on pathological grounds it may be affected. Methods: we measured taste threshold with a Rion TR-06 electrogustometer. A sterile stainless steel electrode was applied to both the chorda tympani (CT) nerve (VII) and lateral circumvallate papilla (VP) (IX). The stimulus current was increased using a single staircase approach until the subject reported a salt or bitter taste. Threshold was measured in decibels (dB). The procedure was undertaken on the following groups: (i) 27 healthy controls, mean age 52 years (18 female); (ii) 19 patients with IPD all conforming to the UK PD Brain Bank criteria. All scored 30 on the Mini Mental test and had a good oral hygiene. Mean age was 69 years (9 female, 10 male), and 13/19 were taking levodopa or dopamine agonist. Results: controls, CT mean threshold was 6.8, 6 dB and for VP, 5.6, 4 dB. Patients, mean thresholds were CT: 15, 10 dB and VP 7.5, 12 dB. Age had minimal effect on Control threshold values. Thresholds were significantly increased in patients for CT ($P > 0.001$) but not for VP ($P > 0.32$). Although the abnormalities were more clearcut for CT than VP this may have related to technical difficulties (in accessing the posterior tongue region). Two IPD drug naive patients had thresholds of 34 dB, which indicates ageusia. Conclusions: taste threshold for CT (VII) was elevated in our patients with IPD. Thresholds from VP (IX) were normal. The CT mediated dysgeusia may relate to the known autonomic dysfunction in IPD. The reason for sparing IX taste is not understood but may relate to technical difficulty.

Poster: Taste: Clinical

Changes in sweet taste in women with gestational diabetes mellitus

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Gestational diabetes mellitus (GDM) is glucose intolerance that occurs during pregnancy. Increased liking for sweet taste in GDM has been shown previously (Tepper and Seldner 1999) and may have consequences for dietary compliance in these women. This study examined taste changes prospectively during pregnancy. To date, 65 pregnant women have been studied, six of whom have developed GDM. Testing was conducted at 12–20, 24–28 and 34–38 weeks gestational age or at similar intervals in non-pregnant controls. Subjects rated sweetness intensity and overall liking of glucose solutions (0.01–0.16 M), as well as strawberry milks, varying in sweetness (0–20% sucrose) and fat content (0–10% fat) using a 15 cm line scale, after an overnight fast. Data were averaged over sessions. Sweetness intensity increased across glucose concentrations, but there were no significant differences among subject groups. Women with GDM gave higher liking ratings to all glucose solutions as compared with either women without GDM or controls ($P < 0.01$–0.001), except for the highest concentration. Women with GDM liked the sweetness of strawberry milks with 5% sucrose and 5% fat more than women in the other groups ($P < 0.05$). No other differences were observed in strawberry milks. These findings are preliminary, but support previous research from our laboratory, showing liking for sweet taste is elevated in women with GDM. Subsequent studies will investigate how these differences in preference relate to endocrine and diet changes during pregnancy.

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Poster: Trigeminal

Nasal irritant sensitivity: inverse relationship between sensory acuity and physiologic reactivity

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We compared nasal irritant sensory acuity and physiologic reactivity in a stratified sample of 52 volunteers ranging from 19 to 68 years of age, including 28 females and 27 allergic rhinitics. We assessed sensory acuity with both CO_2 detection and VOC localization

thresholds, and physiologic reactivity as Cl_2-related increases in nasal airway resistance (NAR). CO_2 detection thresholds and *n*-propanol localization thresholds were were obtained in duplicate using previously published methods. Subjects also underwent exposure to either dilute Cl_2 gas (1.0 p.p.m.) or filtered air by nasal mask for 15 min, with exposures a week apart in counterbalanced order. Subjects had their nasal airway resistance measured in triplicate before, immediately after and 15 min post-exposure, using the technique of active posterior rhinomanometry. The net percent change in NAR attributable to Cl_2 exposure (ΔNAR Net%) was defined as the relative pre- to post-exposure change in NAR on the Cl_2 minus the air exposure day. ΔNAR Net% immediately post-exposure correlated positively with both CO_2 detection and VOC localization threshold ($P < 0.05$). At 15 min post-exposure, ΔNAR Net% correlated positively with CO_2 detection thresholds only. Thus, elevated sensory thresholds (low sensory acuity) predicted a greater congestive response to irritant provocation (high physiological reactivity). This paradoxical result is accompanied by strong age effects on the two outcome variables, with older subject exhibiting lower sensory acuity but greater physiologic reactivity than younger subjects. The implications of this finding in terms of a potentially impaired trigeminal warning function in the elderly have yet to be explored.

Poster: Trigeminal

Retronasal smelling: an oral cavity component

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Retronasal smelling is generally assumed to be purely olfactory, with little or no oral cavity trigeminal component. We tested this assumption by obtaining identifications (ID) of six odorants (anise, cinnamon, coffee, orange, peppermint and strawberry, all alcohol-free food-grade extracts), presented in the oral cavity in vapor phase only, either with normal retronasal smelling (exhalation through anterior nares) or with vapor-phase access only to the oral cavity (exhalation through the mouth with anterior nares closed). With retronasal smelling, 21 subjects correctly ID all odorants ($P < 0.0001$), as did each individual subject ($P < 0.031$). In contrast, with odorant access limited to the oral cavity, only peppermint was ID across subjects ($P = 0.014$), but not the other five odorants ($P > 0.092$). However, one individual could ID all odorants when access was limited to the oral cavity ($P = 0.031$). In a separate experiment, 20 subjects attempted to discriminate the same 6 odorants from blanks, with vapor-phase odorant access limited to the oral cavity. Across subjects, both peppermint and orange were discriminated from blanks ($P = 0.002$, $P = 0.034$), but not the other four odorants ($P > 0.066$). However, five subjects (two male) could discriminate all the odorants from blanks ($P < 0.032$). These data suggest that oral cavity responses to odorants such as peppermint and orange, presumably mediated by the trigeminal system, may often have a role in retronasal smelling, and that in some individuals, odorants not usually classed as trigeminal stimuli may also be effective vapor-phase stimuli for retronasal smelling.

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Poster: Trigeminal

Temporal integration of nasal irritation from ammonia at threshold and supra-threshold levels

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Two experiments examined integration of perceived irritation over short-term (~100–3000 ms) delivery of ammonia (NH_3) into the nasal cavity. Experiment 1 examined trade-offs between time and concentration at threshold-level using nasal lateralization. Subjects attempted to determine which nostril received NH_3 after simultaneous presentation of air into one nostril and NH_3 into the other. Within experimental sessions, the duration of a fixed-concentration stimulus varied to determine the shortest, detectable pulse. Subjects could lateralize increasingly weaker concentrations with longer stimulus-presentations. Experiment 2 examined an analogous trade-off for suprathreshold irritation. Subjects rated irritation from bi-rhinal presentations of NH_3 that varied both in concentration and duration. Rated intensity for a given concentration increased with stimulus duration. Hence, integration, occurred at both threshold- and supra-threshold levels. However, more than a twofold increase in duration was required to compensate for a two-fold decrease in concentration to maintain threshold lateralization or a fixed level of perceived intensity. These results suggest that an imperfect, mass-integrator model can describe short-term integration of nasal irritation from ammonia at both threshold and suprathreshold levels.

Poster: Trigeminal

Quantification of the efficacy of vapors to elicit nasal chemesthesis in anosmics

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The study seeks to clarify the influence of methodological and chemical parameters on the ability of vapors to elicit detection of nasal pungency, or chemesthesis, in anosmic participants ($n = 6$). Twelve neat chemicals were selected based on previous reports that they failed to elicit trigeminal detection, or that they might extend beyond a cut-off point for chemesthesis along homologous chemical series. Vapors, quantified by gas chromatography, were presented from a 1900 ml glass vessel system with tight-fitting nosepieces and from a 237 ml wide-mouth glass flask, employing a three- or two-alternative forced choice procedure, respectively. Results showed higher detectability and confidence of detection with use of the 1900 ml vessels to the point that half the stimuli were detected well above chance. Heating the undetected stimuli to 37°C to increase their vapor concentration made one more stimulus, octane, also detectable. Based on gas chromatographic measurements of concentration and on calculation of expected nasal pungency thresholds, we conclude that the lack of trigeminal impact for the remaining undetected compounds decyl acetate, beta-phenylethyl alcohol, octanoic acid and coumarin does not rest on a restriction of vapor concentration, as for octane,

but on a chemical-structural restriction, e.g. the molecules lack a property to fit into the binding pocket of a receptor.

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Poster: Trigeminal

Chemesthetic responsiveness is independent of thermal taste and chemical taste

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Perception of thermal taste was recently shown to be predictive of higher responsiveness to gustatory and olfactory stimuli. This relationship was hypothesized to be due in part to individual differences in CNS processes involved in flavor perception. Here we report three experiments that tested whether groups of subjects who differ in perception of thermal taste and/or chemical taste also differ in perception of oral chemesthesis. In Experiment 1, subjects identified as 'thermal tasters' (TTs) or 'thermal non-tasters' (TnTs) used the gLMS to rate the intensity of sensations produced on the tongue tip by separate blocks of 100 mM capsaicin and 320 mM menthol stimuli, and by 1.0 M sucrose, 0.56 M NaCl, 0.056 M citric acid and 1.0 mM QSO_4. TTs rated all four taste stimuli significantly higher than TnTs, whereas ratings of burning and thermal sensations (hot, cold) from capsaicin and menthol did not differ between groups. Experiment 2 replicated this finding for capsaicin on the tongue tip and also found no difference between groups when capsaicin was applied to the back of the tongue (circumvallate region). A final experiment categorized high- and low-tasters based on ratings of sucrose sweetness and found no difference in ratings of burning/stinging produced by high concentrations of citric acid and NaCl. We conclude that responsiveness to oral chemesthetic stimuli cannot be inferred from responsiveness to gustatory stimuli, and thus that individual differences in these two modalities are probably determined by different combinations of neurobiological and genetic factors.

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Poster: Trigeminal

Can a single process account for both sensitization and desensitization effects to oral irritation?

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Successive presentation of capsaicin and similar irritants can result in either a greater or lesser response to the second irritant, depending on temporal parameters and the particular irritants. These phenomena, termed sensitization and desensitization, respectively, have been described at levels ranging from ganglion cell responses to subjective judgements of irritation (e.g. Liu *et al.*, 2000). The time scale of sensitization and desensitization occur differ at different levels and likely reflect different mechanisms. We have developed a dynamic mathematical model of the psychophysical response to irritants that describes the behavioral phenomena as predictable outcomes (temporal summation) of the interactions of tonic and phasic processes (Balaban *et al.*, 1999). Thus, it is unnecessary to seek separate physiological mechanisms for at least some behavioral forms of sensitization and desensitization. We consider cases from the literature and suggest strategies for clarifying when mechanisms of sensitization and desensitization are needed.

Poster: Trigeminal

Anterior human nasal mucosa more sensitive to touch

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Objectives: the touch sensitivity pattern of the human nasal cavity mucosa is unknown. Perceptions from this mucosal stimulation during breathing modulate the breathing experience for the person. Methods: the mucosa of 141 nostrils in 76 subjects was stimulated with tiny jets of compressed air at varying velocities to determine the threshold of perception. Nine locations were tested in each nostril and test–retest measures were made. Results: statistically significant ($P < 0.001$) decreases in sensitivity were noted for more posterior nasal locations compaired to the nasal vestibule. The inferior nasal airway under the inferior turbinate was slightly more sensitive than the middle airway. Punctate anesthetic areas were identified in many nostrils.Conclusions: the increased sensitivity of the anterior nasal mucosa may provide more immediate information to the person on nasal airflow. During nasal surgeries, care should be exercised to preserve this nasal mucosa, and to assure airflow to the inferior and middle nasal regions. The significance of the anesthetic areas is unclear, and suggests a varible innervation pattern.

Poster: Trigeminal

Temporal characteristics and regional sensitivity of tingle compounds

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With repeated application to the oral mucosa, some chemesthetic compounds elicit irritant sensations that increase in intensity across trials (sensitization) whereas others evoke a pattern of irritation that decreases (desensitization). Several studies have noted regional differences in irritant sensitivity with some compounds (e.g. ibuprofen) exhibiting stronger irritancy in the throat compared with the oral cavity. Few studies have examined the temporal sensory characteristics or regional sensitivity evoked by tingling compounds. Presently, we investigated whether the perceived tingling sensation elicited by jambu oleoresin (predominant active spilanthol) or a galangal derivative (GR84-8804) changes with repeated application and whether there exists greater sensitivity in the throat or oral cavity. Subjects rated

the tingle intensity of aqueous solutions of jambu or GR84-8804 delivered sequentially, every 30 s, to one-half of the oral cavity for 150 s. Subjects swallowed the last stimulus and rated the perceived throat tingle. Thirty minutes later, subjects again ingested jambu or GR84-8804 and assessed the tingle perceived bilaterally in the oral cavity and throat. Both jambu and GR84-8804 evoked tingling sensations that increased with repeated application. Following a 30 min hiatus, the sensation evoked by jambu was significantly reduced indicating the presence of desensitization, however, no desensitization was indicated following GR84-8804 stimulation. Jambu elicited a tingling sensation that was stronger in the oral cavity, whereas GR84-8804 elicited a sensation that was perceived as more intense in the throat.

Poster: Trigeminal

Trigeminal function in children

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The aim of the study was to investigate trigeminal function in children in comparison to adults. Trigeminal sensitivity was assessed using a lateralization task where subjects are requested to identify the side of the nose to which an odorous stimulus has been presented. This localization is only possible through the trigeminal system. A total of 348 subjects participated (191 female, 153 male; age 5–54 years, mean age 12 years). Eucalyptol (EUC) was tested as a mixed olfactory–trigeminal stimulant; phenylethyl alcohol (PEA) was used a control stimulant with minimal trigeminal impact. In addition, sensitivity to vibration (VIBR) was assessed as a somatosensory control. With regard to all age groups, PEA could not be localized at any age. In contrast, the ability to localize EUC occurred at 5 years of age and stablized at 6 years of age, with no further change afte this. No sex-related differences were found for odor localization with both EUC and PEA. These results not only provide normative data for intransal trigeminal function in children, they also indicate that trigeminal sensitivity is already adult-like at 6 years. In contrast, the ability to localize 'pure' odors like PEA does not change in relation to age.

Slide: Polak Young Investigator Taste Symposium

The gustatory receptor 5a region in *Drosophila melanogaster* reveals integrated genetic networks for gustatory perception, starvation resistance and longevity

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Previous studies have shown that caloric restriction is associated with physiological mechanisms that determine starvation resistance and life span. Trehalose is the principal carbohydrate source for metabolic energy in insects, and in *Drosophila melanogaster* a gustatory receptor for this sugar, Gr5a, has been identified. Transposon insertions in the intergenic region between the *Gr5a* gene and the neighboring *Tre1* locus, which encodes an orphan G-protein coupled receptor, result in sexually dimorphic and antagonistic effects on trehalose preference, starvation resistance and life span. Whole genome transcriptional analysis shows that subtle disruptions in this region result in substantial alterations in transcriptional regulation with significant effects on these traits. These observations provide further evidence that complex chemosensory behaviors in *Drosophila* are determined by dynamic epistatic networks of pleiotropic genes, and that correlations between chemosensory perception, starvation stress resistance and life span can be explained in terms of overlapping genetic networks.

Slide: Polak Young Investigator Taste Symposium

Allelic variation of the *Tas1r3* gene affects taste-evoked responses in the nucleus of the solitary tract of mice

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The *Tas1r3* gene encodes a protein involved in sweet and umami taste transduction, and allelic variation of this gene in mice is associated with high ('taster' phenotype) and low ('nontaster' phenotype) preferences for sweeteners. In order to determine how central gustatory processing is influenced by the *Tas1r3* genotype, we recorded taste-evoked responses of cells in the nucleus of the solitary tract (NST) in mouse strains that are nearly identical except for variation of this gene. We used 129P3/J (129) nontaster mice and a strain of congenic mice with a 129 genetic background and a small donor fragment from C57BL/6ByJ mice that includes *Tas1r3* and confers taster status. Mice were anesthetized, the extracellular activity of single NST neurons was isolated, and a stimulus array that included 100 mM NaCl, 10 mM HCl, 20 mM QHCl, 500 mM sucrose, 10 mM IMP, 10 mM Na-saccharin, and 100 mM D-phenylanine was applied to the oral cavity. Responses across all neurons to sucrose and D-phenylalanine were significantly larger in congenic mice than in 129 mice, but other chemicals evoked similar responses in the two strains. Although mean responses to saccharin did not differ between strains, in congenic mice saccharin's across-neuron profile was correlated more highly with that of sucrose and less highly with those of NaCl, HCl, and QHCL than in 129 mice. Our results suggest that allelic variation of *Tas1r3* affects intake of sucrose and D-phenylalanine by influencing perceived intensity, but affects saccharin intake by influencing perceived taste quality.

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Slide: Polak Young Investigator Taste Symposium

A combinatorial code for bitter taste receptors

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The gustatory system has, in contrast to the olfactory system, little discriminatory power. This is especially evident for bitter tastants where thousands of chemically diverse ligands elicit the same taste quality. Using heterologous expression we identified agonists for 11 of the 25 human TAS2R bitter taste receptors. These receptors are broadly tuned and are therefore sufficient to detect a vast variety of structurally diverse compounds. Moreover, we demonstrate that in analogy to olfactory receptors, TAS2Rs show all three hallmarks of a combinatorial receptor code. First, the majority of the bitter tastants tested activated more than one receptor. Second, many TAS2Rs are activated by several compounds. Third, compounds that activate a certain receptor differ in their rank order of potency. In the olfactory system, the combinatorial code provides the molecular basis of odorant discrimination, because each olfactory neuron expresses only a single receptor. For the TAS2Rs, however, it is unlikely that the combinatorial code contributes to the discrimination among bitter tastants, because all TAS2Rs are expressed in the same set of taste receptor cells. Instead, the observed redundancy can help to avoid certain bitter toxins in case of receptor loss through mutations. Moreover, we speculate that it also contributes to the intensity coding of human bitter perception.

Slide: Polak Young Investigator Taste Symposium

Acid block of TRPM5, a transient receptor potential channel involved in bitter, sweet and amino acid transduction

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TRPM5, a member of the superfamily of transient receptor potential (TRP) ion channels, is essential for the detection of bitter, sweet and amino acid tastes. In heterologous cell types it forms a nonselective cation channel that is activated by intracellular Ca^{2+}. TRPM5 is likely to be part of the taste transduction cascade and regulators of TRPM5 are likely to affect taste sensation. In this report we show that TRPM5, but not the related channel TRPM4, is potently blocked by extracellular acidification. To determine the mechanism by which protons block TRPM5, we generated mutations in His, Asp and Glu residues in all extracellular loops of the channel. Two mutants show significantly reduced sensitivity to protons and a double mutant is nearly insensitive to pH. These data show that variations in extracellular pH acting on an extracellular site of TRPM5 regulate its activity and suggest that pH might regulate TRPM5-mediated taste transduction.

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Slide: Polak Young Investigator Taste Symposium

Nasaccharin both stimulates and inhibits the hTAS1R2–TAS1R3 sweet taste receptor *in vivo* and *in vitro*: evidence supporting an allosteric model of a TAS1R heterodimer receptor

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The sweet taste of Na-saccharin is unlike that of most other sweeteners. While sweetness usually increases monotonically with concentration, Na-saccharin sweetness decreases at high concentrations resulting in an inverted-U shaped intensity function. This same inverted U-shaped Na-saccharin function is evident with Ca^{+2} fluorescence measures of HEK293 cells that express the sweet taste receptor hTAS1R2–TAS1R3. Thus, Na-saccharin appears to serve as an agonist and, paradoxically, as an antagonist to this taste receptor. In support of this idea, high concentrations of Na-saccharin also inhibited several other sweeteners in human subjects as well as in the HEK293 expression assay. We extended the investigation of Na-saccharin's sweet modifying properties by studying its sweet 'water taste': the induction of sweet taste by pure water when it is preceded by an oral chemical stimulus. Interestingly, a strong sweet water-taste was evident following exposure to concentrations of Na-saccharin that inhibit sweeteners. A similar observation has been made with the sweetness inhibitor lactisole (NaPMP), which also elicits a sweet-water taste. We determined that other compounds which elicit sweet water taste are also sweetness inhibitors, suggesting a functional link between the two phenomena. These observations indicate that there are activating and inactivating binding sites on the receptor supporting a two-state allosteric equilibrium model of the TAS1R2-TAS1R3 sweet heterodimer.

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Symposium: Odor Signals From the Immune System: How the Nose Detects Genetic Individuality

Chemosensory detection of histocompatibility phenotypes: responses and functions

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Genes of the major histocompatibility complex (MHC) play a critical role in immune recognition through the presentation of peptide antigens to T-lymphocytes. These genes are the most polymorphic genes known with many hundreds of alleles at some loci. These alleles influence susceptibility to almost all infectious and autoimmune diseases. It has been traditionally thought that some form of pathogen-driven selection is responsible for their unprecedented genetic diversity. However, histocompatibility phenotype can be detected via the chemical senses and a variety of behavioral and physiological responses are influenced by this phenotype including mate choice, kin recognition, individual recognition and selective abortion (Bruce effect). The list of biologically important functions influenced by the ability to chemo-phenotype the MHC of conspecifics includes: production of disease resistant progeny, inbreeding avoidance, kin-biased cooperative behavior and preemptive abortion to prevent costlier infanticide during territorial takeovers by males. The recent discovery that peptides are the odorants and that they are recognized in the vomeronasal organ in a fashion consistent with MHC allele-specific presentation of peptides, suggest that MHC-based behaviors could be central to the evolution of

many of the unique features of histocompatibilty genes. Of all the mechanisms that might explain MHC genetic diversity, the most compelling data support MHC-based disassortative mating preferences. The MHC story is arguably the premier example of a biologically complex system where many of the steps from molecules to physiology and behavior are known.

Symposium: Odor Signals From the Immune System: How the Nose Detects Genetic Individuality

Encoding immune system signals by the mammalian nose: the scent of genetic compatibility

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Work over the past 25 years has shown that genes of the major histocompatibility complex (MHC), which play a well-established role in immune recognition, also influence mating preference and other social behaviors in fish, rodents and humans. These studies led to the notion that MHC molecules are a source of unique individual odors that somehow influence the recognition of individual conspecifics. Less clear, however, are the exact cellular and molecular mechanisms by which this can occur. MHC molecules are part of an elaborate peptide presentation system. Here, we show that small peptide ligands of MHC class I molecules function also as social recognition signals in the mammalian nose, thus providing a novel molecular link between the immune system and the sense of smell. The availability of a large family of social recognition signals involving several thousand structurally defined peptide ligands provides unique opportunities to explore a variety of fundamental olfactory questions including how these molecules are encoded by neuronal populations and their receptors, what the structure–function relationships between peptide ligands and their receptors might be, and how social memories based on the recognition of these signals are formed.

Supported by grants from NIH/NIDCD.

Symposium: Odor Signals From the Immune System: How the Nose Detects Genetic Individuality

Combinatorial coexpression of neural and immune multigene families in mouse vomeronasal sensory neurons

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The vomeronasal organ (VNO) is a chemosensory organ specialized in the detection of pheromones in higher vertebrates. In mouse and rat, two gene superfamilies, V1r and V2r vomeronasal receptor genes, are expressed in sensory neurons whose cell bodies are located in, respectively, the apical and basal layers of the VNO epithelium. Neurons of the basal layer express another multigene family, termed H2-Mv, representing nonclassical class I genes of the major histocompatibility complex. The nine H2-Mv genes are expressed differentially in subsets of neurons. More than one H2-Mv gene can be expressed in an individual neuron. *in situ* hybridization with probes for H2-Mv and V2r genes reveals complex and non-random combinations of coexpression. While neural expression of Mhc class I molecules is increasingly being appreciated, the H2-Mv family is distinguished by variegated expression across seemingly similar neurons, and coexpression with a distinct multigene family encoding neural receptors. Basal vomeronasal sensory neurons may consist of multiple lineages or compartments, defined by particular combinations of V2r and H2-Mv gene expression.

Symposium: Odor Signals From the Immune System: How the Nose Detects Genetic Individuality

Evolution of molecular mechanisms informing about genetic individuality: from interindividual to intercellular discrimination

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The antigen receptors of lymphocytes are generated via somatic recombination. To eliminate from the initial receptor repertoire those that are self-reactive or have undesired specificity, the adaptive immune system depends on an effective quality control system. We propose that, during evolution, the immune system has co-opted a preexisting mechanism for this purpose. Presentation of intracellular peptides at the cell surface via MHC molecules can be viewed as a particular form of functional genome analysis. Structurally different versions of such carrier molecules in each individual would bind to different subsets of peptides from the intracellular pool, dictating the chemical diversity of peptides appearing in MHC/peptide complexes. In this way, MHC peptides mirror genetic diversity. For immune surveillance, MHC/peptide complexes must be linked to individual cells. For interindividual communication, they must be released into the extracellular space. In mice, MHC peptides function as sensory stimuli for a subset of vomeronasal neurons and as individuality signals underlying mate recognition in the context of pregnancy block. This alternative function of MHC peptides may be a shadow of a primordial system informing about genetic individuality that was employed for sexual selection before the emergence of the adaptive immune system. We currently focus on the evolutionary origin of the MHC/peptide system by examining the role of peptides in mate choice decisions of sticklebacks; we also investigate the presence of a similar mechanism in lamprey, a primitive vertebrate without MHC molecules and VDJ recombination.

Poster: Olfactory: Clinical

'Olfactory training' in patients with olfactory loss

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The aim of this investigation was whether patients with olfactory loss would benefit from 'training' with odors in terms of an

improvement of their genral olfactory function. It was hypothesized that olfactory training should produce both an improved sensitivity towards the trained odors and a genereal increase of olfactory function. Material and methods: the prospective study was performed in patients with olfactory dysfunction and controls, matched for age and sex (n = 12). One group of the patients performed the training (n = 47) while another part did not (n = 15). Exclusion criteria for patients were sinusnasal disease. Olfactory training was performed over a epriod of 12 weeks. Patients exposed themselves twice daily to four odors (phenylethyl alcohol: 'rose', eucalyptol: 'eukalyptus', citronellal: 'lemon', eugenol: 'cloves'). Olfactory testing was performed before and after training using the 'Sniffin' Sticks' (thresholds for phenyl ethyl alcohol, tests for odor discrimination and odor identification) in addition to threshold testing for the trained odors. Results: compared with baseline, following training patients experienced an increase in their olfactory function which was observed for the 'Sniffin' Sticks' test score and for thresholds for the trained odors. Similar findings were observed in ehalthy controls. However, olfactory function was unchanged in patients who did not train Conclusions: the present results indicate that the sturctured, short-term exposition to odors may increase olfactory sensitivity.

Poster: Olfactory: Clinical

Odor judgements in patients with alcohol-dependence

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Prior research has demonstrated that alcohol-dependent patients have bilateral impaired olfactory sensitivity, discrimination and identification. The present study investigated olfactory judgements in this alcohol use disorder. Our sample included 30 alcohol-dependent patients and 30 healthy control subjects, well matched for sex, age and smoking status. All subjects were screened for their odor detection threshold ability (sensitivity) and had to judge intensity, familiarity, edibility and pleasantness of 16 odors using visual rating scales. Familiarity, edibility and pleasantness judgements were performed using 16 common everyday odors (real-world items). Compared with controls, patients were significantly less familiar with everyday odors. Patients also showed poorer performance in the judgements about edibility of these odors. Bilateral impairments were present independent of age, gender, smoking and length of abstinence. Results indicate that impaired familiarity and edibility judgements in patients are not attributable to medication or impaired sensitivity. Single odor analyses of the odor of beer revealed a trend towards lower familiarity in patients, and significantly lower scores in the judgements about this odor as an edible (drinkable) item. This study extends previous findings of olfactory deficits in alcoholism, indicating impairments in processes of odor familiarity and edibility judgements in alcohol-dependent patients. Future research using olfactory stimuli (e.g. craving research) needs to consider that alcoholism is associated with a variety of disturbances in olfactory processing.

Poster: Olfactory: Clinical

Japanese odor stick identification test (OSIT-J): comparison of data from US and Japanese subjects

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A new smell test, the Odor Stick Identification Test for Japanese (OSIT-J), has been developed in Japan. To determine if the OSIT-J would be suitable for use in the USA, we administered the test to US and Japanese subjects. Data for individual test odorants as well as composite test scores were analyzed. US and Japanese subjects correctly identified eight of the 13 odorants included in the OSIT-J with scores of 80% or higher. However, for five odorants (e.g. Indian ink, Japanese cypress wood), US subjects' scores were <80% and lower than Japanese subjects, presumably reflecting cultural differences in odor experience. The difference between composite OSIT-J scores for US (77%) and Japanese (93%) subjects was significant ($P < 0.001$). The test time was 7 ± 1 min. When asked their opinions regarding the OSIT-J, US subjects reported the test to be easy, interesting, pleasant and short in duration. These data suggest that the OSIT-J is effective in identifying US subjects with normal smell function. The identification of individual test odorants having a cultural bias is critical when evaluating olfactory function tests for use in different populations.

Poster: Olfactory: Clinical

Recovery of olfactory function following closed head injury or infections of the upper respiratory tract

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The object of this retrospective study was to investigate the change of smell function in patients suffering dysosmia after infections of the upper respiratory tract (post-URTI) or head trauma. A total of 361 patients (228 women, 133 men) were included. Olfactory function was assessed using the 'Sniffin' Sticks'. The mean interval between the first and the last visit was 13.6 ± 11.4 months. Overall, when comparing scores between last and first visit olfactory function improved in 26% of the patients, whereas it decreased in 6% (change of olfactory test score of >6 points). There was a significant influence of the cause of olfactory impairment on the progression: within the post-URTI group (total n = 262) 32% of the patients improved, but only 10% in the post-traumatic group (total n = 99). In 63 and 83%, respectively, no change was observed. In addition, in patients with post-URTI olfactory loss there was a negative correlation between age and change in olfactory function. In general, there were no significant sex-related differences regarding the outcome of the disease. Furthermore, different therapies had no impact on the progression. In conclusion, smell disorders show limited, but significant improvement if caused by an URT-infection, but to a much smaller degree if they are caused by head injury. Importantly, age plays a significant role in the recovery of olfactory function.

Poster: Olfactory: Clinical

Seasonality of post-infectious olfactory dysfunction: retrospective study of 461 patients

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We investigated whether olfactory dysfunction following infections of the upper respiratory tract (post-URTI) has an incidence following the seasonality of URTIs. In this retrospective study, 461 patients (335 female) with post-URTI olfactory loss were examined during a 6 year period (1998–2004). The severity of olfactory dysfunction was assessed by means of the 'Sniffin Sticks' test. Incidence of post-URTI olfactory dysfunction exhibited seasonal fluctuations with deviations from the well known winter seasonality of URTIs. The overall incidence of the disease differed significantly between months ($P < 0.05$). More specifically, March (12.7%) and May (12.6%) were the months with the highest incidence of the disease throughout the year. The lowest incidence was found in September (5.6%). Significant differences were found between these months and months with typically high incidence of URTI as September and December (Mar–Sep, Mar–Dec, May–Sep, May–Dec: $P < 0.05$). This pattern of seasonality was repeated with only slight differences every year. The peak incidence of post-URTI olfactory loss in March may be explained by the high incidence of influenza at this time. However, it is unclear why the incidence of the disease presents a second peak in May, when the incidence of respiratory viruses is relatively low. This raises the question whether climate conditions at this time (low humidity with high temperature) play a critical role in the susceptibility of the nasal epithelia towards certain viral infections during this time of the year, e.g. parainfluenza type III and certain serotypes of rhinoviruses.

Poster: Olfactory: Clinical

Measuring implicit beliefs about odors and health: the 'mysterious machine' task

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Objective: people vary in their beliefs about whether and how odors in the air affect health, which beliefs may affect their reactions to actual emissions. In this qualitative study we used an implicit task to study these beliefs. Method: 12 groups of students ($n = 28$ in total) were asked to design an imaginary machine, which altered the odorous air in the environment in such a way that it no longer caused illness. They had to describe or draw the 'mysterious' mechanism of this machine. This task was embedded in a focus group in which related matters were discussed. Results: some groups designed a machine based on filtering (e.g. by removing heavier molecules from the air, apparently based on the belief that odorous molecules are heavier), or boiling (e.g. reflecting the belief that odors disappear at boiling point). Other participants' machine eliminated odor by removing all bacteria from odorous air. Yet others challenged the assumption that odors alone can cause illness. They wanted to change odor perception, for example by anaesthetizing the receptor cells in the nose or changing the perceiver's beliefs. Conclusion: students indeed endorsed a wide variety of beliefs regarding the relation between odors and health with the most important difference being whether or not they believed that odors are capable of affecting health. In future research we intend to investigate the relationship between belief types, personal characteristics and odor perception.

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Poster: Olfactory: Clinical

Similarities and differences in odor identification and sniff suppression in Parkinson's disease

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Over the last 30 years investigators have reported a large body of evidence suggesting patients with Parkinson's disease (PD) have an olfactory impairment. This study assessed the ability of odors to suppress sniffing as well as odor identification in 39 patients with PD. Patients were assessed using the UPSIT and the newly developed Sniff Magnitude Test (SMT). The SMT is a reliable measure of olfactory function that examines sniffing behavior to quantify olfactory abilities and is minimally dependent on cognitive status and culture. Results showed the SMT and the UPSIT were strongly correlated, and both tests showed patients with PD generally suffer from olfactory impairment (UPSIT: mean = 17.77; SMT: mean = 0.78; $r = -0.55$, $P < 0.001$). However, a surprising number of patients (23%) had impaired identification abilities (SIT < 26) but normal sniff suppression (SMT < 0.70). Other studies have reported that some patients with PD have odor identification deficits but still detect a smell (Ward *et al.*, 1983; Mansoor *et al.*, 1999). These reports are consistent with the dramatic difference between estimated prevalence rates based on odor threshold tests (17–45%) and studies that utilized odor identification tasks (70–82%). The results are discussed in terms of the demands that olfactory tasks place on patients with PD, and possible interpretations of the variability in olfactory performance for people with PD.

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Poster: Olfactory: Clinical

Olfactory functions in asymptomatic carriers of the Huntington disease mutation

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Huntington's disease (HD) is a neurodegenerative disorder initially affecting the basal ganglia and especially the head of the caudate nucleus. Neuropsychological research has indicated that olfactory dysfunction may appear early in HD, prior to the onset of significant motor or cognitive dysfunction. The aim of this study was to examine

whether asymptomatic carriers of the Huntington disease mutation also exhibit olfactory dysfunction. To address this issue we presented an extensive olfactory test battery, comprising tasks assessing olfactory sensitivity, intensity discrimination, quality discrimination, episodic odor memory and odor identification, to a group of gene carriers and non-mutation carriers of the disease. The results showed that gene carriers were selectively impaired in discriminating odor quality, although performance did not differ from controls across the other tasks. The role played by striatum and then in particular the caudate nucleus for olfactory processing in general, and for odor quality discrimination in particular, is discussed.

Poster: Olfactory: Clinical

A 10-item smell identification scale for early detection of Alzheimer's disease

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The current study sought to identify an optimal subset of items from the University of Pennsylvania Smell Identification Tests (UPSIT) that is related to an increased risk of Alzheimer's disease (AD). UPSIT data from 63 controls, 127 minimal to mild cognitive impairment (MMCI) patients and 100 AD patients (n = 290) were analyzed to derive an optimal subset of items that distinguished AD and MMCI converters to AD from MMCI non-converters and controls. Predictive utility for conversion to AD for the 40-item UPSIT, 12-item Brief Smell Identification Test (B-SIT) and derived 10-item subset was further assessed in an expanded cohort of MMCI patients (n = 147). The 10-item scale was better than the UPSIT and B-SIT in classifying subjects according to increasing risk of AD. Survival analyses for data from 147 MMCI patients showed that low scores for the 10-item scale ($P < 0.001$), the UPSIT ($P < 0.03$) and, to a lesser extent, the B-SIT ($P = 0.06$) predicted conversion to AD after controlling for demographic and clinical variables. The 10-item scale was related to an increased risk of AD and strongly predicted conversion to AD in MMCI patients. Independent replication with further validation is needed to ensure the reliability of the present findings.

Poster: Olfactory: Clinical

The effect of gender and the apolipoprotein E epsilon 4 allele on recognition memory for olfactory and visual stimuli in patients diagnosed with Alzheimer's disease and healthy older adults

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Episodic recognition memory for odors, faces and unfamiliar symbols was assessed in apolipoprotein E (ApoE) E4-positive and E4-negative males and females diagnosed with Alzheimer's disease (AD) and healthy age- and gender-matched controls. In controls, males who were E4-negative outperformed E4-positive males in recognition memory for odors and committed fewer false positive errors. However, there were no significant differences between E4-positive and E4-negative female controls. No significant gender or ApoE status differences were detected in recognition memory for faces or symbols in controls. In patients with AD, E4-negative females outperformed E4-positive females in recognition memory for odors and committed significantly fewer false positive errors. However, there were no significant differences between E4-positive and E4-negative males. There were no significant gender or ApoE status differences in recognition memory for faces or symbols in AD patients. The results demonstrate that recognition memory for olfactory stimuli may be particularly impaired in healthy older males with the E4 allele. In patients with AD, odor memory impairments may be less severe in females who are negative for the E4 allele. The results offer new insight into how recognition memory is affected by gender, the E4 allele, and the modality of the stimulus to be remembered in healthy older adults and patients with AD.

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Poster: Olfactory: Clinical

Differences between orthonasal and retronasal olfaction in patients without nasal polyposis

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Most patients loosing olfactory function complain of smell and taste loss. This is mainly due to the absence of ortho- but also retronasal olfactory function. This latter one is mainly responsible for the perceptions of flavor. We report a case series (n = 15) of patients with olfactory complaints without 'taste' complaints. In contrast to the literature, which shows this pattern to occur in case of nasal polyposis, the present patients had lost olfaction after infections of the upper respiratory tract. Psychophysical testing revealed retronasal olfaction to be normal or slightly altered, while orthonasal olfaction was either absent or severely altered. Nasal endoscopy was normal in all subjects. In five patients olfactory event-related potentials could be obtained corroborating the psychophysical findings. Orthonasally no/small responses were found while retronasal OERP were present/larger in amplitude. These clinical observations together with the psychophysical and electrophysiological findings suggest that ortho and retronasal olfaction might be generated by different neuronal populations. These different or overlapping neuronal populations might exhibit differential vulnerability to damages following infections of the upper respiratory tract.

Poster: Olfactory: Clinical

Odorization of the incubator prevents apneas in premature infants

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Leading to a decrease in cerebral blood flow velocity and in cerebral oxygenation, apnea of immaturity represents a major preoccupation for premature infants caregivers. Pharmacological treatments (methylxanthines and doxapram) currently used to treat these apneas are not fully effective and often present undesirable side effects. The present study examines whether exposure to an odor known to modulate the newborn's respiratory rate reduces the incidence of apneic spells. Fourteen premature infants born at 24–28 post-conceptional weeks and presenting apneas resistant to classical treatments were exposed during the second week after birth to the odor of vanilla diffused during 24 h in the incubator. The day before and the day after odorization were used as control for each subject. A significant diminution (44%) of the number of apneas >20 s (or less if associated with immediate hypoxia) was observed during odorization and this diminution was seen in all the subjects. Apneas associated with bradycardias (heart rate <50% of baseline rate) decreased strongly (45%) during odorization and this decrease affected all the infants. No side effects were observed. Odorization of the incubator thus appears of therapeutic value for the treatment of apneas unresponsive to classical therapy and may contribute to preserve the integrity of the developing brain.

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Poster: Olfactory: Clinical

The impact of inflammation on the olfactory epithelium in patients with chronic rhinosinusitis

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Chronic rhinosinusitis (CR) affects an average of 33 million people each year, and is a significant cause of olfactory dysfunction in these individuals. Currently, our goal is to employ various immuno-based and molecular biology techniques to determine the contribution of the inflammatory response to the development of olfactory deficits in individuals suffering from CR, and to identify specific inflammatory components that promote and reflect deterioration and/or changes in the physiology of the sensory epithelium. We predict a correlation between specific inflammatory markers and significant degeneration and/or remodeling of the olfactory epithelium, and that these inflammatory profiles will in turn provide insight into the cause/effect relationship between chronic rhinosinusitis and the degree and duration of olfactory impairment. Preliminary studies thus far have indicated that two cytokines, tumor growth factor beta and IL-1, may play an important role in this phenomonen. Tissue sections from biopsy specimens of CR patients demonstrate an altered cellular structure at the mucosal surface, which appears to be keratinization. Immunocytochemistry revealed intense TGFβ staining in cells near the basement membrane, and in the basement membrane itself. IL-1 receptor staining showed an immunopositive cell population located in the epithelium above the basal lamina, while antibodies specific for the nerve growth factor receptor (P75-NGFR) positively stained cells in the same location, suggesting the possibility that these two receptors are being up-regulated in the same cell population.

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Poster: Olfactory: Clinical

Retronasal transport and active nasal blocking: implications for exposure studies

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Human exposures to airborne chemicals may result in a spectrum of effects on health, and the pathways leading from exposures to responses are frequently unknown. Isolating the nasal cavity from an inhalation exposure can permit blinding the exposure and contribute to identification of mechanisms of action. Nasal clips or plugs have been used previously for this purpose. However, we have found that retronasal transport during oral breathing, with the nares occluded, leads to significant mixing of the inhaled gas with that in the nasal cavity. Using exogenous (helium) and endogenous (argon) tracer techniques, intranasal gas sampling, and analysis via a specially constructed mass spectrometer, both retronasal and anterograde transport in the occluded nose were measured. Rapid transport was observed in the majority of subjects: significant tracer levels were found in the nasal cavity within a few breaths, and levels exceeded 50% of the inhaled tracer concentration within one to several min. Surprisingly, in a few subjects, this retronasal transport was markedly reduced. Simultaneous oral and nasal pressure measurements suggested that this dichotomy was associated with persistent closure of the nasopharynx by the velum in those subjects. Additionally, active nasal blocking using continuous nasal airflow has been investigated using a real-time tracer technique. Implications of these results for human exposure studies will be discussed.

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Poster: Olfactory: Clinical

Assessment of strategies for optimizing Parkinson's disease detection using UPSIT item analysis

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Hawkes and others have suggested that certain items from the University of Pennsylvania Smell Identification Test (UPSIT) may distinguish between patients with Parkinson's disease (PD) and controls better than others. If this is the case, shorter tests for aiding in the diagnosis of PD could be developed that optimize test discriminability. In this study, we examined the contribution of individual UPSIT items for their ability to discriminate between PD and control subjects. UPSIT items from 308 PD and 308 matched normal

controls were evaluated using several different analyses, including one based upon Rasch's one-parameter item response model. Some, but not complete, overlap between items identified by us and by Hawkes as optimal predictors was found. The results are discussed in light of the complexities of odor identification responses, including serial position response preferences within a forced-choice test.

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Poster: Olfactory: Clinical

Olfactory functioning following anteromedial temporal lobe resection

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We investigated olfactory functioning in healthy volunteers and in individuals who had undergone surgery for epilepsy with anteromedial temporal lobe resection including amygdalahippocampectomy. Free and cued identification, odor intensity and valence perception were assessed. Identification was tested using the Sniffin'Sticks set. Intensity and valence were assessed for two pleasant (citral and peach) and two unpleasant odorants (valeric acid and butyric acid), each odorant presented at two different concentrations. All odor stimuli were presented monorhinically. Of particular interest was the analysis of the effects of resection on the level and consistency of judgements of perceived intensity and valence. The results will be discussed in light of previous findings, especially those concerning the role of amygdala in olfactory processing.

Poster: Olfactory: Clinical

Long-term changes in olfactory function in patients with chemosensory disturbances evaluated at the University of Pennsylvania Smell and Taste Center from 1980 to 2004

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Few studies have examined return of function over time in patients diagnosed with chemosensory disorders. In this study, we retested 600 patients previously seen at the Smell and Taste Center for olfactory problems using either the University of Pennsylvania Smell Identification Test (UPSIT) or the 12-item Brief Smell Identification Test (B-SIT) to determine how many individuals regained normal smell function. Although the final analysis of these data is pending, data from 415 of these individuals suggest the following recovery rates for the three largest etiologic groups evaluated: head trauma—9%; upper respiratory infections—25.7%; and chronic rhinosinusitis—23.5%. Influences of such variables as time since problem onset, severity of initial dysfunction, patient age, and other factors on recovery will be discussed.

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Poster: Olfactory: Clinical

The influence of pharmaco-resistant temporal lobe epilepsy and temporal lobe resection on olfaction

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Olfaction is compromised in temporal lobe epilepsy and by temporal lobe resection for intractable disease. In this ongoing study, olfactory and neuropsychological tests have been administered to 21 patients with left- and 29 patients with right-temporal-lobe epilepsy. In most cases, the tests have been administered pre- and post-operatively. The olfactory tests included tests of odor identification, detection, memory and hedonics. The neuropsychological tests assessed a range of functions, including general intelligence, memory and language. A subgroup of the patients received odor event-related potential testing. In general, most measures of olfactory function were compromised on the side of the epileptic focus prior to surgery. Surgery further decreased function on the side ipsilateral to the surgery, and, in some cases, resulted in improvement on the contralateral side. These and other results will be discussed in detail in this presentation.

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Poster: Olfactory: Clinical

Olfactory–trigeminal interaction: evidence from patients with congenital anosmia

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The aim of the present study was to investigate the influence of the olfactory system on trigeminal perception. Intranasal trigeminal sensitivity was assessed in patients with isolated congenital anosmia and healthy subjects on different levels of perception. At the level of the respiratory epithelium patients with isolated congenital anosmia had larger electropyhsiological responses to strong trigeminal

stimuli when compared with healthy controls. No significant difference between the two groups was found for cortical event-related potentials and a psychophysical test used to identify the trigeminal impact of odors. The results of the present study support the concept of peripheral sensory compensation and the idea of a lack of priming of the trigeminal system in the case of olfactory deprivation.

Poster: Olfactory: Clinical

Relationships among three tests of human olfactory function in a clinical setting

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One hundred consecutive patients with complaints of chemosensory dysfunction were administered, as part of their routine evaluation at the Smell and Taste Center, three tests of olfactory function: the University of Pennsylvania Smell Identification Test (UPSIT), the Sniff Magnitude Test (SMT) and the single staircase phenylethyl alcohol odor detection threshold test (PEA-T). The first two of these tests were administered binasally, whereas the PEA-T was administered both binasally and uninasally. Two different odor presentation strategies were employed for the SMT. Spearman correlations among the test measures were generally highest between the UPSIT and PEA measures. Differential performance was noted on the SMT as a function of the odorant species employed and appeared to be influenced by the positioning of the blank within the odorant presentation sequence. Ongoing analyses are examining factors that may optimize SMT's relationship with the other two measures.

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Poster: Olfactory: Clinical

The effect of estrogen and its interaction with the ApoE epsilon 4 genotype on olfactory functioning in nondemented elderly females and females diagnosed with Alzheimer's disease

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Olfactory impairment may be one of the earlier indicators of Alzheimer's disease (AD) that may precede clinical diagnosis. A genotype containing the E4 allele of the Apolipoprotein E (ApoE) may result in increased risk of AD and earlier symptom onset. Studies have reported that estrogen may exert neuroprotective effects on brain areas affected by AD. The current study investigated the effect of Estrogen Replacement Therapy (ERT) on an olfactory threshold test in E4-positive and E4-negative non-hysterectomized females who were either nondemented elderly controls or patients diagnosed with AD. ERT had no effect on odor threshold in the AD patients regardless of E4 status. In contrast, nondemented participants without ERT who were E4-negative performed significantly better on the threshold test than the E4-positive individuals. Performance of the nondemented E4-positive participants with ERT was significantly better than the nondemented E4-positive participants without ERT. Nondemented E4-positive and E4-negative participants with ERT performed at similar rates. These results suggest that ERT may offer protection against loss of olfactory function in E4-positive individuals who may be in the early stages of AD. However, these effects diminish in the later stages of AD.

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Poster: Olfactory: Clinical

Numerical modeling of ortho- versus retronasal airflow and odorant delivery among rhinosinusitis patients

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Chronic rhinosinusitis (CR) with nasal polyposis (NP) can differentially impair ortho- versus retronasal olfactory acuity. Such differences appear to be associated with the presence of mechanical obstruction in the anterior portion of the olfactory cleft. These findings indicate that olfactory loss in NP may result from regional mechanical obstruction or inflammation-induced airway restriction and be dependent on the direction of nasal airflow. As yet, however, no technique can accurately predict the directional impact of nasal obstruction on airflow through the nasal cavity and subsequent odorant delivery to olfactory receptor sites, which is critical to normal olfactory function. Using computational fluid dynamics (CFD) techniques, we can convert patients' CT scans into 3-D numerical nasal models that can quantitatively predict both ortho- and retronasal airflow and odorant delivery patterns. These models are anatomically accurate, preserving any CT findings of airway obstruction caused by CR and NP. Our goal is to correlate model prediction especially in the olfactory region with NP patients retro- versus orthonasal olfactory acuity. In our preliminary findings, CFD modeling of one NP patient's nose showed significant difference in ortho- versus retronasal airflow pattern and olfactory odorant delivery rate in the olfactory region, which correlate well with her better retro- than orthonasal measured olfactory event-related potentials. In the future, such modeling techniques may provide a quantitative evaluation of the functional impact of nasal obstruction to human olfactory perception.

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Poster: Olfactory: Clinical

Sensitivity and specificity of the 3-item Quick Smell Identification TestTM (Q-SIT)

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There is great clinical need for very brief olfactory screening tests, but the few reported in the medical literature have been found wanting (e.g. sensitivity and specificity is rarely reported). The goal of this research was to establish the sensitivity and specificity of the 3-item Quick Smell Identification TestTM (Q-SIT; Sensonics, Inc., Haddon Hts., NJ) in diagnosing olfactory dysfunction. The Q-SIT was administered to a set of 205 consecutive patients (92 men, 113 women, age range 18–88 years) evaluated at the Smell and Taste Center in 2004. As part of their overall assessment, they also received the 40-item University of Pennsylvania Smell Identification Test (UPSIT). Using the UPSIT as an index of function, the Q-SIT was abnormal in 97% (62/64) of the patients with anosmia, 86% (30/35) with severe microsmia, 74% (29/39) with moderate microsmia and 48% (15/31) with mild microsmia. Of the 36 normosmic patients, 64% had a perfect Q-SIT score, 24% (8/36) had one wrong answer and 14% (5/36) had two wrong answers. None of the normosmic patients missed all three items. The Q-SIT had a sensitivity of 80%, a specificity of 64% and a positive predictive value of 91%. These data suggest that a brief 3-item odor identification test can be employed as a rapid first-screen for detection smell dysfunction.

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Poster: Olfactory Development

Role of nitric oxide in neuronal differentiation of the OLF442 olfactory cell line

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Olfactory neurons originate from stem cells, which are part of the basal cells of the olfactory epithelium. After several divisions, these neuronal precursors differentiate into mature olfactory neurons. The OLF442 cell line derives from these neural precursors (MacDonald *et al.*, 1996, *J. Neurosci. Res.*, 45:237–247). These cells express neurofilament and develop processes when grown in low serum conditions. We were able to induce neuronal differentiation in primary cultures of olfactory epithelium basal cells with inhibitors of nitric oxide sintase (NOS; Sülz *et al.*, 2003, AChemS Meeting). In this work we examined the effect of these inhibitors on the differentiation of OLF442 cells. The NOS inhibitors TRIM [1-(2-trifluoromethylphenyl)imidazole] and L-NIL [L-N6-(1-iminoethyl)lysine, DiHCl] decreased cell proliferation and induced the extension of processes and of OMP (mature olfactory neuron marker). ODQ (1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one), a guanylyl cyclase inhibitor, had no effect, indicating a cGMP-independent mechanism. Whole-cell current recordings revealed that the NOS inhibitors caused the expression of a TEA-sensitive, voltage-dependent potassium conductance of the delayed rectifier type. This conductance was absent in untreated cells. Inward currents were not detected. Extracellular ATP had no effect on membrane conductance. These results support the notion that an NO reduction inhibits proliferation and triggers differentiation in OLF 442 cells.

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Poster: Olfactory Development

Olfactory epithelium promotes mitral cell dendritic outgrowth

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Stereotypic connection between olfactory sensory neurons and the projection neurons in the olfactory bulb is the first synaptic relay for olfactory perception. In mice, mitral cell primary dendrites target single glomeruli and form synapses with the olfactory axons. During embryonic development, however, mitral cells develop an elaborate dendritic tree spanning an area of 20–30 glomeruli. Olfactory axons are in close contact with the dendrites of the differentiating mitral cells throughout these stages. To examine whether olfactory nerve regulates the elaboration of the mitral cell dendrites, conditioned medium from E14 olfactory epithelium (OE) was obtained and outgrowth activity was examined in E14 mitral cell culture. Total neurite length of mitral cells was significantly increased in OE conditioned medium compared with that of the control defined medium ($P < 0.001$). This neurite outgrowth promoting activity is detected from E16, E18 and P0 OE conditioned medium assayed with E14 –18 mitral cells. However, mitral cells obtained from P0 and P1 olfactory bulb fail to respond to the OE derived outgrowth activity. These results demonstrate that OE derived activity is present during development; however, mitral cells change their responsiveness at P0. Combining with the observation that developing mitral cells switch from dendritic elaboration to dendritic pruning in early postnatal stages *in vivo*, it suggests that different regulatory programs are present in the embryonic and early postnatal mitral cells. The OE derived activity is heat sensitive and around molecular weight of 30–50 kDa. Further characterization is underway to identify the biochemical nature of this activity.

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Poster: Olfactory Development

Metamorphosis of an olfactory system: hormonal regulation of growth and patterning in the antennal imaginal disc of the moth *Manduca sexta*

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Peripheral olfactory systems of insects undergo metamorphosis, transforming from a simple larval antenna to the highly complex adult antenna mediating diverse chemosensory behaviors. Adult antennae derive from imaginal discs which grow during the larval stage, and undergo neurogenesis and morphogenesis during the pupal stage. We are characterizing patterns of morphogenic activities in the imaginal disc and early developing antenna to identify hormonally regulated events which lead to the patterning of the adult antenna. This study focuses on the antennal disc; disc growth occurs during the final larval instar from a ring of tissue surrounding the base of the larval antenna. We have characterized the spatial patterns of disc growth using an antibody against phosphorylated histone H3 (mitotic marker). Prior to pupation, the disc elongates and everts; we have shown this process is regulated by ecdysteroid hormones, and have observed a subsequent decline in total DNA content suggesting apoptotic events associated with this restructuring. These studies are establishing a foundation for identifying the hormonal regulation of growth and patterning that will give rise to the selection of specific chemosensory phenotypes of adult olfactory sensilla.

Poster: Olfactory Development

Supplementation with retinoic acid leads to recovery of OMP+ neuron population in VAD OE

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Our work has demonstrated that vitamin A deficiency (VAD) leads to a substantial decrease in olfactory marker protein (OMP) mRNA levels in VAD OE of postnatal rats relative to VA sufficient (VAS) controls. Our interpretation is that neuron maturation is impeded when VA levels are reduced *in vivo*. Retinoic acid (RA) is a derivative of VA that is known to affect developmental processes, including cell differentiation and tissue morphogenesis. To determine whether OMP protein is affected in VAD OE in a similar way as OMP mRNA, and to determine whether RA is the active factor causing observed effects on mature olfactory neuron densities in our model system, we produced VAD postnatal rats and determined OMP^+ cell densities using an OMP antibody (kindly provided by F. Margolis), and we supplemented a group of frankly deficient rats with RA by oral gavage for 2 weeks and 6 weeks, and investigated whether the numbers of OMP^+ cells in OE return to control levels. The results of these studies indicate that VAD leads to a significant decrease in the number of neurons expressing OMP protein, and the decrease is equivalent to that observed in OMP^+ cells, as determined previously by *in situ* hybridization. This indicates that VAD does not exert a specific effect on expression of the OMP gene or mRNA transcript. Supplementation of frankly deficient animals for 2 weeks with RA leads to increased numbers of cells expressing OMP protein. Supplementation for 6 weeks leads to a further increase in the numbers of cells expressing OMP, but levels are still below those of age-matched controls fed a VAS diet. We conclude that maturation of neurons to an OMP^+ state is impeded in VAD postnatal rats due to decreased availability of RA.

Poster: Olfactory Development

Glutamate-scavenging glia during olfactory bulb development

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In the developing olfactory bulb, axons of olfactory receptor neurons defasciculate in the external nerve fibre layer (NFL) before undergoing sorting prior to synaptogenesis. As they defasciculate, they lose their olfactory ensheathing cells (OEC) ensheathment, and their terminals become exposed at the boundaries between the outer and inner NFL. It is not known what causes 'like' axons of similar activity to group together and selectively occupy distinct glomeruli. In *Manduca sexta*, central glia migrate out during olfactory lobe formation to participate in targeting. Here, we wanted to determine if central or peripheral glia in mice might sense extracellular glutamate to detect similarly active ORNs and participate in axon sorting prior to synaptogenesis. In immediate postnatal development, astrocytes expressing GFAP and the glutamate transporters GLT-1 and GLAST are restricted to layers of the olfactory bulb central to the glomerular layer. From P1 to P5 and P14, however, astrocytes highly expressing glutamate transporters and NMDA receptors migrate out in a distinct pattern throughout the NFL, all the way to the edge of the cribriform plate, where they associate with bundles of incoming axons. In the adult, when the peak period of synaptogenesis is complete, these glutamate-scavenging astrocytes are still found in the NFL (in lower abundance) but most arrange as clusters around glomeruli. During this period of outgrowth, sorting and synaptogenesis, we also detected expression of mGluR5 in OECs (maximal before P14), which appeared to be down-regulated in the adult. We are currently testing to see if bulbar astrocytes and OECs respond to glutamate gradients *in vitro*.

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Poster: Olfactory Development

Molecular development of the mouse septal organ

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The septal organ, a small island of olfactory neuroepithelium located at the base of the nasal septum, is a distinct chemosensory organ observed in many mammals. By combining cDNA cloning, Affymetrix genechips covering all the mouse olfactory receptor genes and *in situ* hybridization, we recently achieved a relatively complete expression profile of odorant receptors in this area. The septal organ mainly expresses a few defined receptors, which are shared by the most ventral zone of the main olfactory epithelium. The most abundant receptor (MOR256-3) is expressed in ~50% of the cells and the top nine genes together account for the receptors in ~95% of the cells. Expression of these genes follows the one cell–one receptor rule, demonstrated by a thorough combination of the major receptor probes. With well-defined chemoreceptors in the mouse septal organ, we wish to test whether different subtypes

of receptor neurons show differential developmental process. OMP-positive cells appear in the septal organ at ~E16, at least 2 days later than in the main olfactory epithelium. We examined the receptor expression patterns from E16 to P3 using *in situ* hybridization. The top one receptor (MOR256–3) does not appear until E18 and becomes abundant at E20. Two moderately expressed receptors (MOR236-1 and 160-5) dominate at E16. Interestingly, another receptor MOR235-1, the closest counterpart of the MOR236-1, does not appear until E20. By the age of P3, the expression patterns are very similar as in adults. The results indicate that different receptor neurons follow differential developing time course. We are currently investigating whether these receptors are coexpressed by the developing sensory neurons.

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Poster: Olfactory Development

Identification of cell surface antigen expressed by globose basal cells

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The biochemical and cell biological characteristics of the cell surface antigen recognized by mAb GBC-3, a globose basal cell (GBC) marker, are described in this study. GBC-3 labels some, if not all, GBCs by immunohistochemistry, and its immunoreactivity does not overlap with either sustentacular cells (Sus) or horizontal basal cells (HBCs) in the normal rat olfactory epithelium (OE). After epithelial lesion by exposure to MeBr, the expression of GBC-3 antigen increases in the remaining cells, mostly GBCs, and the staining with GBC-3 extends to cells that are also labeled with either Sus or HBC markers. An antigen recognized by GBC-3 is a surface protein, as confirmed by either fluorescence-activated cell sorting (FACS) or live cell staining. Two-dimensional IEF-SDS–PAGE/Western blot shows that GBC-3 recognizes three different proteins of 52, 40 and 37kDa mol. wt. Using mass spectrometry, these proteins were identified as β-tubulin, laminin receptor precursor protein (LRP) and acidic calponin, respectively. Of these, only LRP is expressed on the surface of GBCs, and anti-LRP more closely matches GBC-3 immunostaining than antibodies to the other proteins. Thus, we conclude that GBC-3 reacts with LRP on the surface of live GBCs (and more weakly on immature and mature neurons). We contend that developing and refining GBC-selective markers is critical for identifying and characterizing GBCs, and in turn olfactory tissue stem cells, and mAb GBC-3 is a potentially useful tool to achieve that goal.

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Poster: Olfactory Development

Multipotent stem cells from adult olfactory mucosa

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Multipotent stem cells are thought to be responsible for the generation of new neurons in the adult brain. Neurogenesis also occurs in an accessible part of the nervous system, the olfactory mucosa. We show here that cells from human olfactory mucosa generate neurospheres that are multipotent *in vitro* and when transplanted into the chicken embryo. Cloned neurosphere cells show this multipotency. Multipotency was evident without prior culture *in vitro*: cells dissociated from adult rat olfactory mucosa generate leukocytes when transplanted into bone-marrow-irradiated hosts and cells dissociated from adult mouse olfactory epithelium generated numerous cell types when transplanted into the chicken embryo. It is unlikely that these results can be attributed to hematopoietic precursor contamination or cell fusion. These results demonstrate the existence of a multipotent stem-like cell in the olfactory mucosa useful for autologous transplantation therapies and for cellular studies of disease.

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Poster: Olfactory Development

Immediate early gene expression in the zebrafish olfactory epithelia

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Immediate early genes (IEGs) are transcription factors that are rapidly up-regulated in response to sensory stimuli. They are able to activate downstream genes that can lead to long-term memory formation. We have shown that the zebrafish (*Danio rerio*) is able to form and retain olfactory memories of odorants experienced as juveniles (see Rivard *et al.*, this meeting). In order to explore whether IEGs are playing a role in the formation of these olfactory memories we cloned three IEGs, *egr-1(zenk)*, *c-jun* and *c-fos*, from the zebrafish. Using previously described sequences in zebrafish we cloned *egr-1(zenk)*. We cloned *c-jun* and *c-fos* by homology to other animals. With these sequences we generated mRNA probes for *in situ* hybridization in the developing zebrafish. To determine whether any of the IEGs are up-regulated in response to odorant we are exposing zebrafish to odorant during early development and comparing the IEG expression patterns of odor exposed and control fish. *Egr-1* is not expressed in the olfactory placode during development and it is not up-regulated in response to odorant. *C-jun* is located in the olfactory placode as early as 24 h post-fertilization and we are currently examining *c-jun* expression in odor-exposed fish. We are also characterizing the expression pattern of *c-fos* in odor exposed relative to control fish.

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Poster: Olfactory Development

GnT1 glycosylation is required for axon connectivity with the olfactory bulb

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Sensory neurons in the PNS have a glycan profile that is distinct from CNS neurons. The mAb 1B2 recognizes N-acetyllactosamine (LN) carbohydrates on mature olfactory sensory neurons (OSNs), but does not interact with neurons in the brain. The glycosyltransferase β3GnT1 plays an essential role in LN synthesis. β3GnT1$^{-/-}$ mice lose 1B2 reactivity on OSNs and have severely disorganized OB projections, with most neonatal OMP+ axons failing to establish glomerular contact. This is supported by the analysis of β3GnT1$^{-/-}$ mice coexpressing tau-LacZ or -GFP with specific odorant receptors. P2 axons initially target appropriate OB loci, but fail to penetrate the glomerular layer and are subsequently lost. M72-OSNs exhibit a distinct pathfinding defect; axons in the nerve layer bypass their normal glomerular target and extend over the dorsal OB. This differential affect of β3GnT1 loss on targeting may reflect the heterogeneity of LN expression inherent to individual glomeruli. The OB disorganization in null mice correlates with poor performance on food-finding tasks. Interestingly, β3GnT1$^{+/-}$ mice also display a reduced spectrum of defects found in null mice, including a milder decrease in OE thickness and an up-regulation and redistribution of Notch pathway genes from the basal OE to more apical cell layers. These results suggest that LN glycans play important roles in establishing OB connections, and the subsequent survival and homeostasis of OSN subsets.

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Poster: Olfactory Development

Analysis of Kallmann gene function in the developing zebrafish

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Human Kallmann syndrome is characterized by hypogonadic hypogonadism (deficits in GnRH) and anosmia (loss of sense of smell). Previous analysis of a human fetus mutant for the Kallmann gene (KAL1 or *anosmin1*) revealed a failure of olfactory nerve growth into the forebrain and arrested GnRH cell migration. Though KAL1 has not been found in mouse, zebrafish have two KAL1 homologues, *kallmann1.1* (*kal1.1*) and *kallmann1.2* (*kal1.2*). To identify mechanisms controlling hypothalamic GnRH cell development in the zebrafish we knocked down *kallmann* gene function. We used modified oligonucleotides (morpholinos) to block protein translation of *kal1.1* and *kal1.2*. Strikingly, the knockdown of the *kal1.1* gene, but not of the *kal1.2* gene, caused endocrine GnRH cell loss. Despite this, there was no effect on the neuromodulatory midbrain or nervus terminalis GnRH cells of *kal1.1* morphants. The olfactory nerves of these animals were disrupted but still made connections to the olfactory bulb. To further investigate the effects of *kal1.1* knockdown on the anterior pituitary development, we are injecting *kal1.1* morpholinos in a line of zebrafish that express GFP under the pro-opiomelanocorticotropin (POMC) promoter. Because we have shown previously that hypothalamic GnRH cells arise from the anterior pituitary we predict that *kal1.1* in the POMC-GFP fish will disrupt anterior pituitary development. Our data suggest a crucial role for one isoform of the *kallmann* gene in endocrine hypothalamic GnRH cell differentiation and olfactory system development.

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Poster: Olfactory Development

Differential gene expression in the developing mouse olfactory bulb

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Development of the olfactory circuits requires precise connections among several neuronal cell types within the olfactory bulb (OB). This precise connectivity is achieved through remodeling of the embryonic neuronal morphology during early postnatal stages. Maturation of the synaptic connections within the glomeruli, formation of the granule cell apical dendritic spines, and the pruning of mitral cell dendritic processes occur between P0 and P10. To identify genes regulating these neuronal differentiation events, we compared gene expression profiles between embryonic and postnatal OB using oligonucleotide microarray techniques (Affymetrix). cRNA probes prepared from E16 and P6 OB were hybridized to GeneChip Mouse Genome 430 2.0 array, which contains more than 39 000 transcripts. We have identified 2265 genes that are differentially expressed at these two developmental time points. One hundred and eighty-one transcripts showed more than twofold up-regulation at the postnatal stage (dChip software). Gene ontology studies indicate that transcription factors and signal transduction molecules were the main categories of genes that are up-regulated. Expression patterns of 30 candidate genes were examined by *in situ* hybridization. Four candidate gene transcripts were restrictedly expressed in the mitral or mitral/tufted cells. The expression profiles of selected genes from E14 to P10 were investigated by quantitative RT-PCR. Functions of candidate genes are currently under investigation.

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Poster: Olfactory Development

Molecular cloning and characterization of proneural genes in the olfactory organ of spiny lobsters, *Panulirus argus*

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Proliferation of olfactory receptor neurons and associated cells in adults occurs in mammals and other animals including the spiny

lobster *Panulirus argus* (Harrison *et al.*, 2004, *J. Comp. Neurol.*, 471:72–84). The spiny lobster model is advantageous in having neurogenesis in a discrete locus on its olfactory organ—the antennular lateral flagellum. To identify genes associated with proliferation in adults, we are using two experimental approaches. One is representational difference analysis, to identify transcripts enriched in proliferating tissue (Stoss *et al.*, 2004, *J. Neurobiol.*, 58:355–368). The second, which we report here, is a PCR-based approach to identify spiny lobster homologues of proneural, neurogenic and neurotrophic genes. So far we have identified spiny lobster homologues of two proneural transcription factors: *splhairy*, a homologue of *hairy*; and *splash*, a homologue of *achaete-scute*. *splhairy*'s bHLH domain has 60–80% similarity to other members of the *hes/dpn* family. *splhairy*'s gene expression is restricted to sensory and neural tissues (brain, eyes, antennular lateral and medial flagella, carapace, legs, dactyls; not in abdominal muscle, stomach, hepatopancreas, intestine). The 3′ RACE *splash* is a 1506 bp sequence that encodes a 190 amino acid sequence with 80–90% homology to the bHLH domain of its homologues. We are currently using *in situ* hybridization and immunocytochemistry to examine the cellular location of *splash* and *splhairy*, and PCR to identify additional homologues of proneural and neurogenic genes such as *numb*, *atonal*, *notch* and *delta*.

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Poster: Olfactory Development

Social experience reduces postembryonic neurogenesis in the olfactory system of juvenile crayfish *Procambarus clarkii*

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Olfactory signals present in urine released during fights are thought to play a key role in the formation and maintenance of crayfish dominance hierarchies. New neurons are continuously added to cell clusters in the brain containing cell bodies of olfactory local (cluster 9) and projection (cluster 10) interneurons. Therefore, the crayfish is an excellent model system for studying the effect of social experience on postembryonic neurogenesis in brain regions involved in olfaction. Using *in vivo* incorporation of bromodeoxyuridine (BrdU), we tested the effect of social experience on the production and survival of new cells in cluster 10. Socially naive juveniles were paired for 1, 7 or 14 days and compared with isolate controls. No differences were found in the number of proliferating cells after 1 or 14 days of pairing; however, after 7 days, both dominants and subordinates had significantly fewer BrdU+ nuclei than did isolates. A reduction in the number of mitotic nuclei, visualized using anti-phosphohistone 3 (Ser 10) immunocytochemistry, was likewise seen in paired animals at 7 days only. In separate cell survival experiments, no differences were seen in numbers of BrdU+ cells surviving over 7 or 14 days of pairing or in numbers of mitotic nuclei. We conclude that hierarchy formation, possibly involving social stress, decreases cluster 10 cell proliferation but that this effect declines as the hierarchy stabilizes.

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Poster: Central Taste

Gustatory neural responses in the medial orbitofrontal cortex of the old world monkey

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Anatomical studies suggest that taste neurons should be prevalent within the medial orbitofrontal cortex (mOFC), but electrophysiological studies report that only 2–7% of the cells throughout the OFC respond to taste. This report describes a taste area within and adjacent to Brodmann area 13m of the mOFC where taste neurons are much more concentrated. Taste cells constitute 19% of the population within the 12 mm^2 core of this area, and 8% of the cells in the 1-mm-wide ring around it. Standard chronic recording techniques were used to search for taste-responsive neurons in the mOFC of three awake cynomolgus (*Macaca fascicularis*) monkeys. The battery of taste stimuli included 1.0 M glucose, 0.3 M NaCl, 0.01 M HCl and 0.001 M QHCl. Taste responses were 96% excitatory. The mean breadth of tuning for these cells was 0.79 ± 0.15 (range = 0.28–0.98), similar to that in the anterior insula (0.70), but much broader than in the caudolateral OFC (clOFC; 0.39). The greater breadth of tuning within the mOFC is also reflected in the nearly equal incidence of glucose-best (27%), NaCl-best (25%) and HCl-best (30%) cells. In the clOFC, by comparison, >80% of the cells are glucose-best. Mean response rates to the four basic stimuli were all in the range of 5.2–5.9 spikes/s. Unlike the clOFC, fewer than half of the neurons in the mOFC were modulated by satiety. Both anatomically and functionally, the mOFC taste area appears to occupy an intermediate position between the anterior insula and the clOFC.

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Poster: Central Taste

Functional cholinergic receptors in the nucleus of the solitary tract of the rat

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The rostral portion of the nucleus of the solitary tract (rNST) relays taste information to the forebrain. NST neurons have been characterized by their electrophysiological properties and spontaneous firing patterns, revealing a number of underlying ionic currents. Previous histochemical and ligand binding experiments have indicated the presence of cholinergic mechanisms in the rNST, but there are no functional data on this issue. Here we have extended the investigation of voltage- and ligand-gated ion channels in rat brainstem slices using whole-cell patch recording. Neurons in the rNST appear to possess functional nicotinic acetylcholine receptors (nAChRs), consistent with the involvement of the α7 nAChR subunit. Picospritzer applications of carbachol (10 mM), acetylcholine (ACh; 1 mM) or

choline (10 mM) to rNST neurons produced inward currents blocked by 20–40 nM methyllycaconitine (MLA). In most NST neurons, responses to ACh were completely blocked by MLA, suggesting that α7 nAChR represents the dominant mediator of cholinergic transmission in the rNST. The expression of α7 nicotinic receptors in the rNST suggests that endogenous choline, as well as nicotine and therapeutic nicotinic drugs, may regulate the transmission of taste information at the level of the first synaptic relay. Presynaptic α7 nAChRs could regulate both glutamatergic and GABAergic synaptic transmission in the rNST, as they have been shown to do in several other neural networks, including hypothalamic histaminergic neurons and dopaminergic cells of the ventral tegmentum.

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Poster: Central Taste

Representation of bitter tasting chemicals in the rat NST

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'Bitter' chemicals encompass a heterogeneous group of compounds. Molecular data suggest that a large family of G-protein coupled receptors, the T2Rs, detect this diversity. It is likely that members of this receptor family have narrow molecular ranges but debatable whether this specificity implies discriminability among bitter compounds. We assessed single-neuron responses in the rat nucleus of the solitary tract (NST) elicited by four bitter compounds: 0.01 mM cycloheximide (CY), 7 mM propylthiouracil (PROP), 10 mM denatonium benzoate (DEN) and 3 mM quinine hydrochloride (QHCl). The CY concentration could only be estimated but intensities of the other stimuli are equally and highly effective behaviorally. Responses to two representatives of the other four putative taste qualities were also tested (sucrose and glycine, 'sweet'; MSG and MPG mixed with IMP, 'umami'; NaCl and Na gluconate, 'salty'; and citric acid and HCl, 'sour'), as was parabrachial projection status and receptive field. Surprisingly, bitter chemicals varied in neural effectiveness with QHCl being particularly weak. Across the 11/104 neurons that responded best to a bitter tastant, QHCl elicited lower firing rates than DEN ($P < 0.01$) or CY ($P < 0.03$). CY was the most frequent best stimulus for bitter-best cells but the pattern of responsiveness across bitter stimuli was variable. For all recorded neurons, between-bitter correlations were low ($r = 0.08$–0.6), compared with other qualitatively similar stimulus pairs ($r = 0.86$–0.94) and multidimensional scaling depicted bitter stimuli as loosely clustered but segregated from non-bitter tastants. These results suggest that the specificity of the T2R receptors is not entirely lost in the first-order central relay.

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Poster: Central Taste

Effect of serotonin on membrane properties of neurons of the rat inferior salivatory nucleus

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Neuropeptide-containing fibers have been reported to form synapses with salivatory nucleus neurons (Nemoto *et al.*, 1995, *Brain Res.*, 685). However, little is known about the effects of these neuropeptides on the membrane properties of these neurons. The purpose of this study was to examine the response of salivatory neurons to one of these neuropeptides—serotonin (5-HT). Neurons of the inferior salivatory nucleus (ISN) were retrogradely labeled with a fluorescent dye, Alexa Fluor 568. Brainstem slices were prepared 2-4 days after labeling and whole-cell patch clamp recordings of membrane property performed during superfusion of 5-HT over the slices. 5-HT depolarized the ISN neurons in a dose dependent manner (EC_{50} = 4 μM) sufficient to evoked action potentials in some neurons. At 50 μM 5-HT depolarized the membrane potential from −54 ± 1 mV to −49 ± 1 mV (mean ± SEM, $n = 6$). 5-HT also depolarized in the presence of 1 μM tetrodotoxin, suggesting a direct action of 5-HT on the neurons. In the presence of the 5-HT_2 receptor antagonist ketanserin (10 μM), 50 μM 5-HT only had a minimal effect on the membrane potential (from −53 ± 2 mV to −52 ± 2 mV, $n = 8$). The 5-HT_2 selective receptor agonist α-methyl-5-HT (50 μM) depolarized from −52 ± 2 mV to −47 ± 2 mV ($n = 5$). These results suggest that the 5-HT_2 receptor is involved in depolarization of ISN neurons by 5-HT and that 5-HT may participate in the control of salivary secretion.

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Poster: Central Taste

Responses of gustatory neurons in the geniculate ganglion to L-MSG and linoleic acid

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The purpose of this study was to characterize the responses of gustatory neurons in the rat geniculate ganglion to the taste of umami and fat. We used extracellular single-cell electrophysiology to examine responses of these gustatory neurons to monosodium glutamate (MSG) or linoleic acid (LA), a free fatty acid. Cells first were categorized by their responses to lingual stimulation with the four classic tastants—sucrose, NaCl, citric acid and QHCl—and then were evaluated for their responses to MSG (20, 40, 100 and 300 mM) and LA (11, 22, 44 and 88 μM). We recorded from two sucrose-specialists, 10 NaCl-specialists, seven NaCl-generalists, three acid-generalists and one QHCl-generalist. Neurons responded weakly, if at all, to LA, suggesting that LA alone is insufficient to activate gustatory neurons in the geniculate ganglion. In contrast, virtually every neuron responded to some concentration of MSG. NaCl-specialists, NaCl-generalists and acid-generalists increased firing to MSG in a concentration dependent manner, whereas sucrose-specialists responded primarily to 300 mM MSG. These results suggest that the responses of NaCl-specialists, NaCl-generalists and acid-generalists to MSG is, in part, dependent on the Na^+ ion and may occur through activation of Na^+ channels on the taste receptor cells. On the other hand, sucrose-specialists, which are unresponsive to NaCl, may respond to the glutamate component of MSG, possibly via metabotropic G-protein-coupled glutamate receptors.

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Poster: Central Taste

Intracellular characterization of taste-responsive neurons of the hamster solitary nucleus

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We are using *in vivo* intracellular recording and labeling techniques to examine the taste characteristics, intrinsic electrophysiological properties and morphology of gustatory neurons in the hamster NST. The activity of taste-responsive cells was recorded with intracellular microelectrodes and the tongue was stimulated with anodal current, sucrose, NaCl, citric acid and quinine. Cells were characterized for their responses to current injection and electrophoresis of biocytin was used to label the recorded cell. After recording, brains were sectioned at a thickness of 100 μm in the coronal, horizontal or sagittal plane. Serial sections, processed for biocytin and counterstained with cytochrome oxidase and/or cresyl violet, were examined to reveal the morphology of the labeled cell and its axonal projections. Of the 41 taste-responsive cells recorded thus far, we have successfully recovered 29 labeled cells, including 23 multipolar, four elongate and two ovoid neurons. Although all 41 neurons responded to at least one of the gustatory stimuli, a subset of 24 cells was successfully tested with sucrose, NaCl, citric acid and quinine. Of these, seven responded to only one of the four tastants, nine to two, six to three, and two to all four stimuli, similar to hamster NST cells recorded extracellularly. Among the 29 cells on which we have morphological data, the axonal projections were successfully reconstructed for 12 of them, often showing multiple terminal fields in both the pons and in the medulla in or near brainstem motor nuclei.

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Poster: Central Taste

Coding of bitter stimuli by central gustatory neurons

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It has been proposed that bitter taste is encoded by the activation of discrete neural elements based on molecular findings that many bitter taste receptors (T2Rs) are expressed within the same receptor cells. Conversely, electrophysiological and calcium imaging studies have shown some specificity of taste receptor cells to different bitter substances and indicate that many bitter-sensitive receptor cells also respond to stimuli representing other taste qualities. Here we examined how a variety of bitter stimuli are represented by neural activity in central gustatory neurons. Taste responses (spikes/s) evoked by bathing the tongue and palate with intensity-equivalent concentrations of 19 stimuli categorized as sweet (three), salty (two), sour (two) or bitter (12) were recorded from 51 neurons in the nucleus of the solitary tract of anesthetized rats. Results show that responses to a variety of bitter stimuli were highly correlated across neurons. However, bitter-sensitive neurons, including those that were most effectively activated by bitter tastants, strongly responded to stimuli representative of other taste qualities, especially salts and acids. These data indicate that central neurons that process information from bitter receptors also receive significant input from receptors that mediate other tastes, which has implications for understanding the mechanism by which quality information is represented by the gustatory system in the CNS. Correlations among behavioral responses to bitter stimuli are currently being examined.

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Poster: Central Taste

Response properties of cells in the rat nucleus of the solitary tract following glossopharyngeal nerve stimulation: evidence of modulatory activity and the convergence of input from the hypoglossal nerve

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Evoked responses from electrical stimulation of the glossopharyngeal (GP) nerve were studied in the taste responsive portion of the nucleus of the solitary tract (NTS) of anesthetized rats. Forty-nine adult male rats were anesthetized and prepared for unilateral electrical stimulation of the lingual branch of the GP nerve and for electrophysiological recording from the ipsilateral NTS. In a subset of animals, the cut end of the ipsilateral hypoglossal (HG) nerve proximal to the tongue was also prepared for electrical stimulation. There were 49 NTS cells that showed evoked responses to GP nerve stimulation (median latency = 16 ms). There were 11 cells where pairs of pulses were delivered to the GP nerve at various interpulse intervals: five showed paired pulse facilitation, five showed paired pulse depression and one showed no effect. These data point to a diversity in the nature of GP input to NTS cells. Two additional results were noteworthy: first, electrical stimulation of the GP nerve resulted in long-lasting (~30 s) changes in spontaneous firing rate (either an increase or decrease) in seven (14%) NTS cells with an evoked response to GP stimulation. Second, four of 10 cells that were tested showed evoked responses to stimulation of both the GP and HP nerves. Collectively, these results suggest that oromotor input arising from the HG nerve and relayed to the NTS via the GP nerve may produce long lasting changes in NTS cells that may modulate taste responsivity and/or behavioral reactivity to taste stimuli.

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Poster: Central Taste

Responses in the nucleus of the solitary tract to taste stimuli of similar quality can vary independently across repeated trials

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Previous work from our laboratory has shown that the response magnitude evoked by representatives of the four basic taste qualities in cells in the nucleus of the solitary tract (NTS, the first central relay for gustation) can sometimes show significant variability across repeated trials. In some cells, this variability can result in

ambiguity about the cell's best stimulus, and consequently its role in the coding process. In the present experiment, we studied the variability in response magnitude evoked by tastants of similar quality, i.e. two salty and two sour, across several repetitions. Our initial hypothesis was that if response variability were the result of minute by minute variations in taste sensitivity, then responses to stimuli of similar taste quality should co-vary over time. Rats were anesthetized and prepared for electrophysiological recording from the NTS. Once a taste-responsive cell was isolated, repeated trials of NaCl (0.1 M), LiCl (0.1 M), HCl (0.01 M) and citric acid (0.01 M) were presented and the responses were recorded. Results show that the response magnitudes, defined as the average firing rate over 5 s of stimulus presentation minus the spontaneous firing rate, evoked by the two salts and the two acids co-varied with repetition, but responses to salts versus acids varied independently in some cells. In other cells, evoked responses to similar tasting stimuli varied substantially and independently over repeated trials. These results underscore the idea that the sensitivity pattern across tastants is a dynamic, rather than static, characteristic of a subset of taste-responsive cells in the NTS.

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Poster: Central Taste

Effects of marginal zinc deficiency on NaCl preference and oxytocin secretion in SD rats

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We previously reported that long-term zinc deficiency decreases taste sensitivity at the level of the chorda tympani nerve in rats. We also found that in rats, feeding zinc-deficient or low-zinc diets caused an abrupt increase of NaCl preference even after 4 days' feeding. Therefore, we hypothesized that the dietary signal of zinc deficiency may reflect NaCl preference probably through the central nervous system. Since neurohypophysial oxytocin (OT) is important for the rapid regulation of sodium balance by sodium intake and excretion, we investigated the effects of zinc deficiency on neurohypophysial hormones in rats. Male 4 week-old SD rats were divided into three groups (Zn-Def, Low-Zn and Pair-fed). We measured plasma concentration of OT and vasopressin (VP) in the experimental rats on days 0, 1, 2, 3, 4 and 7 of the experimental period by enzyme immunoassay. In the Zn-Def rats, plasma OT concentration was significantly less than that in the Pair-fed rats after 3 days' feeding. In the Low-Zn rats, plasma OT concentration was significantly less than that in the Pair-fed rats after 4 days' feeding. On the other hand, there was no change of plasma VP concentration. These findings suggest that short-term (marginal) zinc deficiency decreases OT secretion. We also investigated the effect of OT injection on NaCl preference in the experimental rats. After intracerebroventricular injection of OT on days 4 of the experimental period, the Zn-Def rats reduced their NaCl preference. Theses results suggest that there is strong correlation between the increased NaCl preference caused by marginal zinc-deficiency and hypophysial oxytocin system.

Poster: Central Taste

A systems-level representation of appetite

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It has been established that the control of appetite is achieved through the action of peripheral signals on the hypothalamus. Lesion studies in rats and monkeys have demonstrated that appropriate food intake behavior also depends on the integrity of cortical and subcortical structures even if peripheral signaling is preserved. To date, there is no electrophysiological description of how taste and reward areas, and the hypothalamus, work as a system to support energy homeostasis. We provide such a description by simultaneously recording single units in lateral hypothalamus, gustatory cortex, amygdala, orbital frontal cortex and hippocampus to hungry rats before and following central (lateral ventricle) administration of the appetite suppressing peptide, CCK-8. Our results show that a major reorganization of neuronal activity occurs in all these brain areas following CCK-8 administration and that firing rate changes correlate with behavioral adaptation. Central taste areas and hypothalamus seem to work as a distributed system to support food intake.

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Poster: Central Taste

Extensive anatomical overlap of greater superficial petrosal and IXth nerve terminal fields in hamster solitary nucleus

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In mammals, the greater superficial petrosal nerve (GSP) provides afferent input to taste cells located in palatal and nasoincisal mucosa, while IXth nerve (IX) innervates taste cells of the circumvallate papilla. Both nerves terminate in the rostral pole ofthe hamster solitary nucleus (NTS). We sought to quantify anatomical overlap between GSP and IX terminal fields in adult hamster NTS. We used double fluorescent nerve labeling to visualize GSP and LT-IX terminal fields in NTS. GSP and IX were isolated, cut, and labeled with unique dextran amine conjugates. After 1–5 days survival, animals were sacrificed and perfused with 8% paraformaldehyde. Fixed brains were sectioned horizontally and sections examined with with confocal microscopy. Serial optical sections through physical sections of complete terminal fields were saved and analyzed offline. Data so far show that IX terminal field appears ~50 μm dorsal to GSP terminal field, while GPS terminal field extends ~125 μm ventral to that of IX. The dorsal–ventral extent of GSP terminal field exceeds that of IX, but GSP field volume is only ~80% of IX field volume.The two terminal fields overlap extensively in the dorsal–ventral plane (~80% of IX and ~65% of GSP field dorsal–ventral extents). Corresponding volumetric overlap of GSP and IX field volumes is observed: ~50% of GSP field volume overlaps with IX; ~40% of IX field volume overlaps with GSP. These results reveal a rich substrate for convergence of taste afferent input onto common NTS targets, and provide

normative data for studies of development in the hamster central taste system.

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Poster: Central Taste

Ionotropic glutamate receptor expression in preganglionic neurons of the rat inferior salivatory nucleus

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Recent evidence (Fukami and Bradley, 2003, *Soc. Neurosci. Abstr.*, 29) suggests that neurons of the inferior salivatory nucleus (ISN) are activated by excitatory, probably glutamatergic projections originating in the nucleus of the solitary tract (NST). To provide information on the types of ionotropic glutamate receptor subtypes expressed by inferior salivatory nucleus preganglionic neurons in the rat medulla ISN, neurons were examined using fluorescence immunolabeling combined with retrograde neuronal tracing. Neurons of the ISN were labeled by application of the fluorescent tracer AlexaFluor 488 to the glossopharyngeal nerve. After 5 days the rats were perfused with physiologic saline and 4% paraformaldehyde and the brainstems were sectioned horizontally at 50 μm thickness. Molecular biological techniques indicate that ionic glutamate receptors of the NMDA and AMPA subtypes are composed of a variety of receptor subunits. Expression of these subunits on labeled ISN neurons was analyzed using specific antibodies to the subtypes NR1, NR2AB, NR2B, GluR1, GluR2, GluR2/3 and GluR4. All ISN neuron somata were strongly positive for NR1 and GluR1 but unlabeled or only weakly positive for NR2AB, NR2B, GluR2, GluR2/3 and GluR4 receptor subtypes. These results suggest that ISN neurons have both NMDA and AMPA glutamate receptor subtypes.

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Poster: Central Taste

Taste reactivity and NST Fos expression in 'GIN' mice

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The current study evaluated gustatory-evoked oromotor responses and Fos expression in the rostral nucleus of the solitary tract (rNST) in transgenic mice expressing green fluorescent protein in a subset of GABAergic neurons [Oliva *et al.*, 2000; FVB-Tg(GadGFP) 45704Swn/J, 'GIN'] and their parent stain (FVB/NJ). Mice (n = 21) were implanted bilaterally with intraoral cannulas and adapted to a testing chamber. Subsequently, distilled water, 0.3 M sucrose or 0.003 M QHCl (25 μl × 12–20 stimulations/30 min) was infused through the cannulae and behavioral responses were videotaped. Seventy-five minutes after stimulation, mice were anesthetized and perfused, and tissue was sectioned and processed for Fos immunohistochemistry. The predominant 'ingestive' responses to sucrose and water were small mouth movements, ofttten accompanied by grooming. Visible tongue movements were rare. The QHCl response differed dramatically, and included prominent chin rubbing and paw-flailing, along with increased locomotion and jumping. Large amplitude 'gapes' were observed only occasionally. In the rNST, the numbers of neurons expressing Fos-like immunoreactivity (FLI) were elevated following QHCl compared with water and sucrose stimulation, but only in the medial third of the nucleus ($P < 0.006$). In 'GIN' mice, there was a pronounced distribution of GABAergic neurons in the ventral subdivision of rNST. Approximately 15% of NST FLI neurons were GABAergic, but this proportion did not differ according to stimulus. These behavioral and Fos results are similar to those observed in rat and provide an opportunity to investigate gustatory processing in a species amenable to genetic manipulation.

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Poster: Central Taste

Organization of barbel-based facial taste system in weather loach

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Our long–term goal is to understand taste-mediated sensorimotor processing. Our current objectives were (ito) demonstrate suitability of weather loach (*Misgurnus* spp.) for studies of oriented responses to taste stimuli delivered to array of external barbels; and (ii) to reveal the central organization of primary sensory nuclei (facial lobe) that receive barbel inputs. Weather loach possess five pairs of largely non-mobile barbels surrounding the mouth: three maxillary and two mandibular, each covered with taste buds. Cotton swabs soaked with 10^{-3} M solutions of amino acids were applied to the internal or medial maxillary barbels of stationary fish. Stereotypical oriented movements of the head brought the mouth toward or to the stimulus. Fish generally ignored the tactile stimulus without amino acids. The facial lobe of the weather loach is segregated into five lobules on each side. DiI was used to trace the central projections of branches of the mandibular and maxillary nerves that innervate the barbels. The afferent nerve fibers from each barbel terminate ipsilaterally and topographically in a one-to-one relationship in each lobule. The two caudal lobules receiving inputs from the mandibular barbels are less distinct from one another than the lateral and anterior lobules that receive input from maxillary barbels. Consistent with this topology, oral and peri-oral primary afferent fibers likely terminate in the core of the facial lobe where demarcation between lobules is lost. Cytoarchitecturally the lobules show evidence of lamination parallel to the surface of the lobules, suggesting that the barbel representation within lobules may be mapped relative to the surface.

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Slide: Chemosensory Behavior

Toward the olfactory code in the behaving mouse

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The vast majority of our knowledge about the function of neurons in the mammalian olfactory bulb was obtained in anesthetized preparations. We recorded the activity of mitral cells in olfactory bulb of the behaving mouse during an odor discrimination task. Behavioral sessions were followed by anesthesia and recording odor responses of the same mitral cells. We found a striking difference in the neuronal activity between the two states. First, the spontaneous activity in the awake state was much higher. Second, the cell response to an odor was different in anesthetized and awake states. Most of the mitral cells showed responses to odorants in the anesthetized state. Responses by these cells were strongly excitatory. In the awake state, most of these cells did not exhibit any statistically significant odor response. In 10% of these cells the response was weakly excitatory and in another 10% it was weakly inhibitory. Overall, the odor code was much sparser in the awake state than in the anesthetized state. Finally, the firing rate of mitral cells was modulated by non-odorous behavioral events. In the awake state, the differences in firing rate in response to two odor stimuli appear to be modulated by breathing: during exhalation, there is no difference; during inhalation, the difference is most pronounced. During a single inhalation the firing rate of a cell carried up to 0.15 bits of information as to which odor was presented. Presumably, based on signals from this and other cells, the mouse makes its decision regarding the identity and 'meaning' of the odor and selects the subsequent behavior.

Slide: Chemosensory Behavior

Chemotopy predicts olfactory discrimination in zebrafish (*Danio rerio*)

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Odor discrimination depends upon the chemotopic organization of glomeruli within the olfactory bulb. In zebrafish, similar glomerular activation patterns were described for responses to L-Val and L-Ile and to L-Phe and L-Tyr (Friedrich and Korsching, 1997). In addition, a high correlation between mitral cell activities occurred for responses to L-Phe and L-Tyr (Friedrich and Laurent, 2001). These results suggest that zebrafish likely do not discriminate L-Phe from L-Tyr or L-Ile from L-Val. Olfactory discrimination was studied by conditioning individual zebrafish ($n = 10$) with a food reward presented 90 s after the conditioning amino acid solution was injected into the aquarium. After presentation of the conditioned stimuli the zebrafish searched for food more vigorously (video-tracking distance or counting turns >90° during 90 s) than after stimulation with non-conditioned amino acids. We used L-Ala, L-Leu, L-Val, L-Arg and L-Phe as conditioning stimuli; the test stimuli included 17 other L-amino acids tested at 30 μM expected contact concentration. With the exception of (i) L-Tyr on L-Phe-conditioned zebrafish and (ii) L-Ile on L-Val-conditioned zebrafish, the test stimuli were always discriminated from the conditioned stimulus. Differences between individual zebrafish discrimination abilities potentially originated in different individual glomerular activation patterns. Our results indicate that fish do not discriminate amino acids that evoke very similar glomerular activity patterns, but do discriminate those that evoke distinct glomerular activation patterns.

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Slide: Chemosensory Behavior

Chemical signals and chemosensory pathways involved in spiny lobster sheltering behavior

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Caribbean spiny lobsters are gregarious animals that often aggregate in communal dens following nightly foraging excursions. We have shown previously that conspecific urine is sufficient to mediate shelter selection in a laboratory flume. We are currently investigating the species and sex specificity of the aggregation signal, its molecular identity, and the chemosensory pathways involved in its detection. The chemosensory system of the Caribbean spiny lobster (*Panulirus argus*) is organized into two parallel pathways: the aesthetasc/olfactory lobe pathway and the non-aesthetasc/lateral antennular neuropil pathway. These two pathways originate in different populations of antennular sensilla and project to specific neuropils in the brain. Although the pathways are anatomically distinct, their specialized roles in chemically mediated behaviors are not well understood. For example, many food-related behaviors, including food odor localization, discrimination and learning, can be mediated by either pathway. To examine the role of each of these pathways in chemically evoked shelter selection, we performed bilateral ablations of different populations of antennular sensilla and compared the behavior of ablated animals to intact controls. Our results indicate that aesthetasc sensilla are necessary to mediate the response to urine signals, suggesting that this pathway may be uniquely important for processing social odors. We are currently examining the role of non-aesthetasc sensilla in this behavior.

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Slide: Chemosensory Behavior

Mechanisms of sperm navigation in turbulent flow

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Chemical communication between sperm and egg is widely prevalent among taxa with divergent reproductive strategies. Sperm chemoattractants play a pivotal role in fertilization through mediating sperm–egg interactions and increasing gamete encounters. Surprisingly, no study has examined the influence of these signals under realistic conditions. Ubiquitous for all organisms is the presence of fluid motion at the scale of the sperm and egg. Fluid motion may have profound influence on gamete motility and sperm attractant distribution, but such effects are largely undescribed. This study investigated the mechanisms by which sperm navigate to eggs under natural flow conditions. Our discovery of tryptophan as the natural attractant of abalone (*Haliotis rufescens*) sperm offered the opportunity to determine the navigational mechanism used by

sperm. Fluid motion stretched the tryptophan plume around eggs, vastly increasing broadcast distances. Moreover, stretching of the plume caused shallow gradients to dominate plume structure, in contrast to the sharp gradients found in diffusion. Examining the kinetics of sperm behavior revealed that sperm use klinotaxis to orient and swim towards the egg. By integrating the changing concentration over a 200 ms interval, sperm can navigate to a shallow gradient within a temporally and spatially dynamic fluid environment. Moreover, the concentration gradient, not concentration itself, modulated sperm trajectories. Thus, even at microscopic scales, physics tightly constrains the chemical signaling process, dictating sperm navigation.

Slide: Imaging the Neural Code

Effects of feeding on responses in olfactory bulb revealed by fMRI

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Smell is a major means for evaluating food before ingestion and the major contributor to flavor perception of the ingested food, affecting both the type and amount of food intake. Activity patterns in the olfactory bulb (OB) encode all of the peripheral olfactory input and serve as the substrate on which higher olfactory centers perform their functions of abstraction underlying discrimination and perception. Studies of the encoding of food-related odors by odor maps and the relation between olfaction and food intake may provide insight into normal feeding patterns as well as abnormal patterns such as related to obesity. We have used functional MRI (fMRI) to visualize odor-elicited spatial and temporal activity patterns in different OB layers of the rat, under different feeding/hormonal conditions. The patterns in the OB and higher centers elicited by food related odors are specific. The intensity but not the topography of the patterns elicited by food-related odors is affected by the feeding conditions. The effects of feeding on the intensity of the fMRI signal are more significant in the deeper compared with superficial OB layers. Study of the effects of leptin and insulin are in progress. These studies should provide new insights into the role of olfaction underlying flavor perception. If flavor plays a role in the genesis of human obesity, this approach may also give insight into fruitful interventions to restore energy homeostasis.

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Slide: Imaging the Neural Code

Brain mechanisms for extracting spatial information from smell

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Forty years ago, von Békésy demonstrated that the spatial source of an odorant is determined by comparing input across nostrils, but it is unknown how this comparison is effected in the brain. To address this, we delivered odorants on the left or right of the nose, and contrasted an olfactory localization task (in which subjects indicted from which side the odorant was delivered) with an olfactory identification task (in which subjects indicated which of four odorants, propionic acid, amyl acetate, phenylethyl alcohol or eugenol, was delivered) during brain imaging. Subjects were able to perform both tasks above chance [$n = 16$, Ident: accuracy = 74.5%, $F(1,63) = 17$, $P < 0.0001$, Loc: accuracy = 70%, $F(1,63) = 6.56$, $P < 0.0001$]. Olfactory localization but not identification led to lateralized responses in primary olfactory cortex (POC), suggesting the expression of task-specific spatial receptive fields and thus providing a neural substrate for the behavior described by von Békésy. Additionally, localization preferentially engaged a portion of the superior temporal gyrus previously implicated in visual and auditory localization, suggesting that spatial information extracted from smell was then processed in a convergent brain system for spatial representation of multisensory inputs. These results delineate brain mechanisms used to extract spatial information from smell.

Slide: Imaging the Neural Code

Dissociable codes of odorant structure and odor quality in human piriform cortex

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The relationship between representations of odorant structure and odor quality has been a key question in olfactory research for 100 years, though no systematic correlations are yet apparent. Recent studies indicate that physicochemical attributes define response properties in olfactory sensory neurons and bulb. Whether these features are preserved in central olfactory regions is unknown. To determine if human piriform cortex encodes physical or perceptual information, we scanned 16 subjects during an fMRI paradigm of olfactory cross-adaptation. On each trial, subjects made two sniffs in succession and smelled pairs of odorants that varied in structural group (alcohol or aldehyde) or perceptual quality ('lemon' or 'vegetable'), enabling us to dissociate physical and perceptual dimensions of smell. Cross-adaptation in anterior piriform cortex was modulated by odorant functional group, but was insensitive to odor quality. By comparison, posterior piriform cortex cross-adapted to quality, irrespective of structure. The identification of structure-based codes in piriform cortex suggests a preservation of sensory information arising from olfactory bulb, ensuring stimulus fidelity. In turn, quality codes are independent of any simple structural configuration, implying that synthetic levels of neural organization may mediate our experience of smell.

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Slide: Imaging the Neural Code

An application for quantitative analyses and comparisons of rodent fMRI odor maps

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Aims: rodent odor maps are flat images whose optical densities describe the spatial activity patterns in the entire olfactory bulb glomerular layer in animals exposed to specific odor stimuli. We have developed a software application, OdorMapComparer, to carry out quantitative analyses and comparisons of the odor maps. Methods: OdorMapComparer is a standalone window program and written in Java. It loads two images onto the work frame. Image warping employs the thin-plate spline algorithm (Bookstein, 1989). Results: OdorMapComparer allows image transformation including scaling, flipping, rotating and warping so that the images being compared can be appropriately matched to each other. It allows simple subtraction and addition of the two images and statistical analyses including calculation of their normalized correlation. Experiments showed that aliphatic aldehydes induced fMRI odor maps in mouse with activity patterns that are similar in gross outlines but somewhat different in specific subregions. Analyses with OdorMapComparer indicate that the odor map of butanal is more closely related to that of pentanal (with a normalized correlation r = 0.73) than to octanal (r = 0.42), which is consistent with our observation. Conclusions: OdorMapComparer provides a means to carry out quantitative, statistical analyses and comparisons of fMRI odor maps in a fashion that is integrated with the overall odor mapping techniques.

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Symposium: Mapping Olfactory Bulb

Guidelines for odor map-making

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Studies using many methods in many different species over the past 30 years have provided increasing evidence for a universal mechanism in which odors are encoded as spatial activity patterns by differential activation of olfactory glomeruli. There is agreement that these patterns, referred to as odor maps or odor images, encode different odors and different concentrations. However, several principles need to be recognized in order to enable the results from different methods to be correlated with each other and with the underlying molecular basis. First, neural space is not a rigid framework for odor representation. Localization of activated glomeruli by a given odor is a dynamic functional process. Second, no one method is ideal for demonstrating the functional odor maps. The different methods each have their advantages and limitations. It is critical to understand these in attempting to correlate the functional maps from the different methods. Third, for construction of the functional maps it will be important for investigators to agree on rigorous mapping coordinates. A Mollweide type of cartographic projection appears to be a useful approach. Agreement on these guidelines is essential in order to be able to map the results from a given method and relate the maps from different methods into an integrated framework for understanding the spatial and molecular basis for odor detection and discrimination.

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Symposium: Mapping Olfactory Bulb

Testing predictions of a combinatorial identity code

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We propose a set of predictions that one might address before one accepts a model of olfactory coding and then we tested these predictions with respect to a combinatorial identity code by mapping the evoked responses of a wide range of systematically selected odorants across the entire glomerular layer. We found that, in many instances, we could predict the neural response from the characteristics of the odorant molecule, we could predict the nature of the odorant molecule from the neural response and we could predict the perceptual response from the neural response pattern. Furthermore, the neural response that we observed corresponded to the functional anatomy of the glomerular layer. In addition, we observed that odorants that shared certain molecular features also shared neural responses and we found that there was a graded specificity in the glomerular response to similar odorants. Finally, we showed that destruction of a focal area of olfactory bulb activation evoked by an odorant eliminates differential responsiveness to that odorant. These data strongly support the notion of a combinatorial identity code.

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Symposium: Mapping Olfactory Bulb

Molecular-feature clusters in the odor maps of the olfactory bulb

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The glomerular sheet of mammalian olfactory bulb (OB) forms two symmetric maps of odorant receptors (ORs). We used the method of intrinsic signal imaging and systematic panels of stimulus odorants to examine the molecular receptive range (MRR) property of individual glomeruli in a substantial part of the dorsal and lateral surfaces of rat OB. The results showed that glomeruli with similar MRR property gathered in close proximity and formed molecular-feature clusters. Analysis of the molecular features/determinants effective in activating individual glomeruli suggests a systematic, gradual and multidimensional change in the represented molecular features according to the position of clusters in the odor maps. The relationship between the represented molecular features and the perceived 'odor' of effective odorants suggests that the glomerular clusters participate in the representation of odor quality in the OB. Although the shape of the clusters varied across different OBs, each cluster was always located at a stereotypical position in a specific zone of the OB. Characteristic molecular features of glomeruli in most clusters within zone 1 (dorsal zone) were relatively polar parts of odorants including polar functional group(s). In contrast, those in several clusters in zones 2–4 (ventrolateral zones) were hydrocarbon parts or relatively non-polar parts. These results imply a clear difference in the manner of recognizing odorant molecular features/determinants between ORs represented in zone 1 and ORs represented in zones 2–4.

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Symposium: Mapping Olfactory Bulb

Encoding natural scenes in the mouse main olfactory bulb

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Most natural olfactory stimuli contain a large number of distinct components. The principal neurons of the olfactory bulb, the mitral cells, receive their dominant excitatory input on their apical dendrites from a single type of olfactory receptor, but they also receive considerable inputs (primarily inhibitory) on their widespread basal dendrites. We used single unit electrophysiology to investigate whether mitral cells are activated by the integration of many distinct components, or by unique components within complex natural mixtures. Urine consists of over 100 distinct volatiles, and plays an important role in social behaviors. Single-unit recordings uncovered a small population of urine-responsive neurons clustered in the ventral–lateral region of the bulb. Most responded to both male and female urine volatiles, but a fraction responded exclusively to male urine. When we fractionated the mixture using gas-chromatography in combination with electrophysiology, we found that >80% of cells responded to just a single peak of the >100 compounds eluting from the column, although the particular peak eliciting responses varied between cells. This suggests that individual mitral cells act as highly selective detectors for particular volatiles, rather than broadly tuned integrators of diverse inputs. Further experiments using a variety of other natural stimuli (food, predator odors, etc.), or robotic delivery of 100 arbitrary volatiles reveal a similar picture of mitral cell selectivity. Thus we posit that in the context of natural stimuli, each glomerulus, and its associated mitral cell population, acts as a molecular feature detector, indicating the presence of a particular natural compound.

Symposium: Mapping Olfactory Bulb

Natural odor maps in the main olfactory bulb

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Natural odorants are complex mixtures of volatile molecules that bind to a subset of olfactory receptors. Because olfactory receptor neurons (ORNs) expressing a particular receptor target their axons to a small number of glomeruli in the main olfactory bulb (MOB), the response to complex odors is mapped onto a two-dimensional map of activity in the glomerular surface of the MOB. Responses to urine elicit activation of glomeruli in ventromedial and ventrolateral areas of the glomerular layer. In order to understand the mechanisms underlying this response to urine in the MOB, we have used mice whose ORNs are defective for the cAMP transduction pathway (CNGA2 knockout mice). We find that these mice are responsive to urine components, and that these components activate a discrete subset of glomeruli. In addition, the mice respond to urine and urine activates a subset of glomeruli in the CNGA2 knockout mice. Two potential alternate transduction mechanisms appear to be involved in the responses to these naturally occurring compounds. Necklace glomeruli employing the cGMP pathway are responsive to urine components. In addition, a subset of ORNs express the channel TRPM5, a downstream effector of the phospholipase C pathway, and axons from these ORNs target a discrete subset of glomeruli in the olfactory bulb. Our experiments implicate a functionally heterogeneous subset of glomeruli in the response of the main olfactory system to natural odors.

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Symposium: Mapping Olfactory Bulb

Glomerular organization and temporal dynamics of odor maps imaged with receptor neuron-specific probes

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We have used two optical methods to map odor representations by olfactory receptor neuron (ORN) input to the dorsal olfactory bulb. The first uses calcium-sensitive dyes loaded into ORNs, while the second uses a genetically encoded indicator of transmitter release, synaptopHluorin, expressed in all ORNs. Both methods allow mapping of odorant-evoked input to the bulb with single-glomerulus spatial resolution and 0.1–1 s temporal resolution. As with other mapping methods, we find that glomeruli activated by an odorant tend to be clustered in domains, and that the locations of these domains are related to odorant functional group. However, at the level of single glomeruli this chemotopy is loosely organized. For example, a single glomerulus maximally sensitive to ketones is routinely found embedded within a domain defined by its sensitivity to organic acids, and individual glomeruli with diverse odorant specificities can be found adjacent to one another. We also find that maps of glomerular input are temporally dynamic. An odorant can activate different glomeruli with different temporal response patterns. The temporal pattern of input to a particular glomerulus is odorant-specific and consistent across animals. These dynamics are such that maps of glomerular input can change, often significantly, with time. Much of these dynamics occur during a single sniff and are repeated with successive sniffs. These features raise the possibility that the temporal dynamics of glomerular activity maps, in addition to the maps themselves, play a role in encoding or processing odor information.

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Poster: Peripheral Taste Physiology

Regulation of ENaC by intracellular chloride in taste cells

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Epithelial sodium channels (ENaC) contribute to sodium salt taste transduction in a variety of species. Recently, several studies have implicated intracellular Cl^- ions as important regulators of ENaC activity in transporting epithelia (Schrieber *et al.*, 2004, *J. Membr. Biol.*, 199:85), where they directly affect ENaC channels. We have

previously identified a number of transport proteins for Cl^- in taste cells, including several members of the ClC family of Cl^- channels, the cystic fibrosis transmembrane conductance regulator (CFTR) and the Na–K–Cl cotransporter by immunocytochemistry and RT-PCR, and propose they may be involved in gustatory responses to salt and water. To determine if Cl^- regulates the activity of ENaC in taste cells, we performed whole-cell patch clamp recording on C57/6ByJ mouse taste cells using intracellular solutions that were either high (140 mM) or low (10 mM) in Cl^-. Using extracellular amiloride (0.1–10 μm) to determine the magnitude of ENaC-mediated currents, we found that ENaC currents were, on average, 3–4 times larger in low intracellular Cl^- condition. In addition, mouse taste receptor cells appeared to be more sensitive to amiloride in the low intracellular Cl^- condition. Currently, we are using agonists and antagonists of chloride transport proteins to examine the functional role of Cl^- influx and the changes in intracellular Cl^- concentration on sodium influx through ENaC in mouse taste cells.

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Poster: Peripheral Taste Physiology

The possible role of spiking taste receptor cells on gustatory sensory transmission

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A subset of rodent taste cells is known to generate action potentials in response to taste stimuli. Some of these cells express molecular markers for cell types that may transmit taste information to gustatory nerve fibers. The present study investigated the possible role of spiking taste cells on gustatory sensory transmission by examining taste responses of spiking taste cells in isolated mouse fungiform taste buds and comparing them with those of chorda tympani (CT) nerve fibers innervating fungiform papillae. Response selectivity among four taste stimuli (NaCl, HCl, saccharin, quinine) was evaluated as the entropy value of the breadth of responsiveness of each taste cells and fibers. The mean entropy value was not significantly different between fungiform taste cells and CT fibers, indicating that the range of responsiveness of spiking taste cells may be close to that of innervating axons in mice. The dendrogram obtained from data on response patterns of individual mouse fungiform taste cells to four taste stimuli resembled that of CT nerves. Proportions of taste cells predominantly responding to one of four taste stimuli were similar to those of nerve fibers. These results indicate corresponding groupings of taste cells and nerve fibers in mice. This consistency in response characteristics of taste cells and nerve fibers suggest that taste cells generating action potentials may play a major role on transmission of taste information to gustatory nerve fibers.

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Poster: Peripheral Taste Physiology

Polymorphisms of ENaC subunits: absence of relation to mouse strain differences in amiloride-sensitive salt responses

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Amiloride-sensitive epithelial Na^+ channels (ENaCs) are proposed to be involved in salt taste transduction. Electrophysiological studies in C57BL/6 (B6) mice demonstrated that responses to NaCl are inhibited by amilolide in the choda tympani (CT) but not in the glossopharyngeal nerve, suggesting a lack of amiloride sensitivity (AS) in the posterior tongue. The AS also differs among inbred mouse strains. Unlike B6 mice, 129P3/J (129) mice showed almost no amiloride inhibition of NaCl responses even in the CT. In this study, using B6, 129 mice and their F2 hybrids, we examined possible relationships of the AS with mRNA expression levels in fungiform papillae (FP) and single nucleotide polymorphisms (SNPs) of three subunits of ENaC (α, β, γ). The mRNA expression levels of each ENaC subunits examined using RT-PCR analysis were similar in the B6 and 129 strains. Sequencing detected three SNPs in the α-subunit. One of these SNPs resulted in an amino acid substitution, R616W, near the predicted second transmembrane domain in the 129 strain. No SNPs were found in sequences of β- and γ-subunits. Electrophysiological and sequencing analyses in F2 hybrids indicated that there was no relation between the AS and the α-subunit SNP (R616W). These results suggest that neither expression levels of the three subunits of ENaC, nor the SNP in the α-subunit participate in the mouse strain differences in the AS.

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Poster: Peripheral Taste Physiology

Antagonistic actions of neuropeptides CCK and NPY on rat taste receptor cells

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Our laboratory has been investigating expression and physiological actions of neuropeptides in taste receptor cells (TRCs). Previously we reported that the neuropeptide cholecystokinin (CCK) is expressed in subsets of TRCs and that TRCs respond to its exogenous application in excitatory manners that are specifically mediated by the CCK-A receptor subtype. One significant action of CCK was an inhibition of the inwardly rectifying potassium current, KIR. Here we report observations on the expression of a second neuropeptide, neuropeptide Y (NPY). NPY is expressed in a subset of TRCs, which, through double labeling experiments, were demonstrated to be a subset of the CCK-expressing TRCs. To confirm its expression, NPY messenger RNA was localized to taste buds using RT-PCR. Like CCK, exogenous NPY application affected KIR in a concentration-dependent manner (1–500 nM) though rather than inhibiting this current, NPY acted to enhance it by 25–35%. The enhancement of KIR by NPY was blocked if cells were preexposed to the NPY-1 receptor antagonist, BIBP3226. Similarly, this enhancement was mimicked by application of the NPY-1 receptor agonist, [Leu^{31}, Pro^{34}]-NPY, which too was blocked by pre-exposure to BIBP3226. In examining its transduction mechanism, the enhancement of KIR was G-protein dependent but using the blockers U73122 or LY294002 apparently does not involve the enzymes PLC or PI3K, respectively. These results suggest that these neuropeptides, which arise from the same cell, have

strongly antagonistic physiological actions. The expression pattern of NPY and NPY-1 receptors is under study.

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Poster: Peripheral Taste Physiology

Oscillations in chorda tympani responses to sucrose and dulcin

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The existence of oscillations in impulse rate is a characteristic feature of many sucrose-sensitive fibers of the chorda tympani (CT) nerve. Recordings from sucrose-best units (n = 8) of the golden hamster (*Mesocricetus auratus*) CT nerve show periodic bursting with an inter-burst interval of 700 ms based on an analysis of autocorrelations of records binned at 100 ms ($\alpha = 0.05$). The overall rate of activity increases with increase in stimulus concentration, but the oscillation frequency is nearly independent of sweetener (sucrose or dulcin), sweetener concentration or the concentration of an inhibitor such as quinine–HCl. Bursting responses are especially clear in the hamster, but are also seen in published data for other species, including primates. High concentrations of non-sweet stimuli do not show impulse rate oscillations. The 1.43 Hz oscillation may be due to taste-bud circuitry or may be generated by second messengers within the taste cells themselves. Levels of cAMP and Ca^{2+} within taste cells are mutually dependent due to feedforward and feedback loops. Suzuki *et al.* (2002, *Chem. Senses*, 27:789–801) developed a model for a 1.89 Hz oscillation in receptor-cell currents of the rainbow trout olfactory system based on oscillations in intracellular cAMP and Ca^{2+}. Such a model, applied to the hamster gustatory system at the level of the primary afferent nerve, depends on cyclic depolarizations of receptor cells. Oscillations in sucrose-sensitive fibers may be significant in the coding of sweetness by the gustatory system.

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Poster: Peripheral Taste Physiology

Mechanisms underlying adaptation of the response of taste receptor cells to caffeine

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We have previously reported multiple physiological effects of caffeine on rat posterior taste receptor cells (TRCs). Here we examine cellular mechanisms underlying the adaptation of the inhibition of inwardly rectifying potassium current produced by caffeine using whole cell patch clamp recordings. Most responses demonstrated strong adaptation that could be inhibited or facilitated by various pharmacological manipulations. Adaptation requires G-protein involvement and ATP. Second messenger mediated kinase phosphorylation increased adaptation. One key participant appears to be protein kinase C (PKC). Inhibition of PKC by bisindolylmaleimide reduced adaptation while stimulation by phorbol esters increased adaptation. Another key component is extracellular calcium. Nominally free extracellular calcium strongly reduced the number of adapting cells whereas manipulations of intracellular calcium had no effect. At present, the source of extracellular calcium is unknown though it does not appear to be mediated by L-type calcium channels. On the other hand, blocking PIP_2 hydrolysis appeared to enhance adaptation. PLC, blocked by U73122, rather than blocking adaptation, actually increased adaptation. PLC inhibition would results in increased levels of PIP_2. Inhibition of PI-3K, which also uses PIP_2 as a substrate, by LY294002 increased the number of adapting cells. Wortmanin, another potent inhibitor of PI-3K, strongly increased the number of adapting cells. Many of our data point to resynthesis of PIP_2 after receptor-mediated hydrolysis as a key step in the process of adaptation.

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Poster: Peripheral Taste Physiology

Effect of ethanol on the VR-1 variant amiloride-insensitive salt taste receptor

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The effect of ethanol on the amiloride- and benzamil (Bz)-insensitive salt taste receptor was investigated by direct measurement of intracellular Na^+ activity ($[Na^+]_i$ by fluorescence imaging in polarized fungiform taste receptor cells (TRCs) and by chorda tympani (CT) taste nerve recordings. The CT responses to KCl and NaCl were recorded in Sprague–Dawley rats, and in wild type (WT) and vanilloid-receptor-1 (VR-1) knockout mice (KO). CT responses were monitored in the presence of Bz, a specific blocker of the epithelial Na^+ channel (ENaC), VR-1 agonists (resiniferatoxin and elevated temperature) and VR-1 antagonists (capsazepine and SB-366791). In the absence of mineral salts, ethanol elicited only transient phasic CT responses. In the presence of mineral salts, ethanol increased the apical cation flux in TRCs and elicited CT responses that were similar to salt responses, comprising both a phasic component and a sustained tonic component. At concentrations <50%, ethanol enhanced responses to KCl and NaCl, while at ethanol concentrations >50% those CT responses were inhibited. Resiniferatoxin and elevated temperature increased the sensitivity of the CT response to ethanol, and SB-366791 inhibited the effect of ethanol, resiniferatoxin and temperature on the CT responses to mineral salts. VR-1 KO mice demonstrated no Bz-insensitive CT response to KCl and NaCl, and no sensitivity to ethanol, resiniferatoxin, and temperature. We conclude that ethanol increases salt taste sensitivity by its direct action on the Bz-insensitive VR-1 variant salt taste receptor.

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Poster: Peripheral Taste Physiology

Effect of pH and cell volume on the phasic chorda tympani response to acid stimulation

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The relationship between rat fungiform taste receptor cell (TRC) pH (pH_i) and volume and chorda tympani (CT) responses to acidic stimuli was investigated. TRC pH_i and volume were monitored by fluorescence imaging. In TRCs, F-actin and G-actin were labeled with rhodamine phallodin and bovine pancreatic DNase-1 conjugated with Alexa 488, respectively, and imaged using confocal microscopy. CT responses to HCl and CO_2 were recorded in the presence of hypertonic mannitol and probes for F-actin (phalloidin) and G-actin (cytochalasin B) under lingual voltage-clamp. Acidic stimuli reversibly decreased TRC pH_i, induced cell shrinkage, and shifted the equilibrium from F-actin to G-actin. Treating with phalloidin or cytochalasin B altered TRC volume without a change in pH_i. The phasic CT response to HCl or CO_2 (pH 7.4) was decreased by pre-shrinking TRCs with 1 M mannitol or by applying 1.2 mM phalloidin or 20 μm cytochalasin B. In TRCs first treated with cytochalasin B, the decrease in the phasic response to acidic stimuli was reversed by subsequent phalloidin treatment. The pH_i-induced decrease in TRC volume activated a 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB)-sensitive membrane conductance and NPPB decreased the phasic CT response to HCl and CO_2 stimulation. NPPB and hypertonic mannitol were additive in inhibiting the phasic response. We conclude that the calcium-independent transduction events involved in eliciting the phasic CT response to acidic stimuli are: a decrease in TRC pH_i, alteration in the actin cytoskeleton (conversion of F- to G-actin), cell shrinkage, and activation of a basolateral NPPB-sensitive conductance.

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Poster: Peripheral Taste Physiology

Is the vanilloid receptor-1 (VR-1) variant a hamster taste receptor?

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The epithelial Na^+ channel blocker, amiloride, produces as much as a 60% reduction in the integrated chorda tympani (CT) nerve response to NaCl in rats and hamsters. Recent research suggests that a nonselective ion channel derived from the vanilloid receptor 1 (VR-1) gene may be an amiloride insensitive salt-taste receptor. The VR-1 channel transports Ca^{2+} and Na^+ in response to acidic conditions, heat and capsaicin. Known effects of temperature on the VR-1 channel and the VR-1 receptor antagonist SB-366791 were used to investigate the role of VR-1 in post-amiloride responses of the hamster CT. Electrophysiological responses were recorded from the CT of six anesthetized adult, male golden hamsters (*Mesocricetus auratus*; 130–170 g). Taste stimuli [NaCl and KCl (30, 100 and 300 mM), NH_4Cl (500 mM) and sucrose (300 mM)], dissolved in 30 μM amiloride, were presented to the anterior tongue at 4, 21 and 40°C via a gravity flow system (2 ml/s), with or without 1 μM SB-366791 mixed in the stimulus solution. Neural activity was differentially amplified, rectified, integrated and saved on a computer for analysis. Response magnitudes were quantified as the area under an 8-s response curve starting with stimulus onset. Temperature results suggest that post-amiloride sodium transduction occurs via VR-1 derived receptors. However, treatment with SB-366791 produced little change in post-amiloride responses to NaCl. These findings imply that either SB-366791 does not effectively inhibit VR-1 in the hamster or VR-1 does not play a significant role in transducing post-amiloride sodium responses.

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Poster: Peripheral Taste Physiology

Single cell RT-PCR and functional characterization of mouse circumvallate taste cells

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Taste cells are classified into types based on ultrastructural and molecular markers that are presumed to reflect functional classes. Type 2 cells express taste receptors for bitter, sweet and umami and signal transduction proteins associated with these GPCRs, including $PLC\beta_2$. Type 3 cells have synapses and express the synaptic marker SNAP25. We explored the possibility of combining Ca^{2+} imaging with single cell RT-PCR (scPCR) on isolated mouse circumvallate taste cells to determine the molecular identity of functionally identified taste cells. scPCR revealed $PLC\beta_2$ or SNAP25 in 23 and 31% of cells, respectively, consistent with findings from immunostaining data of others. In cells testing positive for either $PLC\beta_2$ or SNAP25, none coexpressed both markers. Taste cells were functionally identified by monitoring Ca^{2+} in response to either depolarization with K^+ or to a mixture of saccharin and cycloheximide. Among functionally identified cells, 27% of cells that responded to the mixture of tastants were positive for $PLC\beta_2$ and none expressed SNAP25. In contrast, 64% of cells that responded to K^+ depolarization were positive for SNAP25 and none expressed $PLC\beta_2$. Our data indicate that there are at least two functional populations of taste cells, one responding to tastants and expressing $PLC\beta_2$, and one responding to high K^+ and expressing the synaptic protein, SNAP25.

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Poster: Peripheral Taste Physiology

Up-regulation of lingual chemokines and adhesion molecules following unilateral chorda tympani nerve section

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Unilateral chorda tympani nerve (CT) section leads to an increase in activated macrophages on both the sectioned and intact sides of the tongue. These macrophages are proposed to release factors that regulate sodium taste function following CT injury. We hypothesize that chemokines and adhesion molecules play a role in macrophage recruitment soon after CT section. SPF Sprague–Dawley rats received unilateral CT or sham section. Rats were euthanized at 6, 12, 24 and 48 h post-sectioning, and frozen sections processed for immunofluorescent staining with antibodies to prominent adhesion molecules. In separate groups of rats, ELISAs (R & D Systems)

were used to analyze relative changes in adhesion molecule expression in tissue homogenates from the sectioned and intact sides of the tongue. By 6 h post-sectioning, vascular ICAM-1 (1:1000; Accurate Chemical and Scientific Corporation) expression is increased. In sham-sectioned rats, lingual blood vessels, macrophages, and even taste receptor cells express MIP-1α (1:500; Serotec), while MCP-1 (1:1000; Serotec) is expressed by blood vessels, mast cells and macrophages. However, denervation induces a dramatic increase in MCP-1 expression that peaks at 24 h post-section. MCP-1 expression is also up-regulated on the intact side of the tongue following CT section, although to a lesser extent. We propose that increases in ICAM, MCP-1 and MIP-1α are essential signals for macrophage recruitment after neural injury in the peripheral taste system.

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Poster: Taste: Neurotransmitters & Modulators

Mouse taste buds release ATP following stimulation by bitter and sweet tastants

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The mechanism used by type II taste cells to communicate with afferent nerve fibers is unclear. Type II cells contain transduction machinery for bitter and sweet taste transduction, yet appear to lack prominent, conventional synapses. ATP is a promising candidate for transmitting information between taste cells or between taste cells and nerve fibers. ATP acts as a neurotransmitter, co-transmitter and neuromodulator in both the peripheral and central nervous systems, and ATP-gated P2X and P2Y receptors are located in taste buds. P2Y receptors are located on taste receptor cells (Kim *et al.*, 2000; Baryshnikov, 2003; Kataoka *et al.*, 2004) and P2X receptors are primarily located on fibers associated with taste buds (Bo *et al.*, 1999; Rong *et al.*, 2000; Cheung and Burnstock, 2002; Stone *et al.*, 2004), although P2X7 may be expressed on both fibers and taste cells (Stone *et al.*, 2004). Previously, we showed a close association between gustducin containing type II cells and P2X2-immunoreactive fibers, suggesting that type II cells may use ATP to communicate to nerve fibers. The goal of the present study is to test whether ATP is released by taste cells following stimulation by bitter and sweet stimuli. To address this question, we used two ATP assays. First, a luminometer was used to detect the release of light following the reaction of luciferase on luciferin in the presence of ATP. Second, 'sniffer' HEK cells expressing ATP receptors were positioned close to taste buds and calcium changes were monitored in the HEK cells following application of tastants to taste buds. Both assays indicate that ATP is released by taste buds following stimulation.

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Poster: Taste: Neurotransmitters & Modulators

The ATPase of taste buds is nucleoside triphosphate diphosphohydrolase-2

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The presence of one or more ecto-ATPase (enzymes that degrade ATP in extracellular space) in taste buds was first shown histochemically (Iwayama and Nada 1967; Barry 1992). Recent studies have established that the ecto-ATPase family consists of various related members now called nucleoside triphosphate diphosphohydrolases (NTPDases; Zimmerman, 2001). Massively parallel signature sequencing (MPSS) from taste epithelium provided molecular evidence suggesting NTPDase2 to be the most likely member present in mouse taste papillae. Immunohistochemical staining verified the presence of NTPDase2 in mouse circumvallate, foliate and fungiform papillae. To examine which taste cell type expresses this enzyme, double label assays were performed using GLAST, PLCβ2 or 5-HT as markers of type I, II or III cells, respectively. Strong NTPDase2 immunoreactivity is present in a large number of cells within each taste bud. Because NTPDase2 immunoreactivity is strongest in association with cell membranes, co-localization with cytoplasmic cell type markers was not straightforward. Careful analysis of these double labeled sections indicates that NTPDase2-ir processes often envelop other taste cells, reminiscent of Type I cells described by others. NTPDase2 appears to be localized to the same membranes as GLAST further confirming that this enzyme is present in Type I cells.

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Poster: Taste: Neurotransmitters & Modulators

ATP and glutamate: paracrine transmitters in mouse vallate taste buds

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In addition to transmitting information from taste cells to gustatory afferent nerve fibers, synapses may occur between cells within taste buds and transmit signals to neighboring taste receptor cells via paracrine mechanisms. Indeed, an abundance of evidence suggests that taste cells can respond to paracrine transmitter candidates such as serotonin (5-HT), ATP and glutamate, among others. We have recently shown that sweet, bitter, and sour tastants stimulate serotonergic taste cells to release 5-HT (Huang *et al.*, 2005, *J. Neurosci.*, 25). However, the cells that respond to taste stimuli (i.e. receptor cells) are believed to comprise a different subset than the cells that secrete 5-HT. Thus, we reasoned that there must be paracrine transmitter(s) that transmit signals from stimulated receptor cells to nearby serotonergic cells, causing the latter to secrete 5-HT. Using biosensor cells to monitor 5-HT release from acutely isolated mouse vallate taste buds (Huang *et al.*, 2005), we show here that glutamate and ATP are two such likely candidates for paracrine transmitters. Namely, taste stimuli (10 μM cycloheximide, 2 mM saccharin), ATP (10 μM) and glutamate (30 μM) all elicited 5-HT release from isolated taste buds. 5-HT release evoked by glutamate was blocked by 10 μM MCPG, a metabotropic glutamate receptor antagonist, but 5-HT release from taste stimulation was unaffected. In contrast, 5-HT release evoked either by ATP or by tastants was blocked by 10 μM

suramin, a P2 receptor antagonist. These data suggest that mouse vallate taste receptor cells communicate with serotonergic taste cells via ATP. Glutamate may also play a role, but the details remain to be elucidated.

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Poster: Taste: Neurotransmitters & Modulators

Neurokinin 1 receptors in lingual salivary gland acinar cells

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The microenvironment of taste receptor cells is very important for taste transduction and introducing taste substances to taste cells. The apical pores of taste buds in circumvallate and foliate paillae are surrounded by the saliva from von Ebner salivary gland (VEG). This study was designed whether neurokinin receptor 1(NK1) is present in von Ebner's acinar cells via immunohistochemistry, and RT-PCR against NK1 and observing changes in intracellular calcium activity. Male Sprague–Dawley rats (200 g) were used for this experiment. For immunohistochemistry, 4-μm-thick sections were used as usual paraffin preparations. Relatively VEG rich tissues were obtained from dissecting 500-μm-thick posterior tongue slices under stereomicroscope view. Single acinar cells and cell clusters were isolated by a sequential trypsin/collagenase treatment and were loaded with 10 μM fura 2-AM for 60 min at room temperature. Fluorescence images of cells at resting state and activated state were recorded and analyzed by CCD imaging system controlled by computer (MetaFluor 5.0, UIP). Immunohistochemistry showed NK1s in VEG acinar cells but not in epithelium nor in surrounding muscles. RT-PCR reveals that the mRNA of NK1 is present in VEG acinar cells. The intracellular calcium activity was increased rapidly in response to bath application of even <1 nM substance P. At 30 nM, L-732,138, an antagonist of NK1, completely abolished the responses elicited by substance P. The responses to carbachol and substance P were also observed when extracellular calcium had been removed, indicating release of calcium ions from intracellular stores. These results suggest that NK1 is an important receptors for secretion of VEG.

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Poster: Taste: Neurotransmitters & Modulators

Glutamate-induced calcium responses in mouse vallate taste receptor cells

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L-Glutamate (L-Glu) is a naturally occurring amino acid in protein-rich food and elicits umami taste. We reported that L-Glu induces transient $[Ca^{2+}]_i$ increases in vallate taste cells of mice (*AChemS XXVI*). Here we elucidate the Ca^{2+} mobilization pathway for these responses. We used Calcium Green-1 dextran-loaded mouse taste cells in lingual tissue slices, confocal microscopy, and immunofluorescence to study this question. Focal application of L-Glu (30 to 500 mM) at the taste pore induced $[Ca^{2+}]_i$ responses in <5% of taste receptor cells. These cells did not respond to depolarization with bath-applied KCl (50 mM). In the absence of Ca^{2+} in the extracellular medium, L-Glu-evoked Ca^{2+} responses were only slightly decreased relative to those recorded in normal Tyrode's medium (control = 10.2 ± 2.1% $\Delta F/F$; Ca^{2+}-free = 9.4 ± 1.8% $\Delta F/F$, n = 9). Depletion of Ca^{2+} stores by thapsigargin (1 μM) abolished responses to L-Glu (control = 12.1 ± 2.4% $\Delta F/F$; thapsigargin = 1.8 ± 1.1% $\Delta F/F$, n = 6). Pretreatment of the lingual slice with U73,122 (10 μM), a phospholipase inhibitor, also abolished L-Glu-induced response (control = 9.2 ± 1.8% $\Delta F/F$; U73,122 = 1.1 ± 0.6% $\Delta F/F$, n = 7). Most glutamate-responsive taste cells were immunopositive for PLCβ2. These observations indicate that L-Glu stimulation initiates a PLCβ2/IP_3 pathway in vallate taste cells and activates Ca^{2+} release from intracellular stores.

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Poster: Taste: Neurotransmitters & Modulators

Co-occurrence of calcium binding proteins and calcium-fluxing glutamate receptors in the primary gustatory nucleus of goldfish

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Primary gustatory afferents utilize glutamate as a neurotransmitter acting on ionotropic receptors of second-order neurons. Many neurons in the primary gustatory nucleus have non-NMDA receptors (AMPA and kainate) that permit passage of Ca^{2+} (and Co^{2+}) ions (Smeraski *et al.*, 2001). Calcium binding proteins (CaBP) play a buffering role for intracellular calcium, thus protecting against glutamate excitotoxicity. Therefore, CaBP might work to maintain intracellular calcium equilibrium in neurons with calcium permeable glutamate receptors. In the present study on the vagal gustatory lobe in goldfish, we used immunohistochemistry to examine the distribution and morphology of neurons with CaBP, including calretinin, calbindin and parvalbumin. Calretinin and calbindin neurons occurred throughout the sensory layer exhibiting round unipolar, horizontal and perpendicular bipolar or multipolar somata. Parvalbumin neurons were mainly round monopolar neurons, especially common in superficial layers. The morphological features of CaBP neurons are similar to those with high Ca/Co permeability (Smeraski *et al.*, 2001). In the motor layer, while parvalbumin labeled many motoneurons and smaller interneurons, calretinin and calbindin labeled external motoneurons and fewer interneurons. All three antibodies recognized fibers in the primary gustatory afferent layer and neurons in the vagal ganglion. Some CaBP neurons also could be labeled by AMPA-induced uptake of cobalt suggesting co-localization of CaBPs and calcium-fluxing ionotropic glutamate receptors.

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Poster: Taste: Neurotransmitters & Modulators

GABA as an inhibitory transmitter in the taste bud

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Immunocytochemical studies have demonstrated that a subset of taste receptor cells (TRCs) in posterior rat papillae is immunopositive for the inhibitory neurotransmitter GABA. We explore the possibility of GABA in taste buds using anatomical and physiological techniques in rat posterior taste buds. Single TRCs label immunopositively for GABA or for its synthetic enzyme glutamate decarboxylase (65/67 kDa). These TRCs are spindled shaped with round nuclei, characteristic of type II and III TRCs. In Western blotting experiments, GAD65/67-immunoreactive bands were observed in posterior as well as anterior tongue tissue. Double-labeling immunofluorescent experiments were performed to study GAD65/67 co-localization with either neural cell adhesion molecule (NCAM), protein gene product 9.5 (PGP9.5) or α-gustducin (αgust). Virtually none of the GAD65/67-immonoreactive TRCs was found to overlap with either NCAM- or PGP9.5-immonoreactive cells, implying that GABAergic taste receptor cells are not type III cells. Only a few GAD65/67-immonoreactive TRCs were also labeled by αgust antibody, suggesting that GABAergic TRCs consist of both αgust-positive type II cells and αgust-negative, PGP9.5-negative type II cells. Patch clamp recordings on acutely dissociated TRCs were performed on whole cell currents, isolated chloride currents, and inwardly rectifying potassium current (KIR) to test for physiological actions of GABA. Whole cell currents demonstrate variable effects whereas inward chloride currents were enhanced by either GABA (10–500 μM) or the GABA(A) receptor agonist muscimol (10–100 μM) in one third of tested cells. KIR was increased in 2 of 15 tested cells, suggesting a possible involvement of GABA(B) receptors.

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Poster: Taste: Neurotransmitters & Modulators

Do TRPM5 expressing cells have classical synapses?

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Although it has been shown that TRPM5 is imperative for the transduction of bitter, sweet, and umami compounds (Zhang *et al.*, 2003; Damak *et al.*, in preparation), it is not yet known how this information is relayed to the nervous system. We set out to determine if cells that express TRPM5 also express proteins involved in synaptic transmission, such as the presynaptic SNARE protein SNAP-25 and voltage-gated Ca^{2+} channels (VGCCs). To identify cells that contain TRPM5 we used transgenic mice expressing green fluorescent protein (GFP) from the TRPM5 promoter. Using immunocytochemical methods we looked for SNAP-25-like immunoreactivity (LIR) in GFP expressing cells of the palate, foliate, and circumvallate papillae. We found no evidence of SNAP-25-LIR in any GFP positive cells, indicating no overlap between TRPM5 expression and taste cells expressing the synaptic marker SNAP-25. We also used Ca^{2+} imaging techniques to determine if GFP positive cells respond to depolarization with an increase in intracellular Ca^{2+} indicative of VGCCs. When depolarized, these cells had no Ca^{2+} response, although most responded with increased intracellular Ca^{2+} to the PLC activator 3M3-FBS. These data suggest that TRPM5-expressing taste cells use a means other than classical synapses to communicate with the nervous system.

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Poster: Taste: Neurotransmitters & Modulators

A quantitative study of syntaxin-1 immunoreactivity in rat taste buds: colocalizations with α-gustducin, PLC2, synaptobrevin-2, PGP 9.5 and 5-HT

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Previous studies from our laboratory have demonstrated that taste cells with synapses display immunoreactivity to antisera directed against the SNARE proteins SNAP-25 and synaptobrevin-2, and the presynaptic membrane protein syntaxin-1. In order to learn more about the functional nature of taste cells with synapses, we determined the extent of colocalization of syntaxin-1-like-immunoreactivity (LIR) with proteins that are believed to be involved in gustatory signal transduction (α-gustducin, PLCβ2, synaptobrevin-2, PGP 9.5 and 5-HT). Our preliminary results indicate that ~15% of the taste cells in rat circumvallate taste buds display syntaxin-1-LIR. Syntaxin-1-LIR cells also display immunoreactivity to PLCβ2 (11%), synaptobrevin-2 (99%), PGP 9.5 (72%) and 5-HT (72%) antisera, respectively. No α-gustducin-LIR, however, is present in syntaxin-1-LIR cells. Only 4% of PLCβ2-LIR cells display syntaxin-1-LIR, while syntaxin-1-LIR is present in 66% of synaptobrevin-2-, 75% of PGP 9.5- and 94% of 5-HT-LIR cells. These results suggest that syntaxin-1 is present in type III taste cells; while some (if not all) type II taste cells lack the syntaxin-1.

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Poster: Taste: Neurotransmitters & Modulators

Immunoreactivity to sensory transduction and synaptic protein markers in the taste buds of mouse and rat circumvallate papillae: a quantitative comparison

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Much of the electrophysiological, ultrastructural and immunocytochemical studies on rodent taste buds have been carried out on rat taste buds. In recent years, however, the mouse has become the species of choice for molecular and other studies on taste buds. Do rat and mouse taste buds have the same cell types, sensory transduction markers and synaptic proteins? We have begun a series of experiments to determine whether the mouse and rat have the same molecular machinery for taste signal transduction and synaptic function. In the present study we have used antisera directed against PLCβ2, α-gustducin, 5-HT, PGP-9.5 and synaptobrevin-2 to determine the percentage of taste cells expressing these markers in taste buds in both rodent species. Our results suggest that there are significant differences ($P < 0.05$) between mouse and rat taste buds in the percentage of taste cells displaying immunoreactivity for all five markers. Rat taste buds display significantly more IR than mice for

PLCβ2 (31.8% versus 19.6%), α-gustducin (18% versus 14.6%), and synaptobrevin-2 (31.2 versus 26.3%). Mice, however, have more cells that that display IR to 5-HT (15.9 versus 13.7%) and PGP 9.5-LIR (14.3 versus 9.4%). These results suggest that there may be a higher percentage of type II cells and a lower percentage of type III cells in rat circumvallate taste buds when compared with mouse circumvallate taste buds. These differences indicate that rats and mice are indeed different in their gustatory processing.

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Poster: Taste: Neurotransmitters & Modulators

Presynaptic Ca^{2+}-fluxing glutamate receptors on primary afferent terminals in goldfish

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The primary gustatory nucleus in goldfish has a stereotyped structure in which primary gustatory afferents terminate in a distinct laminar pattern. Ionotropic glutamate receptors are crucial for transmission of primary gustatory information and some are permeable to Ca^{2+} ions. We used the capability of Co^{2+} to pass through Ca^{2+}-fluxing glutamate receptors to study their distribution in the primary gustatory nucleus of goldfish. In these Co^{2+} uptake studies, the layers of primary afferent termination exhibited fine grain punctate labeling suggestive of axonal terminals or dendritic spines. Transection of the vagus nerve eliminated this punctate staining in a time course consonant with primary afferent degeneration. The primary afferent fibers (and vagal ganglion cells) are immunoreactive for calbindin and calretinin, two Ca^{2+} binding proteins (CaBP). Unilateral vagal nerve section followed by cobalt uptake or immunohistochemistry for CaBP showed that both the cobalt punctuate label and the immunoreactivity for the CaBP corresponding to primary afferents is present 4 days after nerve cut, but is diminished at 6 days and disappears after 8 days degeneration. We will utilize EM-immunocytochemistry for CaBP to characterize the ultrastructure of the puncta in the layer of termination of the primary afferents. These results support the presence of Ca^{2+} fluxing glutamate receptors on the presynaptic terminals of primary gustatory terminals providing an avenue for modulation of release of glutamate with gustatory stimulation.

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Poster: Taste: Neurotransmitters & Modulators

Regional localization patterns of glycoconjugate vary among papillae in various mammalian animals

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It has been reported that sensitivities to various tastants are different depending on the region of the tongue. In addition, it has been reported that the expression patterns of various molecules involved in taste perception, including taste receptors, are different between the anterior and posterior part of the tongue. We conducted lectin histochemical studies to elucidate whether these differences exist in papillae of a wide variety of animals. We examined five species of animals, each of them from different orders: dogs (Carnivora), mice (Rodentia), horses (Perissodactyla), cows (Artiodactyla) and monkeys (Primate). We examined the expression patterns of 24 types of lectins in three types of papillae on the tongue: circumvallate, foliate and fungiform. We confirmed that cows doesn'*t* possess foliate papillae. The number, size and the distribution of papillae differed among species. Our results demonstrated that: (i) some lectins showed positive reactions in these animals' papillae; (ii) the lectin reactivity varies among species examined; and (iii) there are great varieties of localizations and densities of positive reactions among the papillae even in the same type of species. Our findings suggest that the different localization patterns of the glycoconjugates among lingual regions are common among various types of animals, and they may be involved in the different sensitivities of taste.

Poster: Taste: Neurotransmitters & Modulators

Maillard peptides are a clue to the kokumi taste of the typical Korean soy sauce, ganjang

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Ganjang is used as a primary food seasoning in Korea. It confers continuity and mouthfulness to the taste of food when used sparingly; this taste effect is very similar to what is called kokumi in Japan. Recent studies have suggested that maillard peptides—peptides that react with aldehydes or xylose in aged cheese or model systems—enhance kokumi. The objectives of this study were to investigate the kokumi of ganjang and the possible contribution of maillard peptides to this taste. The taste profiles of ganjang aged for one to four years were evaluated in a model soup system using the descriptive analysis method. All of the ganjang scored for umami, kokumi, mouthfulness and continuity, which became stronger with aging. Aging also caused the gradual development of browning, which was measured as the absorbance at OD 450 nm, and increases in the total, free and bound amino acids (a.a.). Ganjang of different ages were fractionated by ultrafiltration to give F-I (mol. wt < 500), F-II (mol. wt 500—10 000) and F-III (mol. wt > 10 000). Only F-II conferred the kokumi taste; it had the strongest absorbance at OD 450 nm. In F-II of ganjang that was at least 3 years of age, there was a marked change in the kokumi and bound a.a., which decreased to 72–75%, with conspicuous decreases in Lys (48–58%), Arg (50–53%) and His (40–44%). In conclusion, ganjang confers kokumi, and aging increases this taste. The formation of maillard peptide, which has a mol. wt of 500–10 000, might contribute to creating the kokumi of ganjang.

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Poster: Ion Channels, Rafts & Pumps

Olfactory physiology and behavior of mice with alterations in metabolism or weight

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Diet-induced obesity and metabolic disorders have been recently shown to affect memory and sensory input. Mice heterozygous for the insulin receptor ($IR^{+/-}$), homozygous null for the melanocortin receptor 4 ($MC4R^{-/-}$) or Kv1.3 potassium channel ($Kv1.3^{-/-}$) were tested in environmental chambers designed to assess metabolism and behavior (locomotion, ingestive behaviors, oxygen consumption and respiratory quotient). $Kv1.3^{-/-}$ mice had increased locomotion, irregular ingestive behaviors and weighed less than wild type controls (wt) even though they exhibited the same caloric intake. $MC4R^{-/-}$ mice weighed 2–3 times that of $Kv1.3^{-/-}$ mice whereas $IR^{+/-}$ mice were slightly greater in weight without decreased locomotion. $Kv1.3^{-/-}$ mice displayed increased olfactory ability whereas $IR^{+/-}$ mice showed no deficits in gross anosmia. $IR^{+/-}$ mice, but not $Kv1.3^{-/-}$ mice, showed impairment in both short- and long-term object recognition testing (memory). Mitral neurons from $IR^{+/-}$ and $IR^{-/-}$ mice had a decreased amplitude of the Kv1.3-contributing current with a correlate decrease in Kv1.3 expression. Analogous experiments in $Kv1.3^{-/-}$ mice demonstrated a slowing of inactivation kinetics, an increased action potential firing, but no change in IR expression. Currently we are generating double mutant lines to test whether weight, olfactory and memory phenotypes of obese mice can be rescued in the K channel-null background.

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Poster: Ion Channels, Rafts & Pumps

Adaptor proteins perturb olfactory bulb K channel modulation by receptor tyrosine kinases

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Receptor tyrosine kinases (RTKs), modulate ion channel activity and create adaptor protein docking sites by phosphorylating their targets at specific tyrosine residues. Kv1.3 is a voltage-gated *Shaker* channel that carries 60–80% of the outward current in mitral cells of the olfactory bulb (OB) and has been shown to be a substrate for tyrosine phosphorylation by two RTKs, the insulin receptor kinase (IR kinase) and neurotrophin B (TrkB). Activation of these RTKs in the OB results in current suppression of the Kv1.3 channel, thereby affecting neuronal excitability. We now show that adaptor proteins post synaptic density 95 (PSD-95), nShc and Grb10 disrupt the RTK-evoked Kv1.3 current suppression. Specifically, PSD-95 disrupts insulin-evoked Kv1.3 current suppression via IR kinase, Grb10 relieves BDNF-evoked Kv1.3 current suppression via TrkB kinase, while nShc eliminates Kv1.3 suppression via both RTK pathways. PSD-95 alone significantly alters the biophysical properties of Kv1.3 in an activity-dependant manner, and most strongly suppresses Kv1.3 current by 48%. A channel mutant lacking the canonical C-terminal PDZ binding domain and a PSD-95 mutant lacking the membrane-associated guanylate kinase (MAGUK) were constructed by site-directed mutagenesis and cDNA truncation, respectively. These mutants, along with previously constructed nShc and TrkB mutants, will help determine the mechanism by which phosphorylation-dependent Kv1.3 channel modulation is disrupted by adaptor proteins.

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Poster: Ion Channels, Rafts & Pumps

Differential modulation of Kv1.3 and Kv1.5 channels by BDNF and insulin signaling in the olfactory bulb

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Previously we have shown that olfactory bulb neuron (OBN) current properties are modulated by activation of the neurotrophin receptor TrkB, by brain-derived neurotrophic factor (BDNF), to increase tyrosine phosphorylation of the potassium channel Kv1.3. In addition, the insulin receptor (IR) present in the olfactory bulb can also modulate Kv1.3 by tyrosine phosphorylation. Unexpectedly, using heterologous expression (HEK 293 cells), we found a 2.1-fold ($n = 4$) increase in channel protein expression under Kv1.3 and TrkB co-transfection conditions in the absence of BDNF. Protein expression of another *Shaker* olfactory bulb channel, Kv1.5, showed a 2.8-fold ($n = 6$) decrease when co-transfected with TrkB. Unlike Kv1.3/TrkB interactions, Kv1.5 reciprocally induced a 2.3-fold ($n = 6$) decrease of TrkB protein levels. These data suggest a phosphorylation-independent mechanism of regulation, allowing the TrkB receptor to differentially modulate the α-subunit composition of these voltage-gated channels. In addition, the overall phosphorylation of Kv1.5 is unchanged in the presence of TrkB or IR, even under BDNF or insulin stimulation. This contrasts with our observed phosphorylation-dependent modulation of Kv1.3 via activation of these same kinases. These results suggest that TrkB and the IR may alter the excitability of OBNs through combinatorial means altering *Shaker* channel properties through addition of negative charge (phosphorylation) and differentially influencing α-subunit cellular expression or trafficking.

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Poster: Ion Channels, Rafts & Pumps

Arachidonic acid stimulates a Ca^{2+}-activated K^+ current in mouse vno neurons

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The binding of certain odorants to their receptors can activate the PLC pathway in mouse vomeronasal neurons. One of the products of PLC pathway, arachidonic acid (AA), elicits Ca^{2+} transients through a putative TRPC2 channel. Our preliminary data suggested that these Ca^{2+} transients elicited via PLC pathway open

a large-conductance Ca^{2+}-activated K^+ (BK) channel. Using Fura-2 Ca^{2+} imaging, we found 50 M AA elicited Ca^{2+} transients in ~40% neurons (4/10 cells). Using gramicidin perforated patch-clamping recordings, we found repetitive pulses of 50 M AA increased the outward current at more depolarizing potentials in the majority of cells (20/22 cells). Substitution of bath Ca^{2+} with Ba^{2+}, or 1 mM Cd, eliminated the increased outward current, indicating that it was Ca^{2+}-dependent. In addition, this outward current was blocked by 20 mM TEA, indicating it was the K^+ current. There are three types of Ca^{2+}-activated K^+ channels categorized by their K^+ conductance: SK (small conductance), BK (large conductance) and IK (intermediate conductance) channels. In our preliminary studies, an SK channel blocker (1 M Apamin) failed to block the AA-induced increase in outward current. However, a BK channel blocker (100 nM iberiotoxin) was able to block it. These preliminary data suggested the existence of BK channels in mouse vomeronasal neurons. BK channels could play important role(s) in shaping the odorant responses. For example, they may help to re-polarize the neurons after the action potentials. Further experiments will be done to determine the role(s) of the BK channels in VNO odor responses.

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Poster: Ion Channels, Rafts & Pumps

Single Ca^{2+} activated Cl^- channels in chemosensory cilia of toad olfactory receptor neurons

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Olfactory cilia contain cyclic nucleotide-gated channels (CNG), Ca^{2+}-dependent Cl^- channels (Cl_{Ca}) and Ca^{2+} activated K^+ channels (K_{Ca}). Odor responses consisting on increases in action potential rates involve the CNG and the Cl_{Ca} channels. Only the K_{Ca} channels had been previously recorded at the single-channel level from the ciliary membrane (Delgado *et al.*, 2003, *J. Neurophysiol.*; Delgado and Bacigalupo, 2004, *Eur. J. Physiol.*; Castillo *et al.*, 2005, *FEBS Lett.*). In this work we show, for the first time, a single-channel study of ciliary Cl_{Ca} channels in membrane patches excised from toad olfactory cilia. This channel strongly depends on intraluminal $[Ca^{2+}]$, exhibiting a $K_{0.5}$ = 0.38 μM ($V_{pipette}$ = −30 mV; n = 3). The channel is also voltage-dependent, with a $V_{0.5}$ = −53 mV (15 μM Ca^{2+}). Two open conductance states are most frequently observed, of 11 and 22 pS under symmetrical Cl^- (120 mM; n = 7). This channel has a similar Ca^{2+}-dependence than the Cl_{Ca} channel previously studied from macroscopic measurements on dendritic knob membrane patches and intact single cilia. However, the conductance of the unitary channel recorded directly on the cilium is 1–2 orders of magnitude higher than that estimated by noise analysis in macroscopic studies.

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Poster: Ion Channels, Rafts & Pumps

Effect of adenylyl cyclase inhibitors on electroolfactogram responses in rat

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Biochemical measurements of odor-induced second messenger production in rat olfactory cilia preparations lead to the hypothesis that odors stimulate either cAMP or IP3 production (Breer and Boekhoff, 1991, *Chem. Senses*, 16:19). This hypothesis has been challenged by a variety of experiments that included a study by Zufall and colleagues in mouse demonstrating inhibition of the electroolfactogram (EOG) response to odors thought to increase IP3 production by inhibitors of adenylyl cyclase (to block cAMP production) (Chen *et al.*, 2000, *J. Physiol.*). In a recent study from our laboratory we studied the responsiveness of the main olfactory epithelium and bulb to a variety of odors in mice defective for the olfactory cAMP pathway (CNGA2 knockout mice). Our studies were consistent with the studies of Zufall and co-workers: CNGA2 knockout mice were unresponsive to odors such as lilial that had been postulated to stimulate IP3 production by Breer and Boekhoff (Lin *et al.*, 2004, *J. Neurosci.*, 24:3703). However, we did find other odors (2-heptanone and 2,5-dimethylpyrazine) that CNGA2 knockout mice were responsive to, and whose EOG responses in wild type mice were only partially inhibited by cyclase blockers. In this study, we perform a thorough characterization of the effect of adenylyl cyclase inhibitors on EOG responses to diverse odorants in rat.

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Poster: Ion Channels, Rafts & Pumps

Functional role of lipid raft microdomains in cyclic nucleotide-gated channel activation

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Cyclic nucleotide-gated (CNG) channels are the primary targets of odorant-induced signaling in olfactory sensory neurons (OSNs). Compartmentized cyclic nucleotide signaling is necessary to ensure rapid and efficient activation of these non-selective cation channels. However, relatively little is known about the subcellular localization of CNG channels or the mechanisms of their membrane partitioning. Here we report that the alpha subunit of the olfactory CNG channel, CNGA2, but not the photoreceptor CNGA3, associates with cholesterol-rich lipid raft microdomains in heterologous expression systems and in rat olfactory epithelium. However, CNGA2 does not appear to associate with caveolin in these two systems. Cholesterol depletion, using methyl-β-cyclodextrin, abolished prostaglandin E_1-stimulated CNGA2 channel activity in intact cells. Surprisingly, isoproterenol-stimulated channel activity was not significantly affected. Recordings from membrane patches excised from CNGA2-expressing HEK-293 cells revealed that cholesterol depletion dramatically reduced the apparent affinity of homomeric CNGA2 channels for cAMP while only slightly reducing the maximal current. Recent data suggest that CNGA2 channels are functionally dependent upon a discrete pool of cellular cholesterol. Our results show that olfactory CNG channels target to lipid

rafts and that depletion of raft lipid dramatically alters the function of CNGA2 channels.

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Poster: Ion Channels, Rafts & Pumps

Lipid rafts organize chemosensory signaling in *Paramecium*

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Lipid rafts, cell surface membrane microdomains, organize signaling molecules. Rafts are insoluble in detergents, and enriched in cholesterol, glycosphingolipids and GPI-anchored proteins. Lipid rafts seem to organize chemosensory signaling proteins in *Paramecium*. Using biochemical criteria, we identified lipid rafts in cilia and cell body membranes. Triton X-100-insoluble ciliary membrane fractions distribute in sucrose or Optiprep gradients with densities characteristic of lipid rafts in other systems. The proteins in these fractions include the GPI anchored surface antigens, the GPI anchored folate chemoreceptors, and tubulin. The plasma membrane calcium pumps (PMCAs) are absent from all fractions. Likewise, Triton X-100-insoluble fractions of cell body membranes distribute in sucrose or Optiprep gradients with densities characteristic of lipid rafts. GPI-anchored surface antigens, folate chemoreceptors, tubulin and the PMCAs distribute differently among the light density fractions. Perturbation of the cholesterol disrupts chemoresponse, but has relatively little effect on the density of lipid rafts. Methyl beta cyclodextrin (MBCD) treatment of whole cells reduces membrane cholesterol by ~60%. However, this reduction does not perturb the general distribution of proteins in the lipid raft fractions of density gradients. Interestingly, MBCD perturbs chemoresponse to glutamate and folate when whole cells are treated with MBCD prior to behavioral testing. We take these results to implicate lipid rafts or special lipid microdomains in the organization of the signaling molecules for *Paramecium* chemoresponse. These molecules include the PMCAs and chemoreceptors, such as the folate chemoreceptors.

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Poster: Ion Channels, Rafts & Pumps

The organization of plasma membrane calcium pumps in *Paramecium*: implications for signal transduction

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Plasma membrane calcium pumps (PMCAs) in *Paramecium* appear to play a role in chemosensory signal transduction. Several lines of indirect evidence implicate specific PMCA isoforms in two chemoresponse pathways, in which they carry a sustained hyperpolarizing conductance. Of 24 PMCAs in the *Paramecium* genome, isoforms 2–4 are most closely related to each other and to the human isoform 4b. An antibody that recognizes their similar C termini shows that they localize to base of cilia, probably at the ciliary necklace particles, with the folate chemoreceptor, GPI anchored surface antigens, and other surface proteins that characterize lipid rafts in other systems. Immunoprecipitation of the isoforms 2–4 co-precipitates calmodulin and tubulin. We have previously demonstrated that calmodulin binds to isoforms 2–4, and calmodulin regulation is well established for the mammalian PMCAs. The co-immunoprecipitation of tubulin is consistent with our view that the PMCAs are closely associated with cytoskeleton at the bases of the cilia. Isoform 3 is readily soluble in Triton X-100, and isoforms 2 and 4 are not, and are found in very specific lipid raft fractions of sucrose gradients. Over expression studies have implicated Isoform 2 in the chemoresponse to folate, and isoform 3 is generally involved in chemoresponses with the exception of responses to ammonium. These data indicate that the isoforms 2–4 are different in their physical chemical properties and their roles in chemoresponse while at the same time they co-localize to the bases of cilia where chemosensory signaling molecules are located.

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Poster: Ion Channels, Rafts & Pumps

Paramecium ryanodine receptors localize to mitochondria and contribute to depolarization-mediated swimming behavior

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Ryanodine receptors (RyR) are intracellular calcium-release channels normally found on the endoplasmic or sarcoplasmic reticulum in metazoans. Because caffeine and 4-chloro-m-cresol stimulation of *Paramecium* results in an increase in intracellular calcium concentration and subsequent trichocyst discharge, it is possible that a calcium channel similar to the ryanodine receptors found in mammalian skeletal muscle and other tissues may be found in *Paramecium*. Although exposure to ryanodine showed no phenotypic effects in exocytosis experiments, ryanodine receptors might still be involved in calcium events associated with swimming behavior. When cells were treated with the ryanodine receptor antagonists ryanodine or dantrolene, backward swimming responses initiated by KCl depolarization were reduced. We subsequently labeled *Paramecium* with fluorescent-tagged ryanodine and were able to visualize specific labeling of putative ryanodine receptors. Surprisingly, fluorescent-labeled ryanodine did not co-localize to either the plasma membrane associated alveolar sacs or to endoplasmic reticulum, but to mitochondria. Treatment of *Paramecium* with low (10 nM) concentrations of the oxidative phosphorylation uncoupler carbonyl cyanide phenylhydrazone (FCCP) caused depolarization-induced backward swimming behavior in high KCl concentrations comparable to dantrolene and ryanodine treatments. We have recently identified at least three different RyR sequences from the *Paramecium* genome project and are currently amplifying one for use in RNAi experiments.

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Poster: Human Sensory Perception

Brain response to putative pheromones differs between homo- and heterosexual men

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Introduction: the testosterone 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. In a recent PET-study we found that smelling of AND and EST activated sexually areas of anterior hypothalamus, and that this activation was differentiated with respect to sex and compound. Methods: identical PET experiments were now carried out in twelve homosexual men, using EST, AND, and four unfamiliar odors (butanol, cedar oil, lavender oil and eugenol). Passive smelling of odorless air (AIR) was the baseline condition. Results: the activation pattern was related to sexual orientation rather than biological sex. Like heterosexual women but unlike heterosexual men, homosexual men activated hypothalamus with AND, with a maximum in medial preoptic area/anterior hypothalamus (in animals highly involved in sexual behavior). In contrst, common odors were processed similarly in all three groups of subjects, engaging only the olfactory brain (amygdala, piriform, orbitofrontal and insular cortex). Homosexual men did not differ from heterosexual men with respect to hormone levels. Comments: our brain reacts differently to the two putative pheromones compared with common odors. The present data suggest a link between sexual orientation and hypothalamic neuronal processes.

Poster: Human Sensory Perception

Dissociating somatosensory and olfactory cues as inputs to the olfactomotor system

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The flow of air is greater into one nostril than into the other because there is a slight turbinate swelling in one. This difference in airflow optimally sensitizes each nostril to different odorants. Here we found that this effect was evident only when individually tested nostrils are restricted to the same sniff duration (as would be the case in a normal birhinal sniffing) (n = 19, difference across nostrils in perception, $P < 0.007$). When sniff duration was not restricted, subjects spontaneously sniffed longer in the low-airflow nostril, thus equating its perception with the high-airflow nostril (difference across nostrils in perception, $P = 0.20$). This olfactomotor compensation can rely on one of two cues: either a somatosensory cue of reduced airflow leading to increased sniff duration, or an olfactory cue of reduced odor leading to the increased sniff duration. To dissociate between these two possibilities we will report results obtained with a special olfactometer that allowed subjects to sniff freely with both nostrils, but receive odor in only one nostril.

Poster: Human Sensory Perception

PROP and retronasal olfaction

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Retronasal olfaction is linked to taste. Bull (1965) noted anecdotally that patients with severed chorda tympani nerves reported reduced flavors; anesthesia of the chorda tympani reduced retronasal olfaction (Snyder *et al.*, 2001). However, intensification of retronasal olfaction is produced commercially by intensifying taste (Noble, 1996); retronasal olfactory sensations are also intensified in PROP supertasters (Duffy *et al.*, 2003). To test an odorant presented both orthonasally and retronasally, we distributed strawberry fruit roll-ups to 600 lecture attendees. They rated strawberry sniffed as the package was opened; held their noses, chewed the candy and rated sweetness; then opened their noses and rated the strawberry flavor and sweetness. They also rated the bitterness of PROP paper (~1.6 mg PROP). Ratings were done with the general Labeled Magnitude Scale. Classifying by PROP ratings, the 25% with lowest ratings were nontasters and the 25% with highest ratings were supertasters. In ANOVA, sweetness was most intense to supertasters and least to nontasters (previously shown); sweetness was most intense accompanied by strawberry flavor (intensification of sweetness by retronasal olfaction); strawberry flavor was most intense to supertasters and least intense to nontasters (supporting previous associations between PROP and retronasal olfaction). Interestingly, supertasters perceived the orthonasal strawberry odor as more intense than did the medium and nontasters (see Kirkmeyer and Tepper, 2003), although the effect was smaller than for retronasal strawberry odor. This agrees with the suggestion that orthonasal intensification may reflect generalization from previous experiences with intensified retronasal odorants (Small, 2004).

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Poster: Human Sensory Perception

Odor characteristics predict odor similarity

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Humans compare and contrast odors based on intensity and quality descriptors. To further understand how odor quality affects human perception of odor similarity, we had 19 subjects rate the pairwise similarity of nine different odorants (acetophenone, amyl acetate, diphenyl oxide, ethyl butyrate, eugenol, guaiacol, heptanal, hexanoic acid and phenyl ethanol) of comparable perceived intensity. Intersubject odorant similarity ratings agreed ($r = 0.67$, $P < 0.01$), indicating the presence of a common basis for perception of similarity among subjects. To probe the source of this common basis, we performed principle component analyses on the 146 odorant-descriptors given for the 160 odorants in Dravnieks' *Atlas of Odor Character Profile*.

We first computed the pairwise metric distances between the nine test odorants listed above within the one-dimensional odor perceptual space represented by the first principle component. We then computed the correlation between this metric and the subjected-rated similarity of all odorant pairs. The resultant expected negative correlation (greater distance between odors predicts reduced similarity) was surprisingly strong and significant ($r = -0.73$, $P < 0.01$). We then systematically added eigenvectors (axes of odor space) and found that the correlation reached an asymptote at six principle components ($r = -0.95$, $P < 0.01$), beyond which correlation did not strengthen. Thus odor similarity judgements for our test odorants were very well predicted by a six-dimensional odor perceptual space.

Poster: Human Sensory Perception

Adaptation and identifying components of olfactory mixtures in humans

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Humans have a limited ability to identify odor qualities of individual odorants presented in multi-component mixtures (Livermore and Laing, 1996, 1998). Very little is known about the effects of adaptation on odorant mixture perception. We tested whether identification improves after selective adaptation to components of mixtures. The four components (labels) were 1 M isopropyl alcohol (alcohol), 5 mM vanillin (vanilla), 0.5 mM phenethyl alcohol (rose) and 1 mM L-menthol (mint). Trained subjects ($n = 14$, 18–35 years of age) were presented with pairs of stimuli to sniff, and identify from a list of labels. Tests were done on separate days either before (control) or after a 5 s adaptation to odorants containing 0–3 components. As the mixture became more complex, identification became more difficult ($P = 0.0001$). However, a target stimulus within a mixture was easier to identify after adaptation to non-target stimuli ($P = 0.0001$). Improvement in the ability to recognize a component in a mixture after non-target adaptation was greater the more complex the mixture ($P = 0.02$). The accuracy with which the four stimuli were identified before adaptation differed ($P = 0.003$), but the improvement resulting from adaptation to non-target stimuli did not differ. Thus, distinct odors, lost in multi-component mixtures, rematerialize with the same quality after adaptation to other mixture components.

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Poster: Human Sensory Perception

Lifespan changes in source memory for olfactory stimuli

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The present study investigated source and item memory for odors in children (7–10 years), young adults (18–24 years) and older adults (65+). During the study phase, one male and one female experimenter (sources) randomly presented either 16 odors or 16 objects (control stimuli) to the participant. Presentation alternated between sources so that each source presented eight stimuli. Once the 16 stimuli were presented, the sources exited and a third experimenter began the test phase. To assess item recognition memory, a stimulus from the study phase and a novel stimulus were presented to the participant who was asked to choose the stimulus presented during the study phase. The experimenter presented a stimulus and asked whether the male or female experimenter had presented the stimulus during the study phase. No significant differences were detected in item memory for odors or objects in all age groups. However, results indicated that source memory for odors was poorer in children and older adults than in young adults. Children and older adults demonstrated a similar magnitude of impairment in source memory for odors. It has been suggested that the frontal lobes play a critical role in source memory and odor memory; a brain region that continues to develop throughout childhood and degenerates in older adults. Therefore, source memory for odors may be particularly affected in children and older adults because of immaturity of the frontal lobes in children and age-related changes in the frontal cortex in older adults.

Poster: Human Sensory Perception

Transformations in odor percept identity as a function of intensity

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Anecdotal evidence suggests that some odorants, such as indole, undergo a dramatic shift in percept when sampled at high versus low concentrations. Such a concentration-dependent transformation is not only an hedonic transformation, but also a transformation in percept identity. We set out to characterize potential changes in odor percept, using the odorants indole and vanillin. We first used a maximum-likelihood adaptive staircase to determine subjects' thresholds for the detection of indole diluted in mineral oil and for vanillin diluted in water. We then provided each subject with the concentration step in the series that was one step higher than the mean threshold for each odorant. Each subject performed an odor quality evaluation on the sample provided using the 146 descriptors from the Dravnieks Atlas of Odor Character Profiles. Following quality evaluation of this low intensity sample, each subject repeated the evaluation on a high intensity sample. A Kolgorov–Smirnov goodness-of-fit test showed that ratings of low intensity indole versus high intensity indole were significantly different ($P < 0.005$), whereas those of low-intensity vanillin versus high-intensity vanillin were not ($P = 0.2$). A Wilcoxon test revealed that the effect of concentration on percept was different for vanillin and indole ($Z = 2.15$, $P < 0.03$).

Poster: Human Sensory Perception

The responsiveness of fMRI signal to odor concentration

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The purpose of this study was to examine fMRI signal in response to different odor concentrations. This is essential for the study of human olfaction and associated diseases. The odor concentrations used for fMRI studies were first determined psychophysically outside of MRI scanner. The subjects were asked to rate odor intensity/pleasantness as three different concentrations of lavender (0.032, 0.10 and 0.32%) were presented in a randomized order with an olfactometer. The fMRI studies were performed with participants (n = 8, age = 27.3 years, 5 female) using an olfactory intensity paradigm on a 3T Philips MRI. The UPSIT was administered to participants to determine olfactory ability. During the study, respiration of the subjects at the rate of 10 cycle/min was controlled by auditory prompt to synchronize odor administration and image acquisition (T_R = 3s). Each odor concentration was presented during inhale period (3 s) of the respiratory circle followed by a 45 s resting period with three repetitions. The above three odor concentrations were presented from the weakest to the strongest. The perception of odor strength was validated with a post-study questionnaire. Statistical analysis comparing the activation from different strengths revealed significant bilateral activation differences in the primary olfactory cortex and insula. These findings establish the groundwork for understanding the unique hemodynamic response to odor concentration, which is important for studying olfactory deficits and neurodegenerative diseases, such as AD.

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Poster: Human Sensory Perception

Emotional familiarity and the detection of emotional chemosignals

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Previous research shows that humans can distinguish between chemosignals from different emotional states (Chen and Haviland-Jones, 2000; Ackerl *et al.*, 2002; Chen and McClintock, in preparation), and that experience enhances sensitivities to nonsocial odors (Dalton *et al.*, 2002) and emotional chemosignals (Chen and McClintock, in preparation). In the present study, we further examined factors that influenced the detections of human chemosignals. Female participants evaluated chemosignals collected from their husbands/boyfriends, as well as from unfamiliar male and female donors. The sweat samples were collected from the underarm regions while the donors were under neutral and emotional states of happiness and fear. Female participants were asked to identify the different smell on triple-forced choice tasks where happiness (or fear) served as the target and the neutral state as the control. Preliminary analysis shows that the proportion correct for the detection of happiness in husbands/boyfriends surpassed that in male and female strangers in six out of nine subjects, and in eight out of nine subjects for fear. Our results provide further support that experience enhances detections of emotional chemosignals, and shed light on the role of physical and emotional familiarity in this context.

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Poster: Human Sensory Perception

Characterization of fMRI hemodynamic response in human central olfactory system

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The hemodynamic response to odors is significantly different from those by other sensory stimuli because of a rapid habituation. Characterization of this phenomenon unique to olfaction is important for olfactory fMRI paradigm design and data analysis. We performed an olfactory fMRI study with a paradigm designed specifically to investigate this question. Six healthy young subjects participated in the study. The subjects were trained to breathe following audio instructions to synchronize the respiration with odorant delivery and image acquisition to ensure accurate onset and duration of stimulation. The respiration was closely monitored. During fMRI, lavender odor was delivered to the subject's nostrils through Teflon tubing at strength of 0.32% and flow rate of 8 l/min. After a 60 s baseline, the stimulation lasted for 21 s and then repeated for three times which interleaved with 45 s recovery periods. Statistical analysis with standard hemodynamic model detected activations in primary olfactory cortex and other secondary brain structures (i.e. orbitofrontal cortex, etc). However, when the hemodynamic response was modeled with different length of stimulations (12 s, 18 s and 21 s), the shorter the stimulation yielded stronger activation map under the same confidence level ($P <$ 0.001), indicating a rapid decay of hemodynamic response after onset. These data suggested that (i) the current hemodynamic model obtained form other sensory stimuli may not fit olfaction and a habituation factor must be incorporated in the model; and (ii) olfactory stimulation paradigm should be built based on brief stimulations with precise synchronizations of data acquisition and respiration.

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Poster: Human Sensory Perception

The effect of magnetic field on olfaction

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Functional magnetic resonance imaging is increasingly used as a tool to elucidate the neural substrates of olfaction. However, it is possible that the magnetic field used in functional imaging may itself affect olfaction. It is known that the direction and strength of a magnetic field have an effect on visual perception tasks. Likewise, the strong magnetic field of an MRI scanner can induce phantom gustatory perception. Anecdotal observations in our laboratory suggested that olfactory intensity perception was enhanced under a strong magnetic field. To address this possibility, we employed the University of Pennsylvania Smell Identification Task (UPSIT), administering it to 20 subjects in and out of a magnetic field (4 Tesla Varian). In addition to identification, subjects rated stimulus intensity and pleasantness on a visual analog scale. There was no effect of the static magnetic field on the subject's ability to accurately identify the odor

($P = 0.3359$). However, in the 4 T static field, odors were perceived as both more intense ($P = 0.0023$) and pleasant ($P < 0.0001$). To further probe this effect, we are now parametrically varying the magnetic field to 4, 2 and 0.5 T, while delivering odorants with an air dilution olfactometer. The parametric effects on perception of odorant intensity and pleasantness will be assessed, and discussed in light of possible mechanisms underlying this effect.

Poster: Human Sensory Perception

Inhibition of bitter taste by adenosine 5′-monophosphate in non-tasters and supertasters of PROP

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The perception of bitterness is complex; a wide range of structurally divergent compounds are bitter, and human sensitivity to bitterness varies markedly. Two techniques may be useful for exploring these differences. First, the ability to taste 6-*n*-propylthiouricil (PROP) is an inherited trait that may predict sensitivity to other bitter substances. Second, the common nucleotide adenosine 5′-monophosphate (AMP) inhibits bitter taste signaling pathways *in vitro* and may inhibit human bitter perception as well. This study examined the association between PROP status and bitterness perception, and determined the efficacy of AMP as a bitterness inhibitor. Healthy adults were classified as non-tasters (NT; $n = 30$) or supertasters of PROP (ST; $n = 30$) using a filter paper method. Subjects rated suprathreshold concentrations of caffeine, epicatechin, L-phenylalanine, naringin, PROP and quinine. Na-AMP was used at 0, 0.01 and 0.02 mmol/l in each stimulus series. All compounds were more bitter to ST than to NT across concentrations ($P < 0.02$–0.001) except for quinine, which was more bitter to ST only at the highest concentration ($P < 0.01$). AMP reduced the bitterness of caffeine at all caffeine concentrations ($P < 0.01$) by an average of 26.4%. This effect was not AMP dose-dependent and was similar for NT and ST. AMP at 0.02 mmol/l reduced the bitterness of concentrated quinine. These data demonstrate that PROP status is associated with greater bitterness perception in all but one of the compounds tested (quinine) and that AMP is an effective bitter blocker in caffeine with limited efficacy in quinine.

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Poster: Human Sensory Perception

The interaction between evaluative and passive response to taste in the human brain

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The goal of this study was to assess whether cognitive and affective evaluation of taste would influence brain response compared with passive tasting. Subjects were presented with tastants or tasteless blanks and required to perform one of four different tasks while being scanned with fMRI: detection (D—'is there a taste'), identification (ID—'what is the taste'), affective judgement (AJ—'how pleasant is the taste'), and passive tasting (PT—'randomly press'). Comparison of taste versus tasteless activated insula and opercular regions in ID, AJ and PT but not D. The effect of task on response to taste and tasteless were analyzed separately. Irrespective of whether the subject was receiving a taste or tasteless solution, all three tasks (D, ID, AJ) activated the cerebellum relative to PT. Task affected response to taste and tasteless differently. When tasting, a hierarchy of activation was observed such that performing D recruited no additional areas, ID recruited the insula/operculum and anterior cingulate cortex and AJ recruited the insula/operculum, anterior cingulate cortex and midbrain. These additional responses were not observed with tasteless. These findings show that evaluation interacts with the gustatory response. They also accord with previous studies of olfaction in providing evidence for a role for the cerebellum in active exploration of sensory experiences (Sobel *et al.*, 1998; Savic *et al.*, 2000; Zelano *et al.*, 2004).

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Poster: Human Sensory Perception

Differential brain patterns during intensity and pleasantness evaluation of taste stimuli

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A few studies have investigated the correlation between taste brain activations and perceived intensity or pleasantness, but the physiological substrate of the evaluative task itself is unknown. The present study examined differences in brain activation evoked by intensity and pleasantness evaluation of the same stimuli. Subjects participated in two event-related functional MRI sessions, during which they were presented with six taste stimuli randomized within and between runs. Each session was composed of two runs of 24 min each: during one, subjects evaluated the intensity of the stimuli and during the other, they evaluated their pleasantness. Subjects made ratings on Labeled Magnitude Scales by pointing to a screen with a joystick. Results showed that evaluating intensity of taste stimuli produced activation in ventral insula, thalamus, superior and medial frontal gyrus, rolandic operculum and left medial/posterior dorsal insula. Pleasantness evaluation produced activation in postero lateral orbitofrontal cortex, medial frontal gyrus, ventral insula and anterior dorsal insula. Orbitofrontal activation may relate to the reward/motivational value of the stimuli. Activation in the rolandic operculum and medial/posterior insula may relate to more pronounced activation of primary regions during intensity evaluation, as previously suggested in the case of olfaction.

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Poster: Human Sensory Perception

The impact of aroma on perception of age

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While the determination of age has been primarily mediated visually, the ambient odor may also have an influence. To determine this, thirty-seven subjects (age average of 28 years, ranging from 13 years to 71 years) in a single blinded, randomized fashion, estimated the age of models in 20 photographs while wearing either blank masks or masks impregnated with a grape, cucumber or grapefruit aroma. The grape odor ($P = 0.198$) and the cucumber odor ($P = 0.244$) had no significant effects. The grapefruit aroma reduced subjects' perception of overall models' ages by an average of ~3 years ($P = 0.025$) and of female models' age by 5 years ($P = 0.053$). Possible mechanisms whereby the grapefruit aroma created a rejuvenating effect with relationship to perceived age includes induction of positive affect, sexual arousal, anxiolysis, change in cognitive set and odor-induced visual distortion. The perceived rejuvenating effect of the grapefruit aroma may have utility in facilitating intergenerational communication or act as an adjuvant like botulism toxin, in cosmetic and pharmaceutical usage to impact perception of age.

Poster: Human Sensory Perception

Children's hedonic response to alcohol odors are related to parental drinking and smoking habits

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Odors can acquire specific meaning early in life which can persist throughout the lifetime. The present study focused on 292 children aged 3–8 years to determine whether the hedonic response to odors was related to the drinking and smoking habits of their mothers. Age-appropriate tasks were used to assess children's liking, identification and preference for a variety of odors ranging in hedonic valence and familiarity, one of which was the odor of beer. The children's mothers completed a variety of questionnaires to assess their cigarette and alcohol use and answered questions about their reasons for drinking to determine an index of the extent to which individuals consume alcohol to change their state of mind or reduce dysphoric feelings or both, hereafter referred to as 'escape drinking'. Preliminary analysis revealed children whose mothers are escape drinkers and smoke cigarettes were significantly more likely to dislike the odor of beer when compared with children of mothers who were non-escape drinkers. Such findings are consistent with our previous research and suggest that early learning about alcohol is based on sensory experiences at home and anchors it to the emotional context in which their mothers drink and smoke.

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Poster: Human Sensory Perception

More than hedonics: cognitive measures impacted by some odors

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In a study investigating the influence of synthetic odor on style of writing personal events, 60 men and 60 women were exposed to a no-odor control or to peri-threshhold odors, either naturally representative (e.g. gardenia) or created (e.g. fine fragrance). Those in the 'natural' group had higher Flesch–Kincaid (FK) scores ($F = 3.25$, $P < 0.04$) and used more positive emotion words ($F = 3.72$; $P < 0.03$). Use of cognitive words (e.g. 'thought') was correlated with FK scores ($r = 0.29$, $P < 0.04$). Word counts (productivity) did not differ by odor group. Only in the control group did women write more than men ($F = 4.75$, $P < 0.01$). Women used more depressive words ($F = 5.41$, $P < 0.02$), but there was no odor effect. Self-reports of emotionality or vividness were not affected by odor. Considering our previous studies, different types of hedonically pleasing odors may affect emotional or cognitive processes independently.

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Poster: Human Sensory Perception

The molecular basis of individual recognition scents used in different contexts

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Two highly polymorphic gene complexes contribute to individual scents of rodents, although the components used for individual recognition depend on context. MHC odours are important in mate choice, both for the selection of mates of dissimilar MHC type and for recognition of familiar mates required to maintain pregnancy after fertilization. The major urinary proteins (uMUPs) define individual scent mark owners in the context of competitive signaling among males, but the role of MHC type in individual recognition among competitors was not known. We tested this by examining territory owner responses (i) towards urine according to the scent owner's genetic difference to the territory owner (MHC, genetic background, both, none) or genetic match to a familiar neighbour; and (ii) during interaction with a cage-mate, a genetically identical male, a male of different MHC or a male with a different genetic background. Urine of different genetic background always stimulated greater scent marking than own while that of different MHC type failed to stimulate countermarking. MHC odours stimulated increased investigation only when the scent matched both MHC and genetic background of a familiar neighbour. In a competitive context, MHC-associated odours are neither necessary nor sufficient for scent owner recognition. However, MHC odours do stimulate close contact investigation when scents belong to a familiar competitor. We should not be surprised that the scent advertising individuality is the product of multiple genes, nor that the gene clusters contribute differently to 'individual recognition' according to context.

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Symposium: Presidential Symposium: Obesity: Biological Determinants of Ingestive Behavior

Hormones and neuropeptides involved in energy balance

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The increased incidence of obesity in the world is occurring simultaneously with an explosion of new knowledge on the neurobiological controls over food intake. This overview will cover where and how several categories of chemical messengers related to energy homeostasis are sensed, and how they are integrated to influence an individual's tendency consume more or less food. An important point is that biologic controls are exerted on meal size rather than on when meals occur since the latter are based more on learning and opportunity. Two types of signals are key to meal size. Satiety signals, like cholecystokinin or CCK, are secreted from the GI tract and indicate the quantity and quality of calories consumed. They act on receptors on sensory nerves that project to the hindbrain. Increasing (or decreasing) their activity prior to a meal reduces (increases) meal size. Adiposity signals (leptin and insulin) circulate in direct proportion to body fat and are passed through the blood-brain barrier to stimulate receptors mainly in the ventral hypothalamus. Increasing (or decreasing) their activity in the brain reduces (increases) body weight. Transmitters in the ventral hypothalamus include those that are mainly catabolic (e.g. aMSH) or anabolic (e.g. NPY), and these are directly stimulated by adiposity signals and local nutrients (glucose and lipid), and their output is integrated with satiety signals. When an individual gains (loses) weight, adiposity signals increase (decrease) and the control system develops increased (decreased) sensitivity to satiety signals. Smaller (larger) meals are then consumed until weight is restored.

Symposium: Presidential Symposium: Obesity: Biological Determinants of Ingestive Behavior

Neural mechanisms controlling food intake and body weight: mind versus metabolism

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A leptin-sensitive neural network centered in the hypothalamus has been identified as the homeostatic control system for the regulation of food intake and energy balance. While this system is remarkably powerful in defending the lower limits of adiposity, it is weak in curbing appetite in a world of plenty. Another extensive neural system that processes appetitive and rewarding aspects of food intake is mainly interacting with the external world. Polymodal sensory representations of foods, food cues and their specific reward expectancies are acquired, stored and recalled in circuits including areas of the prefrontal cortex, amygdala and ventral striatum. Gustatory, visual and olfactory signals converge on single neurons in areas of the orbitofrontal cortex and basolateral amygdala after passing through processing steps in the respective sensory channels. The amygdala is particularly important for learning stimulus–response and response–reinforcement associations, the orbitofrontal cortex for maintaining such associations, and the nucleus accumbens for translating them into goal-directed behavior. This non-homeostatic system is constantly attacked by sophisticated signals from the environment, ultimately resulting in increased energy intake in many genetically predisposed individuals. Identification of the neural pathways that mediate this dominance of cortico-limbic processes over the homeostatic regulatory circuits in the hypothalamus and brainstem will be important for the development of behavioral strategies and pharmacological therapies.

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Symposium: Presidential Symposium: Obesity: Biological Determinants of Ingestive Behavior

Chemosensory controls of food intake

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Obesity results when energy intake exceeds energy expenditure. The wide availability of highly palatable and energy-rich foods is one factor thought to promote overeating. Food palatability is determined by innate taste biases (e.g. sweet tooth) and learned flavor (taste, odor, texture) preferences. In rodents, the post-oral actions of nutrients (carbohydrate, fat, protein) condition strong and persistent flavor preferences. This is revealed in studies that pair the intake of flavored solutions with intragastric nutrient infusions. Post-oral nutrient feedback can reverse innate taste aversions and overcome genetic difference in taste preferences. Some but not all learned flavor preferences are associated with increased hedonic reactions and consumption. Nutrients differ in their ability to reinforce flavor preferences which suggests the involvement of specific post-oral chemosensors rather than a 'calorie' detector. Consistent with this idea, nutrient conditioning occurs in food-sated as well as deprived animals and with hypocaloric nutrient infusions. Little is known about the visceral chemosensors, including recently identified taste signaling proteins, that mediate post-oral nutrient conditioning. Flavor–nutrient conditioning has been reported in children and adults, but its overall importance to human food preferences and obesity remains to be established.

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Symposium: Presidential Symposium: Obesity: Biological Determinants of Ingestive Behavior

Reward mechanisms and hedonics of feeding behavior

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Animals eat for a variety of reasons including energy needs, time of day, social interactions, stress and reward. The rewarding properties of food motivate animals to seek food and to ingest more food than is needed to maintain a non-obese body weight. Many neuroactive compounds and brain regions are involved in reward-induced consummatory behavior. Endogenous opioids are amongst the most studied neuroregulators thought to be involved in food-related reward. These substances are synthesized or have receptors in many brain regions associated with hedonics and/or energy regulation, including the hindbrain, the parabrachial nucleus, hypothalamic sites, the amygdala, the ventral tegmental area and the nucleus accumbens. Early publications suggested that opioids selectively induced intake of fat; however, other data suggest that opioid

antagonists are particularly potent in reducing intake of any preferred diet, independent of its macronutrient composition. In addition to opioidergic pathways, dopaminergic circuits are involved in consumption of highly palatable nutrients, including fat and sugars. Consumption of a palatable food has been shown to release dopamine in the shell of the nucleus accumbens and systemic blockade of mu_1 opioid receptors blocks this food-induced dopamine release. A good deal of data has been collected indicating that there is a neural network involving opioids, dopamine and other neuroregulators located in multiple brain sites that integrates eating motivated by the rewarding qualities of food.

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Poster: Olfactory & VNO Receptors

Functional analysis of a stably expressed human olfactory receptor

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Recombinant expression of G protein-coupled receptors (GPCRs) in heterologous systems is widely used for high throughput screening of ligands. Heterologous expression of olfactory receptors (ORs) is different to other GPCRs since the recombinant receptors are not readily transported to the cell surface. Consequently, the percentage of functional ORs that are accessible to odorants is low. In the present study, the well-characterized human OR17-40 was chosen to investigate different *in vitro* expression systems. Transient expression of OR17-40 was compared with a system where the OR protein was stably and constitutively expressed. An inducible expression system was used to study the influence of OR17-40 expression levels on the receptor activation of ligands. The same expression systems were used to examine changes of the receptor activation profiles over time dependent on their OR17-40 expression levels. Cryopreservation of different OR expression systems was also investigated. This showed that the presence of functional OR protein after thawing depended strongly on OR expression levels in the cells. Based on these data, OR17-40 was stably expressed in a heterologous system, resulting in a robust *in vitro* expression system for ligand screening. Activation of the receptor with odorant molecules was studied by monitoring fluxes of the internal calcium concentration in a microtiter plate format. Ligand specificities of OR17-40 were consistent with data in the literature and receptor activation by agonists occurred in a dose-dependent manner. A series of structurally related molecules was tested for their ability to activate OR17-40.

Poster: Olfactory & VNO Receptors

Bombykol and bombykal receptors in the silk moth, *Bombyx mori*: a molecular mechanism of sex-pheromone reception

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Sex pheromones released by adult female moths are detected by narrowly-tuned olfactory neurons in the conspecific male antennae. The silk moth, *Bombyx mori*, is a perfect model for the pheromone study because of the simplest pheromone communication system, wherein a single chemical compound, called bombykol, elicits the full array of sexual behaviors. We herein show that two male specific olfactory receptors (ORs), BmOR1 and BmOR3, are mutually exclusively expressed in a pair of adjacent pheromone sensitive neurons in male antenna and are coexpressed with a highly conserved OR family receptor, BmOR2. Two voltage clamp recordings of *Xenopus* oocyte expressing these ORs demonstrated that BmOR1 was specifically tuned to bombykol, while BmOR3 was a specific receptor for bombykal, an oxidized form of bombykol. Coexpression with BmOR2 promotes the functional expression of BmOR1 and BmOR3, and confers pheromone-stimulated cation channel activity. The same ligand-stimulated cation current was also observed when general ORs in *Drosophila* were coexpressed with Or83b or BmOR2. These results implicate that both odorant and pheromone signaling pathways are mediated via a common mechanism in insects.

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Poster: Olfactory & VNO Receptors

Ectopic expression of olfactory receptor genes in heterologous tissues

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Although olfactory receptors (ORs) have their main function in olfactory epithelium, ectopic OR expression has been reported in several non-olfactory tissues, including testis. We analyzed database transcriptional levels for hundreds of human and mouse OR genes. Olfactory epithelium had a fairly high summated OR expression level, with most ORs roughly equal. In contrast, tissues such as mouse thyroid and salivary gland had very high ectopic expression in a small (10–30) 'highlight' gene subset. At least in some cases (e.g. OR51E2/ PSGR, previously reported as prostate-specific) this may result from genomic co-localization. Testis showed neither appreciable summated OR expression, nor a substantial number of OR 'highlights'. Only ~10% of the ORs previously reported as expressed in human male germ cells showed here enhanced tissue transcription, and these did not include ORs previously implied in sperm chemotaxis. The OR ectopic expression patterns are consistent with a neutral expression model, controlled by random drift, independent of functionality. This is supported by the complete lack of correlation in microarray tissue expression between mouse and human and by the similar ectopic expression seen for OR genes and pseudogenes. Considerable care should thus be exerted when offering a functional interpretation for ectopic OR expression.

Poster: Olfactory & VNO Receptors

Functional expression and characterization of mouse odorant receptors in *Xenopus* oocytes

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We are using *Xenopus* oocytes as a heterologous expression system to allow functional expression and characterization of mouse odorant receptors. The receptors were tagged with the N-terminal 20 amino acids of rhodopsin and coexpressed with $G\alpha_{olf}$ and the cystic fibrosis trans-membrane regulator. Odorant induced current responses were measured under two-electrode voltage clamp. Oocytes expressing OR-S6 receptors responded to application of nonanedioic acid with an EC_{50} of 4.5 ± 1.0μm (mean ± SEM, $n = 8$). To further characterize this receptor, we tested dicarboxylic acids with varying carbon chain lengths. OR-S6 was activated by 100 μm octanedioic acid, decanedioic acid and undecanedioic acid, with response amplitudes that were 37 ± 10, 52 ± 5 and 6 ± 2% of the response to 100 μm nonanedioic acid, respectively (mean ± SEM, $n = 8$). Hexanedioic acid, heptanedioic acid and dodecanedioic acid failed to activate OR-S6. Screening of additional compounds revealed 5-oxononanedioic acid (100 μm) as a ligand for OR-S6 (13 ± 4% of the response to 100 μm nonanedioic acid). We are currently pursuing the expression of additional receptors using the oocyte system. We are also currently testing the role of accessory proteins (RTP1/RTP2/REEP1) in promoting functional expression of odorant receptors in Xenopus oocytes.

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Poster: Olfactory & VNO Receptors

Olfactory responses in the proboscis of *Anopheles gambiae*

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Understanding of the olfactory system of the malaria vector mosquito, *Anopheles gambiae*, could provide a better approach to control olfactory based human host (blood meal) preferences. Recently, 79 olfactory receptor (OR) genes have been characterized in this mosquito. Among these, AgOR7, the anopheline ortholog for a highly conserved subfamily of insect odorant receptors, is thought to play an important role in olfactory signal transduction. AgOR7 is expressed in most of the olfactory neurons in antenna, maxillary palp and the proboscis of female mosquitoes. Furthermore, RT-PCR analyses in this study indiate that several OR genes are also expressed in the proboscis, supporting the hypothesis that this appendage (together with the antennae and maxillary palps) plays a functional role in olfactory responses. Olfactory responses from each T2 proboscis sensillum containing AgOR7 expressing neurons were characterized electrophysiologically indicating that there are narrowly tuned olfactory reponses to human sweat compounds such as butylamine and isovaleric acid further suggestive that a restricted set of ORs are indeed functional in this appendage. Also, the central projection patterns of proboscis neurons reveals arborization to the ventral antennal lobes, which is the initial olfactory processing center in the insect brain. Taken together, these data strongly support the hypothesis that in addition to its primary role in gustatory chemosensory processes the proboscis is the site of an accessory olfactory pathway. It is tempting to speculate that this appendage could detect critical olfactory information from human skin at extremely close range that is critical in the terminal processes of mosquito blood feeding behaviors.

Poster: Olfactory & VNO Receptors

Antibodies recognizing olfactory receptor subtypes

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Olfactory receptors are considered as multifunctional elements of olfactory sensory neurons; they are supposed to mediate the response to adequate odorants and to play an important role in target finding of the axons, and may even contribute to a monoallelic expression of one receptor type per cell. Efforts to explore the features of distinct receptor subtypes have always been hampered by the fact that specific receptor antibodies were not readily available. In the present study, we have tried to generate subtype-specific antibodies. In a first approach, antibodies raised against a peptide, characteristic for the mouse OR37 receptors were assessed. Immunohistochemical analyses of nasal whole mount preparations revealed staining of individual cells; their clustered distribution matched that previously visualized by *in situ* hybridization. In tissue sections analysed by confocal microscopy, intense fluorescence was visualized in the cell body, dendrite and most notably the cilia of individual sensory neurons. In a second set of experiments, antibodies generated against mOR256-17 were assessed; this OR-subtype is expressed in the medial zone and *in situ* hybridization suggests it may also be expressed in cells within the cribriform mesenchyme during development. In fact, in distinct developmental stages, immunoreactive cells were visible in the cribriform mesenchyme, in particular the membrane of these cells was labelled. Moreover, the notion that receptor proteins may be present in the axons of olfactory neurons was confirmed; very distinct axon bundles and glomeruli were visualized by the antibodies. These results strongly suggest that the newly generated antibodies may represent useful tools for studying olfactory receptor proteins.

Poster: Olfactory & VNO Receptors

Odorant receptor heterodimerization in the olfactory system of *Drosophila*

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In insects one extremely conserved member of the odorant receptor (OR) family is expressed in nearly all olfactory neurons in addition to the conventional ORs. We used a combination of electrophysiological and biochemical approaches to investigate the role the ubiquitously expressed receptor DOR83b plays in the olfactory system of *Drosophila melanogaster*. Bioluminescence resonance energy transfer experiments and Western blotting were performed to demonstrate that conventional insect ORs can dimerize with DOR83b in a heterologous HEK293 expression system. Moreover, OR heterodimerization had functional consequences in calcium imaging measurements, the threshold dropped and the number of cells responding significantly increased. We reduced DOR83b

expression in the fly by RNA interference and observed, that the amplitude of electroantennograms was reduced compared with control flies. The observed residual responses likely reflect incomplete knockdown of DOR83b mRNA, as suggested by the small residual staining in in-situ hybridization experiments. Our experiments show, that DOR83b forms heterodimeric complexes with other odorant receptor proteins, which strongly increase their functionality. We are currently investigating protein interactions with the conserved C-terminal regions of DOR83b to gain further insight into its function in olfactory signal transduction in insects.

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Poster: Olfactory & VNO Receptors

Structural determinants of citronellic odorant recognition by the human olfactory receptors OR1A1 and OR1A2

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The structural determinants of human olfactory receptors (OR) that are important for odorant recognition have remained obscure, so far. Recently, Man *et al.* (2004) predicted a set of 22 positions that constitute a generalized odorant binding pocket (Man *et al.*, 2004, *Protein Sci.*, 13:240–254) for mammalian OR, based on the notion that amino acid positions that are functionally significant would be conserved in orthologs, but variable in paralogs. However, until now, none of the predicted odorant-interacting amino acid residues have been put to the test in site-directed mutational and functional expression experiments with OR. Recently, we have identified Olfr43 from mouse chromosome 11 as an OR specific for citronellal and related odorants [Shirokova *et al.*, 2004, *J. Biol. Chem.*, December 14 (Epub ahead of print)]. The orthologous gene to Olfr43 in human, OR1A1, and its paralogous gene OR1A2, are located in a well characterized sythenic region on human chromosome 17. We have de-orphaned both human OR, and show that the orthologs Olfr43 and OR1A1 displayed a similar EC50-based odorant profile, centred around citronellic terpenoid structures, but also aliphatic aldehydes. Based on the predictions by Man *et al.*, we have combined site-directed mutagenesis, and functional expression of the two human OR, and their mouse ortholog, with a rhodopsin-based homology model. By this, we identified (i) amino acid residues that are necessary for the responsiveness of the orthologs OR1A1 and Olfr43 toward their odorants; and (ii) amino acid residues that are sufficient to account for differences in the odorant profiles of the paralogs OR1A1 and OR1A2.

Poster: Olfactory & VNO Receptors

Functional expression of sea lamprey olfactory receptors

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Sea lampreys represent the most primitive extant vertebrates and presumably retain more features of the earliest vertebrate ancestor than any other living group. Extensive electrophysiological and behavioral studies indicate that sea lampreys respond to a small number of odorants compared with other fishes, including a single amino acid, several bile acid pheromones and a few steroids. We have used molecular cloning techniques to isolate several candidate olfactory receptors from sea lampreys. Two subfamilies, each with a small number of members, are similar to putative olfactory receptors isolated from the European river lamprey (Dryer and Berghardt, 1998, *J. Neurobiol.*, 37:383–392; Freitag *et al.*, 1999, *Gene*, 226:165–174). A third subfamily, most closely related to vertebrate V2Rs, has also been identified. Here we report the functional expression of putative olfactory receptors in a mammalian cell line (HEK 293 cells) and calcium imaging screening for ligands of these molecules. Two sea lamprey receptor clones, SLOR1 and SLOR2, representing different subfamilies have been tentatively identified as receptors for 3-ketopetromyzanol sulfate, a sex attractant released by mature males, and for 15-alpha-hydroxytestosterone, respectively. By matching ligands with receptor molecules of a known nucleotide sequence, the sea lamprey is one of a very few model systems in which a connection between stimulus identity, nucleotide sequence, cell physiology and behavioral effects can be made.

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Poster: Olfactory & VNO Receptors

Assessing the importance of olfaction for sea turtles by using allelic variation to show selection on olfactory receptor genes

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Little is known about the importance of olfaction in the life of a sea turtle. Behavioral studies have shown that sea turtles can distinguish odors under water and in the air which suggests that olfaction might play a role in locating feeding sites, nesting beaches, and in mating interactions. One way to assess the importance of a system to the life history of an animal is to see how active selection has been on the genes involved in that system. This can be accomplished by assessing the allelic variation of a gene across populations of the animal. Low allelic variation suggests strong selection thus high importance of a gene. By cloning and sequencing OR genes from blood DNA several sea turtle OR genes were identified. Two of these genes showed remarkable conservation between Loggerhead, Leatherback, and Green Sea Turtles. An allelic variation study was conducted on these two genes plus several more of the sea turtle OR genes found. Blood samples were obtained from 20 Atlantic, 10 Pacific and 18 Mediterranean loggerhead plus 18 Pacific green and 18 Atlantic leatherback turtles. Allelic variation for the two conserved genes was zero within populations and extremely low (1–6 amino acids different) between populations and species. Variation was also low in the other genes surveyed, suggesting that olfaction is indeed very important for these animals.

Poster: Olfactory & VNO Receptors

Relationships between transduction pathways and the chemosensitivities of goldfish olfactory receptor neurons

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Fish olfactory receptor neurons (ORNs) are known to use one of two transduction cascades, one which employs adenylyl cyclase (AC)/cAMP, the other phospholipase C (PLC)/inositol 1,4,5-triphosphate (IP_3). Although it is known that different types of fish ORNs express different odorant receptors and G-proteins, whether ORNs with different sensitivities use different transduction mechanisms is unknown. We addressed this question using *in vivo* single-unit recording from 90 goldfish ORNs which we exposed to the AC activator forskolin, as well as a food (amino acids) and two pheromonal odors (sex steroids and prostaglandins). While half the ORNs responded to forskolin in an excitatory manner, half did not respond. No forskolin-insensitive ORN responded to a pheromone, but 31% of them (14 of 45) detected amino acids. In contrast, 62% of the forskolin-sensitive ORNs (28 of 45) responded to either a pheromone or the food odor. To examine whether forskolin-insensitive ORNs employ a PLC/IP_3 pathway we tested the PLC activator imipramine. We found that imipramine-sensitive ORNs only responded to amino acids (4 of 30). These results suggest that goldfish have two types of ORNs, one which uses AC/cAMP and detects either feeding cues or sex pheromones (likely ciliated ORNs), and another which uses a PLC/IP_3 and is specialized for the detection of feeding stimuli (likely microvillar ORNs). *In vitro* assays are planned to test these results.

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Poster: Olfactory & VNO Receptors

Location of ligand binding sites on odorant binding proteins using photoaffinity probes and mass spectrometry

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Previously we have reported that the Asian elephant sex pheromone, *Z*7-dodecen-1-yl acetate is bound to serum albumin (a 68 kDa α-helical protein) and is excreted in this sequestered form in the urine of preovulatory females. In sampling the alkaline urine spots male elephants mix this excretion with acidic trunk mucus releasing volatile pheromone in a pH-mediated fashion that transitions eventually to olfactory receptors in the VNO via the flehmen response. A portion of this free pheromone is 'mopped-up' by copious odorant binding protein (OBP, an 18 kDa β-barrel lipocalin). Binding was demonstrated to both proteins by passive attachment using both a GC-based volatile odorant binding assay and on polyacrylamide gels using a radiolabeled pheromone analogue and autoradiography. We also used covalent attachment to the diazoacetate photoaffinity analogue (*Z*7-dodecen-1-yl diazoacetate) using both cold and tritiated forms. Utilizing archival polyacrylamide gels (between 2 and 5 years old) we have now performed a proteomics analysis on excised Coomassie-stained protein spots of OBP and albumin samples reacted with the diazoacetate analogue and examined for covalently attached adducts with an ion-trap mass spectrometer. Pheromone analogue fragments were detected attached to several tryptic peptides that map isn close proximity to suspected binding site residues based on 3-D homology models of these proteins. This demonstrates the applicability of mass spectrometry to help analyze ligand-protein associations.

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Poster: Olfactory & VNO Receptors

Odorant specific requirement for arrestin function in *Drosophila melanogaster*

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Olfaction largely mediates the strong anthropophilic trait characteristic of the primary malaria vector, *Anopheles gambiae*. The ability to modulate olfactory sensitivity is necessary to detect and discriminate amongst a multitude of odor stimuli. Desensitization of activated odorant receptors has been postulated to occur when arrestins bind to the receptors and inhibit further signaling. A paucity of *in vivo* data on olfactory desensitization prompts use of *Drosophila melanogaster* genetics to investigate the role of arrestins in regulating olfactory signaling pathways. Physiological analysis of peripheral olfactory sensitivity reveals decreased responsiveness to a panel of chemically distinct odorants in flies deficient for arrestin1 (arr1), arrestin2 (arr2) or both. These phenotypes are manifested in odorant- and dose-dependent fashions. Behaviorally, arr2 deficient *Drosophila* display decreased overall mobility whereas arr1 mutants are impaired specifically in olfactory-based orientation towards attractive odor sources. As the olfactory deficits vary according to chemical identity and concentration, they indicate that a spectrum of arrestin activity is essential for odor processing. Arrestin mutant phenotypes are hypothesized to be a consequence of down-regulation of olfactory signaling to avoid cellular excitotoxicity. Importantly, a functional characterization has been elucidated for the *A. gambiae* homologue, AgArr1, via transgenic expression of AgArr1 in $arr1^1$ mutant fruit flies. Taken together, these data clearly indicate that arrestins are required for wild type olfactory function and add another level of complexity to peripheral odor coding mechanisms that ultimately impact olfactory behavior.

Poster: Olfactory & VNO Receptors

Mediators of sperm motility–odorant receptor expression profiles of human sperm

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Fertilization is still one of nature's best kept secrets. Despite a century of research we still lack a comprehensive understanding how mammalian sperm cells navigate inside the female body, locate, and finally fertilize the egg. Recently, we showed that *in vitro* activation of the odorant receptor hOR17-4 by a variety of floral odorants (e.g. bourgeonal) mediates both chemotaxis and chemokinesis in human sperm cells. Given an estimated number of up to 40 different testicularly expressed odorant receptors (ORs), a detailed characterization of further members of this 'unconventional' group of ORs is critical to gain new insight into their role in reproduction. Here, we report cloning, recombinant expression and functional characterization of a novel human testicular OR. Using a combination of imaging and behavioral assays, we show activation of sperm by cognate receptor ligands and describe a specific receptor-mediated motility pattern. Comparative analysis of different OR-induced signaling pathways as well as of cell-specific receptor expression profiles are subject of current research.

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Poster: Olfactory & VNO Receptors

De-orphaning, functional characterization and cAMP signalling of five human V1R-like receptors

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The human vomeronasal organ (VNO) is an anatomical entity which is generally considered to be vestigial or non-functional. However, five potentially functional genes have been identified from human genome searches, coding for receptors that are related to the family of V1 receptors of the apical part of the mouse VNO. These five human receptors have been termed hV1RL1-5, one of which has recently been demonstrated to be expressed in the human main olfactory epithelium (MOE). Since nothing was known about their function, all five human V1RL-receptors had to be addressed as true orphan receptors. We found that four out of five V1RL-receptors are expressed in the human MOE. All five receptors have been rho-tagged, and expressed at the plasma membrane level of HeLa/Olf cells. We screened the human V1RL receptors against a collection of 47 odorants from different chemical classes. We de-orphaned all five human V1RL-receptors, and found that they responded to a variety of odorants in a combinatorial way, thus behaving like olfactory receptors from MOE. Testing chemically related odorants with variable sizes and functional groups, specific odorant profiles can be attributed to all human V1RL receptors. Our findings may support the notion that the human MOE has adopted pheromone perception. V1RL-receptors in the human MOE may function as odorant or pheromone receptors.

Poster: Olfactory & VNO Receptors

DNA structural changes are associated with odor sensing in novel DNA-based fluorescent sensors

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Our laboratory has developed an artificial nose that exploits, in principle, 22 attributes of biological olfaction. This device has the potential to contribute to several fields, including land-mine detection, food-spoilage detection and lung cancer detection by providing a rapid, non-invasive screening tool. One important feature is the use of broadly-tuned sensor arrays to achieve odor discrimination. In the first such experiments of which we are aware, we show that dried, 20–30 base, single-stranded DNA–Cy3 conjugates respond in a broadly tuned, sequence-dependent fashion to odorant molecules. DNA–Cy3 conjugates have the combinatorial potential to provide large arrays of novel sensors. Preliminary work has produced sets of DNA–Cy3 sensors for examination using a variety of techniques including a novel use of DNA microarray technology as a odor sensing tool. Using these sensors, it has been shown that (i) DNA–Cy3 sensors have the necessary properties of sequence dependence and rapid, reversible odor detection to be used as sensors in the artificial nose; (ii) odor detection is concentration dependent; and (iii) DNA tertiary structure, as detected by circular dichroism, in solution, and FRET, in solid-state, are altered by odorant molecules. Based on these and other data, we believe that the change in fluorescence seen with odors is due to odorant molecules altering DNA tertiary structure. Interactions between Cy3, the DNA molecule, and the odorant chemicals thus alter the local environment of the Cy3 flurophore resulting in changes in fluorescent intensity. These interactions are modulated by DNA sequence via mechanisms still under investigation.

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Poster: Chemical Ecology and Social Recognition

The determinants of dominance in crayfish: the role of social communication

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Crayfish commonly modify their behavior in the presence of a conspecific. This is particularly true in the context of aggressive exchanges, where group members form a web of social relationships. Although hierarchical structures are a widespread phenomenon, the dynamic processes which produce them remain poorly understood. In particular, it is the exchange of information during agonistic interactions that give rise or can alter the outcomes of those interactions. In crayfish, dominance is thought to be largely determined by physical superiority, encounters are purely dyadic without incidences of coalitions, fighting behavior is highly stereotyped and the outcome of encounters can be judged reliably. Here we present our recent evidence that shows that dyadic encounters are dependent upon a number of other factors, including the exchange of chemical information during encounters. Results are obtained through behavioral studies and a combination of urine

visualization and DPIV measurements. Factors that influence dominace include the controlled release of urine and the manipulation of sensory information currents. These results provide an initial understanding of the complex associations that exist between different levels of organization, including sensory processes, dispositions for aggression, dominance status and underlying sensory mechanisms.

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Poster: Chemical Ecology and Social Recognition

The intrinsic and extrinsic factors involved in the social behavior of crayfish

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Fighting success and dominance in crayfish depends on a variety of extrinsic and intrinsic factors. Several intrinsic factors improve the chances of a crayfish becoming dominant, such as physical superiority, larger weapons, being in the reproductive form, having a previous positive social experience and having an increased serotonin function. Extrinsic factors that lead to increased dominance include dominance pheromones, the appropriate visual and mechanical signals, and ownership of valuable resources. While it is fairly easy to list the types of factors that influence aggression and dominance relationships between crayfish, there remain many unanswered questions at both the proximate and ultimate level of causation. In particular, it is important to understand how these factors interact to determine dominance within a crayfish. We ran a series of social behavioral trials measuring the dominance status of crayfish under different extrinsic and intrinsic factors. These include the presence of shelters, chemical signals, previous history, level of starvation, and naive controls. The results of these studies show that chemical signals are likely the most important determinant of dominance in crayfish.

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Poster: Chemical Ecology and Social Recognition

Chemosensory signals in stream habitats: implications for ecological interactions

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The combination of varying aquatic habitats and flow regimes creates a complex stimulus environment from which sensory information can be extracted. Previous studies with crayfish in artificial stream settings have shown that altering the temporal and spatial structure of an odor plume modifies orientation behavior. Exposure to more temporally complex odor signals enables crayfish to locate food more efficiently. In order to link these studies to a more natural setting, we examined how odor signals are dispersed in three physically different habitats by simultaneously measuring flow patterns and odor plume characteristics. Flow measurements were taken using an Acoustic Doppler Velocimeter (ADV), and *in situ* odor plume measurements were taken using the Epsilon electro-chemical system. ADV measurements showed that the flow in the gravel and transition areas had more turbulent energy than the sand habitat. These changes in the turbulent flow had profound effects on the fine-scale distribution of the chemical signal. In the sand habitat, the concentration of odor pulses was significantly higher than in the gravel or transition habitats. In addition, the odor pulses had slower temporal characteristics in the gravel habitat than the transition and sand habitats. These results support previous laboratory work indicating that there is habitat specific chemosensory information available for organisms to use.

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Poster: Chemical Ecology and Social Recognition

Social communication and analysis of status specific urine in the crayfish, *Orconectes rusticus*

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Social communication is vital for the formation of social status in crayfish. Communication uses various sensory modalities that play a role in establishing these relationships. In crayfish, chemoreception has been found to play an important role in the development and maintenance of social hierarchies. During an agonistic bout, urine is released from the nephropores and is propelled in the anterior direction toward a conspecific by use of the fan organs (maxillae and maxillipeds). Conspecifics detect chemical signals with two pairs of antennules. Previous work has been done on the frequency and duration of urine releases during agonistic bouts between crayfish. The results show that dominant crayfish have a longer duration and more frequent release of urine than subordinates. There may be differences in the chemical composition of urine in dominant and subordinate animals, perhaps due to intrinsic variability. Status specific urine was collected and stored at –15°C. The active constituents of status specific crayfish urine are separated through the use of dialysis testing and alcohol separation. The social status and previous social experience of crayfish alters the response of conspecifics. Therefore, social status alters the composition of chemicals within urine. Consequently, it appears as if crayfish can alter the behavior of conspecifics by releasing urine during a fight, and that this urine may be a true indicator of social status and fighting ability.

Poster: Chemical Ecology and Social Recognition

Evolution of social communication in flowing systems

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The physics of environments can structure how animals send and receive signals. Habitat specific physics constrain signal transmission and provide a mechanism for evolution of sensory biases. This experiment investigates how the use of chemically-mediated social signals are influenced by environmental fluid dynamics. If environments place constraints upon chemical communication, then we would predict that crayfish would develop behavioral adaptations for effective communication in either the lentic or lotic environments in which they are found. We hypothesize that chemical communication during crayfish agonistic behavior is influenced by the environments they inhabit. *Orconectes rusticus* and *virilis* were collected from stream and lake habitats. Aggression assays were performed under lotic and lentic treatments. Flow rate was 10 cm/s for lotic and 0 cm/s for lentic treatments. Dominant river crayfish were found to spend significantly more time upstream of a subordinate in lotic conditions. Lake crayfish positioned themselves randomly in both treatments regardless of status, as did the river crayfish under lentic conditions. These results support our hypotheses that crayfish living in river habitats are adapted to utilize flow in communication, whereas lake dwelling crayfish are not. Furthermore, the temporal dynamics of the agonistic interactions were influenced by flow. These results add to a growing global theory of communication within an evolutionary framework.

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Poster: Chemical Ecology and Social Recognition

Arginine: a potent prey attractant to predatory newts in mountain streams

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Chemoreception of aquatic animals has been well-studied in the laboratory, but rarely in the field. The California newt, *Taricha torosa*, in natural stream habitats is an excellent model system for exploring behavioral responses to prey odors. Here, we selected 13 amino acids for field bioassays based on their concentrations in prey tissue extracts. Bioassays were calibrated for stimulus dilution by means of fluorescent dye releases and flow-through spectrofluorometry. Moreover, hydrodynamic properties of stream flows were determined using an electromagnetic current meter. Of all amino acids tested, only arginine, alanine and glycine were significantly attractive (relative to stream water controls). These three substances caused free-ranging newts to turn upstream and swim towards the odor sources. Additional experiments showed that arginine was the most effective attractant, evoking plume-tracking behavior at concentrations as low as 10 nM. In subsequent trials, nine arginine analogs were tested, but each compound failed to elicit a significant response. Even subtle changes to the arginine molecule destroyed all bioactivity. For example, addition of a single carbon to the side chain, esterification of the α-carboxyl group, or minor substitutions to guanidinium, eliminated attractant effects. Within its natural habitat, the California newt thus exhibits keen sensitivity and narrow tuning to the free amino acid, arginine, a chemical signal of its prey.

Poster: Chemical Ecology and Social Recognition

A blend of three novel sulfated steroids released by larval sea lamprey functions as a potent pheromone for migratory adults

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The sea lamprey (*Petromyzon marinus*) begins life in freshwater streams, which it then leaves to parasitize lake or oceanic fishes before eventually re-entering streams to spawn. Our previous studies have demonstrated that adult lampreys locate spawning streams using a migratory pheromone released by stream-resident larval lampreys. Last year we reported the isolation of three behaviorally active compounds from larval holding water, one of which was the bile acid, petromyzonol sulfate (PS). Here we report the results of our analyses of the unknowns. The least abundant of these is a novel disulfated steroid with 28 carbons and a molecular weight of 590 amu (590). The other has 34 carbons, two sulfates, and a mass of 704 amu (704). EOG recording from adult lampreys found 590 to be detected at concentrations ranging down to 10^{-12} M, while 704 was detected at 10^{-13} M. Behavioral testing confirmed these sensitivities while EOG cross-adaptation suggested that all three compounds are detected by independent receptor sites. When tested in a two-choice preference maze, 704 alone was less active than the complete (natural) mixture, even when added at concentrations 2.5× higher than natural concentrations. Based on these results we conclude that the pheromone functions as a blend. Experiments are planned to test the precise importance of the blend so that it might be developed for use in sea lamprey control.

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Poster: Chemical Ecology and Social Recognition

Timing and duration of odor exposure is critical for successful olfactory imprinting in sockeye salmon (*Oncorhynchus nerka*)

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The final freshwater stages of salmon homing migrations are governed by the olfactory discrimination of home-stream water. Prior to their seaward migration, juvenile salmon learn (imprint) site-specific odors associated with their home stream, and later use these retained odor memories to guide the final phases of their homing migration. Imprinting is critical for successful homing and salmon that do not experience their natal water during appropriate juvenile stages are more likely to stray to non-natal sites. To determine the critical developmental periods for successful homestream imprinting, we exposed juvenile sockeye salmon (*Oncorhnychus nerka*) to a mixture of imprinting odorants at distinct developmental stages: (i) alevins/emergent fry; (ii) smolt exposure; and (iii) control. The smolt exposure group was further divided into three groups with different

exposure durations (6 weeks, 1 week, 1 day). Successful imprinting was assessed by behavioral testing and EOG analysis of maturing adults. A total of 334 adults were tested for behavioral responses to the imprinting odors in two-choice mazes. Alevin-exposed and 6 week smolt exposure fish spent significantly more time in the odor scented arm, 60.1% ($P = 0.038$) and 58.5% ($P = 0.043$) respectively, compared with control fish that tended to avoid the imprinting odors (45.4%). Fish exposed to odors for 1 week or 1 day as smolts spent the majority of time in the odor arm (52.0 and 50.7%, respectively) but responses were not significantly different from controls ($P > 0.05$). We observed no effect of treatment on EOG sensitivity to the imprinting odor mixture or the individual component odors.

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Poster: Chemical Ecology and Social Recognition

Intraspecific chemical signaling in the sea hare *Aplysia californica*: defensive secretions also contain conspecific alarm cues

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The sea hare *Aplysia californica* releases ink and opaline when attacked by predators. These secretions chemically defend *A. californica* from predators such as spiny lobsters and anemones, facilitating escape upon attack. In addition to modifying predator behavior, ink and opaline also affect conspecifics by functioning as alarm cues. When juvenile *A. californica* are presented with ink or opaline from other individuals, they exhibit alarm behaviors such as head withdrawal, moving away from the stimulus, and escape locomotion. Thus, the release of secretions by a sea hare that has been attacked signals to nearby conspecifics that a predator is nearby and evasive behaviors should be produced. The alarm response is specific to ink and opaline, as neither *A. californica* haemolymph nor shrimp odor elicit the response; however, alarm response is not species specific, as ink and opaline from the congener *A. dactylomela* elicit escape behavior in *A. californica* individuals. Further, *A. californica* respond to ink from the octopus *Octopus bimaculoides* and the squid *Loligunculus brevis*, suggesting that these alarm cues are conserved among ink-producing mollusks. Utilizing bioassay-guided fractionation, we have tracked down the active molecules in *A. californica* ink and opaline that elicit these responses. We have determined that three compounds in both ink and opaline separately elicit alarm behaviors in juvenile conspecifics. We are currently in the process of identifying these molecules.

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Poster: Chemical Ecology and Social Recognition

Neuronal control of antennular grooming in the spiny lobster, *Panulirus argus*

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In decapod crustaceans, the antennules bearing olfactory and chemomechanosensory sensilla are groomed by mouthpart appendages—the third maxillipeds—in a stereotyped behavioral pattern called antennular grooming behavior (AGB). In spiny lobsters, *Panulirus argus*, AGB is elicited by L-glutamate, and this chemosensory response is exclusively mediated by asymmetric setae (AS) that accompany the more numerous olfactory sensilla (aesthetascs) located on the lateral flagella of the antennules (Schmidt and Derby, 2005, *J. Exp. Biol.*, 208:233–248). The aim of this study was to further examine the neuronal control of chemically elicited AGB. In two sets of experiments, we tested the effect on chemically elicited AGB of unilateral removal of either the AS or of the eyestalk containing a major portion of the brain, the lateral protocerebrum. We found that upon unilateral removal of AS, AGB of the affected antennule was virtually eliminated whereas the AGB of the unoperated antennule remained unchanged ($n = 9$). Subsequent unilateral eyestalk removal caused a significant increase of chemically elicited AGB of both antennules independent of their position with respect to the ablated eyestalk. These results support the notion that chemically elicited AGB is likely mediated by the lateral antennular neuropils, since they receive only ipsilateral sensory input and contain antennular motoneurons that usually have no or only sparse contralateral projections (Schmidt and Ache, 1996, *J. Comp. Physiol. A*, 178: 579–604). But the results also show that higher brain centers located in the lateral protocerebrum are involved in the control of AGB.

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Poster: Chemical Ecology and Social Recognition

Does the frequency of occurrence of odorants in the chemical environment determine olfactory sensitivity? A study with acyclic monoterpene alcohols in three species of nonhuman primates

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Using a conditioning paradigm, the olfactory sensitivity of five spider monkeys, three squirrel monkeys and three pigtail macaques for six acyclic monoterpene alcohols was assessed. Geraniol, nerol, linalool, citronellol, myrcenol and lavandulol were chosen as these odorants have been shown to differ markedly in their frequency of occurrence in plant odors, allowing us to address the question whether abundance in the chemical environment is a determining factor for a species' sensitivity to a given odorant. We found (i) all three primate species to have a well-developed olfactory sensitivity for acyclic monoterpene alcohols; (ii) that the squirrel monkeys were significantly more sensitive for members of this class of odorants than the other two species, and were able to detect all six odorants at concentrations below 0.1 p.p.m.; and (iii) a lack of positive correlations between olfactory sensitivity in the three primate species, and the abundance of the acyclic monoterpene alcohols in flower odors and etheric oils. Thus, the present results do not support the hypothesis that a species' olfactory sensitivity for members of a given chemical class may be related to the frequency of occurrence of such odorants in its chemical environment. Rather, they suggest that differences in the behavioral relevance of odorants and/or differences in the degree of structural similarity may account for both within- and between-species differences in olfactory sensitivity.

Poster: Chemical Ecology and Social Recognition

Increased conspecific and heterospecific vocalizations in response to an avian social odor

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The crested auklet (*Aethia cristatella*), a seabird with a citrusy odor, co-occurs with its smaller, unscented relative, the least auklet (*A. pusilla*). Both nest and interact in deep, rocky crevices. In the dark, sub-surface habitat: (i) odor and vocal cues may be emphasized; and (ii) the larger crested auklet may be a superior competitor. We recorded vocalizations of crevice-dwelling adults, as each was exposed to a randomized series of three scent tests: (i) synthetic crested auklet odor; (ii) banana odor (a novel scent); and (iii) ambient air (control scent). To avoid disruption, we inspected each crevice once (at the end of the three-scent series) to assess if it was vacant or if a bird had remained in place for all tests. In both species, birds that had vacated crevices by the end of the test series tended to vocalize longer in response to crested auklet scent, compared with those that remained ($14 \leq df \leq 21$, $1.96 \leq t \leq 1.84$, $0.039 \leq P \leq 0.079$). We detected no such pattern for banana scent ($P > 0.30$). We also found a general difference between species: least auklets vocalized longer than crested auklets, regardless of scent treatment ($25 \leq df \leq 27$, $2.69 \leq t \leq 2.99$, $0.0061 \leq P \leq 0.011$). Our data suggest that auklets prone to vacating crevices were more vocal toward crested auklet odor than those that remained. Male crested auklets often eject crevice occupants aggressively. For birds prone to vacate, crested auklet scent may signal the approach of an aggressor. Our conclusion is consistent with captive data in which odor correlates positively with male aggression in crested auklets. For least auklets, our data are the first to suggest that birds may employ heterospecific odor recognition.

Poster: Chemical Ecology and Social Recognition

Chicken embryos are capable of habituating to an avian social odor

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Although chemosensory learning in embryos occurs in every class of vertebrate, including birds, details of how the process works are unclear. Olfaction in domestic chickens (*Gallus domesticus*) is thought to begin on embryonic day (ED) 19, when a chick pips the egg's air sac and begins breathing. However, olfactory receptors are capable of functioning even earlier (on ED13). We tested whether chick embryos exhibited chemosensory learning prior to ED19. We incubated 18 eggs from ED10 to ED18 in scented air containing *cis*-4-decenal and octanal, key compounds of a social odor from crested auklets (*Aethia cristatella*; Hagelin *et al.*, 2003). An identical control group was not exposed to auklet odor. Prior to air sac pipping (on ED 18) we quantified the behavioral responses of each embryo to three scents [auklet odor, mint (a novel scent), and deionized water]. Embryos responded more to auklet and mint odors than to water. However, embryos incubated in auklet odor also exhibited fewer 'high magnitude' behavioral responses (e.g. kicks, body movement, turning head) when tested with auklet scent, compared with controls ($Z = -2.11$, $P = 0.035$). We conclude that chemosensory learning, in the form of habituation, can occur in avian embryos before they breathe. Further, compounds in crested auklet social odor have the potential to affect avian chemosensory learning prior to hatching. The behavioral habituation we observed is consistent with familiar odors causing reduced distress in newly hatched chicks. Our methods provide a means for future studies to quantify chemosensory responses in bird embryos during different stages of development.

Poster: Chemical Ecology and Social Recognition

Social behavior and odor function of a tangerine-scented seabird

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The social function of tangerine scent in crested auklets (*Aethia cristatella*) is unknown. We monitored a captive population at the Aquarium of the Pacific in Long Beach, CA for two breeding seasons. Specifically, we investigated the relationships between sex, social rank and two odor compounds thought to play a role in ectoparasite resistance (decanal and *cis*-4-decenal; Douglas *et al.*, 2004). We also tracked the odor of birds over time to determine whether scent declined at the end of the breeding season, coincident with the loss of other secondary sexual ornaments. Intra- and inter-sexual aggression and courtship revealed that the auklets maintained a stable, linear hierarchy; social ranks remained remarkably consistent between years ($n = 14$, $F = 28.6$, $P = 0.0002$). Decanal correlated positively with male rank ($n = 5$, $F = 21.16$, $P = 0.019$) during 2003 only. Neither compound correlated with female rank in either year ($P > 0.18$). Odor declined significantly in the population by the end of the breeding season (decanal: $n = 10$, $Z = -2.85$, $P = 0.004$; *cis*-4-decenal: $n = 10$, $Z = -2.86$, $P = 0.004$). A positive relationship between male rank and decanal concentration in 2003 is consistent with decanal acting as a male indicator trait; ectoparasites are known to find decanal aversive in a dose-dependent fashion. End-of-season scent loss also suggests that odor behaves similarly to other secondary sexual traits and may serve as an olfactory ornament. Auklet displays are multimodal and involve many ornaments. Like other ornate birds, auklets may have the flexibility to shift between traits, focusing on some more than others, depending on season or circumstance.

Poster: Chemical Ecology and Social Recognition

The influence of predator odors on maternal behavior of rodents

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Risk of predation can significantly disrupt the behavior of potential prey. Chemosensory detection may be an important aspect of predator avoidance strategy for many mammals. In our earlier studies we examined the influence of predator chemical cues derived from feral cat urine on reproductive output of rodents: rats, mice and voles. Animals responded to predator chemical cues with reduced litter size and skewed sex ratio. The reduction in litter size in rodents exposed to predator urine was attributable to suppressed progesterone levels affecting the implantation of embryos. Exposures of mice (*Mus musculus*) to urine from feral cats (*Felis catus*) under semi-natural conditions significantly affected survivorship of offspring. We tested the hypothesis that predator chemical cues may disrupt maternal behavior in rats and mice. Exposures of mice and rats to cat urine during pregnancy did not affect the time of retrieval of pups by females 3 days after litter drop when pregnant females were housed singly. Also it does not affect the rate of infanticide in rats and mice towards their own pups. At the same time, exposures to cat urine significantly ($P < 0.001$, $n = 40$) increased the rate of infanticide in mice directed to litters of other females when animals were kept in enclosures. In rats (*Rattus norvegicus*) we observed the same effect under laboratory conditions when pregnant females were housed 3–4 per cage ($P < 0.001$ $n = 42$).

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Poster: Olfactory Behavior

Olfactory recognition in canines

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To assess the nature of olfactory cues canines use in identifying humans, a retriever was trained to recognize a target's scent. After smelling three boxes containing T-shirts impregnated with human scent, the dog would exhibit a sit/stay response on recognition of the target. The distracter scents were from humans not related to the target. The dog was trained to a 95% identification criterion with correct responses rewarded only 90% of the time. After training, probe trials were conducted in which the scents from the target's relatives or from humans who bathed in the target's soap were used in place of the target's smell. Probe trails were inserted into a testing session ~20% of the time (the other 80% were the target recognition trials). Responses to probe trials were never rewarded. Although the dog did not give the recognition response above baseline for any of the target's family members, she gave the recognition response more often when some but not all non-related humans used the target's soap. To determine if added smells could reduce target identification, a T-shirt was collected after the target used a different soap. The dog did not recognize the target with this new added smell. Later, when the target returned to the original soap, the dog again made the appropriate identification. Since genetic similarities did not produce identification confusions, these results support the hypothesis it may be relatively easy for the dog to separate humans based on smells related to genetically controlled metabolic factors. On the other hand, added smells like those from bath soap can produce identification confusions at least with this testing paradigm.

Poster: Olfactory Behavior

Behavioral consequences of noradrenaline signaling blockade in the main olfactory bulb of adult mice

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In rats, the role of noradrenaline (NE) signaling in the main olfactory bulb (MOB) has been characterized for early preference learning, a behavior where neonatal rats learn to prefer an odor associated with stroking. Blocking β adrenergic receptors in the MOB blocks early preference learning. Adult rodents utilize a more complex neural system allowing for increased behavioral flexibility. Thus, the role of NE modulation in the MOB would be expected to take on a subtler role in a behavioral context. This study's objective was to determine the behavioral consequences of NE signaling blockade in the MOBs of adult mice performing two-odor go/no-go discrimination tasks of varying difficulty. Water-deprived mice received bilateral 2 μl injections of vehicle (saline), phentolamine (α blocker, 14 mM) or alprenolol (β blocker, 14 mM), or a combination of the two drugs immediately preceding the behavioral task. The odor pairs used were of varying molecular similarity and hence difficulty. Animal groups receiving saline, alprenolol or phentolamine alone did not differ statistically in the number of trials to discrimination for all odor pairs tested. The injection of both drugs resulted in an odor pair-dependent effect, ranging from complete blockade for similar odors to no disturbance when compared with saline-injected controls. We conclude that blockade of NE signaling in the MOB of adult mice does not impair two odor discrimination behavior *per se*, but does impair the ability to discriminate similar odors.

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Poster: Olfactory Behavior

Epigenetic imprinting of Rasgrf1 affects associative odor learning in neonatal mice

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The Rasgrf1 gene has been proposed to couple synaptic activity to durable learning-dependent changes in synaptic strengths. Inactivation of Rasgrf1 affects neuronal excitability and impairs long-term memory formation in adult mice. Rasgrf1expression is imprinted, i.e. it is expressed exclusively from the paternal allele in the neonatal mouse brain, transitioning to biallelic expression on postnatal day 10 (PN10). The effects of this imprinting are not known; indeed, to date there is no known behavioral phenotype attributable to epigenetic imprinting. We have identified the regulatory domains that control the imprinting of Rasgrf1, and have generated mice in which the neonatal expression of Rasgrf1 is ectopic (i.e. biallelic, maternally imprinted, or absent). Using a positively reinforced neonatal associative learning paradigm, we have shown that mouse pups at PN3, PN5 and PN10 will exhibit a preference for a conditioned odor after a single pairing, although

additional training trials increase the measured degree of preference. We applied this paradigm at PN8 to mice expressing all four patterns of Rasgrf1 imprinting. Neonates expressing Rasgrf1 biallelically exhibited stronger preferences for the conditioned odorant than wild type (paternal monoallelic), while null mutants exhibited no learned preference, and pups expressing only the maternal allele did not differ from wild type. These data indicate that Rasgrf1 imprinting influences neonatal odor preference learning, and support inclusive fitness models respecting the evolutionary utility of genomic imprinting.

Poster: Olfactory Behavior

Ontogeny of odor discrimination: intensity modulation of olfactory acuity emerges postnatally

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Olfactory acuity can be modulated by several factors including past experience, expectation and stimulus intensity. Although imaging data appear to predict reduced olfactory acuity as stimulus intensity increases, behavioral data suggest just the opposite—enhanced odorant discrimination with increasing intensity. In light of these data, it has been argued that contrast enhancement in the olfactory system is dynamic, depending on olfactory bulb local interneuron function to modulate olfactory acuity with respect to intensity. If this is the case, then neonatal rats, with severely limited numbers of juxtaglomerular and granule interneurons, should exhibit predictably different patterns of intensity modulation of olfactory acuity than mature rats. PN7, PN14 and mature rats were used as subjects. Odorant discrimination was determined using a cross-habituation task with odor-evoked heart-rate orienting responses as the behavioral measure. This task requires no prior training, and can be expressed by animals during the first postnatal week; thus it is ideal for the study of odor discrimination ontogeny. A homologous series of ethyl esters were used as stimuli at 75, 150 and 300 p.p.m. In PN14 and mature rats, olfactory acuity increased with increased stimulus intensity. In PN7 rats, however, acuity was greatest at the lowest intensity and was stable or reduced as odor intensity increased. The emergence of high-dimensional contrast enhancement circuitry within the olfactory bulb can account for these developmental changes.

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Poster: Olfactory Behavior

The role of centrifugal projections to the olfactory bulb in olfactory processing

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While activity in the olfactory bulb (OB) and piriform cortex is closely tied to physical stimulus features, abundant physiological, neurochemical and activity-dependent histological data indicate that neural activity in the olfactory bulb is also strongly modulated by experience. This fact suggests an important role for centrifugal projections to the olfactory bulb in the processing of olfactory stimuli. These centrifugal projections include, but are not limited to, projections from pyramidal cells in secondary olfactory cortical, frontal cortical and hippocampal structures, in addition to neuromodulatory inputs from the locus coeruleus, the HDB and the raphe nucleus. To date, little is known about the roles of cortical and hippocampal feedback projections to the OB for olfactory processing. Some studies have suggested that the bulbo-cortical loop is crucial for maintaining the oscillatory dynamics of the olfactory bulb (Gray and Skinner, 1988; Neville and Haberly, 2003), but it is not clear how changes in bulbar dynamics affect odor perception. We are using electrical lesions of the olfactory peduncle, sparing the LOT, to investigate how reduced centrifugal feedback to the olfactory bulb affects odor learning and perception in rats. Our experiments suggest that centrifugal feedback does not play a major role in non-associative odor learning and discrimination, as tested in an olfactory habituation task.

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Poster: Olfactory Behavior

Characterization of compounds in anal gland secretion of nutria (*Myocastor coypus*)

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Nutria, or coypu, are invasive rodents that are ravaging wetlands across their introduced range. Nutria have a sexually dimorphic (male larger) anal gland (AG) used in scent marking behavior. This study looked for a similar dimorphism in compounds produced by the AG. AGs were dissected from nutria killed by licensed trappers within 6 h of death and frozen at –20°C for later analysis. After thawing, AGs were homogenized and extracted with pentane or dichloromethane. AG extract was analyzed using coupled GC-MS. Female AG extract ($n = 2$) contained no detectable compounds. Male AG extract ($n = 3$) contained 15 compounds that were characterized with further MS analysis. Of the 15 compounds, six are fatty acids of known structure. Nine of the compounds are partially characterized farnesene isomers. Each male AG extract GC had the same compounds present, but in differing proportions. This suggests that nutria can use AG secretion to identify individual males. The discovery of farnesene in male nutria AG is also interesting in light of its established role as a dominance pheromone in male mice (Ma *et al.*, 1999). Such knowledge of compounds used in nutria chemical communication could be useful in developing coypu-specific attractants.

Poster: Olfactory Behavior

Exposure to odors improves olfactory discrimination in adult rats

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A number of electrophysiological experiments have shown that odor exposure alone, unaccompanied by behavioral training, changes the response patterns of neurons in the olfactory bulb (Wilson *et al.*, 1985; Buonviso *et al.*, 1998; Buonviso and Chaput, 2000; Montag-Sallaz and Buonviso, 2002). More specifically, odor

exposure reduced the number of mitral cells which responded to odorants with increased firing rates while increasing the number of mitral cells which were inhibited by the presence of odors. As a consequence, across mitral cells in the olfactory bulb, individual odors should be better discriminated due to previous exposure. In accordance with this hypothesis, we tested how odor exposure affects the spontaneous discrimination of pre-exposed and novel odors. Experimental rats were exposed to the (+) and (–) enantiomers of limonene for three consecutive weeks. Spontaneous discrimination between the enantiomers of limonene, terpinen-4-ol and carvone was tested before and after the odor exposure period using an olfactory habituation task. We found that experimental rats spontaneously discriminated only between the enantiomers of carvone before the exposure period whereas they spontaneously discriminated between all three enantiomer pairs after exposure to (+) and (–) limonene. This experiment suggests that odor experience changes perception in the manner we predicted based on other groups' electrophysiological experiments.

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Poster: Olfactory Behavior

A simple shift in peripheral olfactory specificity is associated with divergent male moth behavioral preference

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Male moths locate females through attraction to a specific blend of female sex pheromone. Species isolation may occur through male attraction or antagonism to pheromone components of closely related species. Stabilizing selection acts to prevent mating mistakes in this way, and produces a strong preference for a specific pheromone blend. Given this constraint, how do novel features in pheromone communication systems arise as new species diverge? The moth species *Heliothis virescens* and *Heliothis subflexa* can hybridize under laboratory conditions, and female progeny may be backcrossed to parental strains to analyze the inheritance of characters including olfactory preference in males. These species have similar pheromone blends, however *H. virescens* males respond only to blends containing Z9–14:Ald, while male *H. subflexa* instead require Z9-16:Ald (in addition to Z11–16:OH). We have identified behavioral phenotypes that segregate for Z9–14:Ald/Z9–16:Ald preference in a 1:1 ratio in backcross males. Genetic analyses (QTL) indicated a significant correlation between odor preference and the presence of a single autosome. Sensory neurophysiology in males of known behavioral phenotype and genotype indicated that the preference was associated with the specificity of olfactory receptor neurons (ORNs) for Z9–14:Ald and/or Z9–16:Ald. Thus, a simple change in ORN odorant specificity might account for a major change in male behavioral preference. In contrast, preliminary results show that the inheritance of other divergent traits, such as Z11–16:OAc-mediated antagonism which likely involve both peripheral and central olfactory characteristics, are more complex.

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Poster: Olfactory Behavior

Genetic basis of kin recognition

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Differentiating kin from non-kin enables organisms of many species to allocate resources or altruistic behaviour towards related conspecifics. Based on olfactory preference tests, we have shown that shoaling juvenile zebrafish recognize and prefer full siblings over unrelated conspecifics. Recognition of the odor of even unfamiliar full sibs demonstrates that the kin recognition mechanism is based on phenotype matching. This implies that zebrafish larvae become imprinted on an olfactory template for kin and compare it with that of unrelated individuals later in life. Our goal in the current project was to determine behaviorally the critical developmental stage for imprinting on kin odor and subsequently characterize olfactory receptor gene expression during this developmental window. We raised zebrafish larvae in (i) groups of full siblings and (ii) isolation after varying lengths of exposure (0, 4, 7 or 15 days) to groups of full siblings. All animals were tested for olfactory kin recognition at the age of 21 days. Larvae were able to recognize kin if they spent the first 7 and 15 days post-fertilization with full siblings while larvae were unable to recognize kin if they matured in total isolation or if they were exposed to kin for only 4 days. These results show that zebrafish require exposure to kin odor to become imprinted and that they can not use their own odor (self phenotype matching) as an olfactory template. Using quantitative real time PCR, we found significant differences in the expression of olfactory receptor genes between larvae that grew up in kin groups or in isolation. This may indicate that exposure to kin odor modifies the expression of olfactory receptor genes.

Poster: Olfactory Behavior

Critical role of urine-derived familiarity of odor in the aggressive behavior of mice

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In group-living mammals, distinguishing intruders from their group members is important for protecting their territory. In mice, although it has been suspected that male urine contains aggression pheromones which induce aggressive behavior, familiarity seems another important factor for controlling aggressive behavior. In this study, the role of olfactory recognition in territorial aggression was investigated. An intact ICR male mouse (resident) was grouped with a castrated DBA mouse (cage-mate) and a female ICR mouse for establishing territory in a resident–intruder paradigm. The resident showed aggression to an unfamiliar castrated DBA mouse (UFC) but not to its cage-mate. Then we examined the role of urine odor in the recognition of familiarity. When a part of the body of the UFC was swabbed with the cage-mate's urine, the resident showed few attack-bites to the UFC. Similar effect was observed

when an intact male intruder was swabbed with the cage-mate's urine. On the other hand, the resident did not show any aggression toward its cage-mate even when it was swabbed with the UFC's urine. Finally, we changed the food in an attempt to modify the urine profile. The resident did not show aggression to the UFC carrying the odor of the cage-mate's previously collected urine. These results suggest that the information about familiarity conveyed by urine odor diminishes aggressive behavior by the resident male mouse.

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Poster: Olfactory Behavior

Effect of learning on fos-activation patterns and neurogenesis in the olfactory system

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Mice are model organisms for olfaction studies due to their adept learning of olfactory-based associations. We examined the effect of associative learning on neurogenesis and neuronal activity patterns in the olfactory system. One week prior to training, mice were injected with 0.1 mg/g BrdU. All mice were presented with two pots of sand, one scented with isoamyl acetate and the control containing mineral oil. Fifteen mice were trained to dig in the scented pot for a sucrose tablet and 14 control animals, exposed to the same pots, were not rewarded. After 135 trials, all mice in the experimental condition dug in the scented pot at least 70% of the time. One week following the end of training (4 weeks after BrdU injection), mice were isolated in an odor-free chamber for 12 h, exposed to 1.0 Pa isoamyl acetate for 1 h and then sacrificed. After removal and post-fixation of the brain, brains were divided midsagitally and each hemisphere was sectioned at 40 μm for later immunohistochemical labeling (right hemisphere BrdU, left hemisphere c-Fos). There was a trend toward a greater density of BrdU-positive cells in the granule layer of the OB of experimental mice, while in the glomerular layer this trend was reversed with control animals, demonstrating a greater density of BrdU labeling. These trends were not significant. Additionally, we analyzed and will discuss the pattern of neuronal activation, indicated by Fos immunoreactivity, for the olfactory bulb and several secondary olfactory structures.

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Poster: Olfactory Behavior

Odors as signals: human odortypes

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Body odors help to regulate social, sexual and endocrine response of many species, including humans. Our research, conducted for over 25 years mainly using mice, has demonstrated that genes in the major histocompatibility complex confer upon individuals a specific unique body odor which we have termed the animal's odortype. We have now expanded this research to humans with a program to determine whether HLA-based odortypes can be used to identify individual people. To do this, we have recruited, HLA-typed and collected urine and sweat samples from each of >70 of these individuals. The donors have been grouped into HLA supertypes (a grouping that reflects similarities in the binding pockets of the HLA molecules) for bioassay and chemical analyses. As one component of our program, we are training mice in a Y-maze to discriminate urine and sweat volatiles based on their HLA supertype. We show that mice distinguished between urine samples of two individuals and that they generalized these responses to urine samples collected from the same two individuals 8 years earlier, indicating long-term individual odor stability. In some but not all cases, mice generalized responses of training samples to other pairs of samples based on similarities of HLA supertype. These data provide evidence that MHC type in humans, as in mice, is involved in provisioning an individual with a distinct, genetically-based volatile body odor.

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Poster: Olfactory Behavior

A re-examination of odor mixture quality and its assessment

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Odor mixtures are perceived as different from (configural) or the same as (elemental) their components. Recent studies propose that structural or perceptual similarities of components predict configural properties of binary mixtures. We evaluated this in rats using four binary mixtures with varying structural similarity [eucalyptol/benzaldehyde, eugenol/benzaldehyde, octanol/octanal and (±)-limonene]. The range of ratios tested for each mixture was determined by the components' vapor pressures. In the first part of the study we use a digging task to evaluate recognition intensity. In the second part, we translate this to a computer-controlled task using an olfactometer for odor delivery and a lever press response. This allows assessment of component recognition in individual animals and individual trials, better control of the odor stimulus and the possibility of assessing component responses electrophysiologically. Component odors are substituted for the odor mixture in a fraction of unrewarded CS+ trials in a partial reinforcement CS+/CS– go/no-go paradigm. The number of trials in which a rat responds to a given component odor correlates with number of s of digging in the other task. Three results are presented: (i) no mixture maintains purely elemental or configural properties for all concentration ratios; (ii) structural similarity or dissimilarity does not predict configural or elemental perception; and (iii) overshadowing is significant in all odor sets.

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Poster: Olfactory Behavior

Olfactory preferences in the zebrafish

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We have devised a series of experiments to ascertain whether zebrafish form and retain olfactory memories of odorants experienced as juveniles. We have shown that zebrafish exposed to the odorant phenylethyl alcohol have a preference for the odor relative to their unexposed control siblings (Rivard *et al.*, this meeting). Concerning olfactory memory as exemplified by salmonids, clearly the odor trace they follow to their natal stream is a complex bouquet of chemicals. We proposed that a relevant odor to the fish is the trace odor of dead fish from the year before (in the case of the salmon). To test this idea we made dead fish odor (DFO) by euthanizing zebrafish and allowing them to decompose in aerated aquaria. Juvenile fish were exposed to the DFO (10 days after preparation) for 3 weeks of development, while control siblings were exposed to system water. DFO solution was maintained during the time the fish grew to adulthood. Adult fish were tested in a Y-maze at 6–8 months of age. The fish preferred the arm of the maze baited with DFO regardless of whether they experienced the odor as juveniles. This suggested that zebrafish have an inherent preference for DFO. To further test this preference we made DFO from two strains of zebrafish (*Danio rerio*) as well as a closely related (goldfish: *Caraussius aurautus*) and a distantly related (cichlid: *Julidochromis marlieri*) species. We then exposed adults to these odors in the Y-maze. To date we have found no innate preference for the DFO of conspecifics, but this may be an age-dependent effect. We are currently repeating these experiments.

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Symposium: Receptors Symposium: I Unraveling smell

L. Buck

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Symposium: Receptors Symposium: I Pheromone receptors in *Drosophila*

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In *Drosophila*, a large family of gustatory receptors genes (Grs) expressed in taste neurons is thought to mediate the recognition of sugars, toxic compounds and pheromones, thereby controlling feeding and mating behaviors. One of these genes, GR68a, was shown to be expressed sex-specifically in neurons of ~10 male-specific bristles in the foreleg. Inactivation of these neurons or down regulation of Gr68a RNA by RNA interference in males results in a large reduction of male courtship activity (Bray and Amrein 2003). Specifically, the loss of courtship activity occurs during the second step in courtship, whereby the male taps the female's abdomen to receive pheromone input, implying that GR68a functions as a pheromone receptor.

We have now extended our analysis of potential pheromone receptors and their role in courtship to include a Gr family closely related to Gr68a, namely Gr39a.a to Gr39a.d. We generated a strain in which all four of these genes were deleted. In addition, we also created a knock out of Gr68a by homologous recombination. Behavioral studies of courtship and feeding behavior of males from these fly strains, lacking various members of this putative pheromone receptor family, will be presented.

Symposium: Receptors Symposium: I

Semi-monoclonal expression of the odorant receptor transgene

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The mouse has >1000 odorant receptor genes clustered at nearly 40 different loci on many different chromosomes. Using YAC transgenic mice, we have been studying the expression and projection of the *MOR28* gene cluster located on chromosome 14. We have identified a 2 kb homology (H) region 75 kb upstream of the *MOR28* gene, which is highly conserved between the mouse and human. Deletion of the H region abolished the expression of all the OR genes in the *MOR28* cluster. When the H region was relocated closer to the cluster, the number of olfactory sensory neurons (OSNs) expressing the proximal OR gene was greatly increased. When the H-region was attached to the *MOR28* minigene of 13 kb (*H-MOR28*), nearly 90% of OSNs in ventro-lateral region of the OE expressed the minigene. Surprisingly, >200 ectopic *MOR28* glomeruli appeared on the olfactory bulb. OSNs expressing the differently tagged endogenous *MOR28* co-inervated into many different ectopic glomeruli for the *H-MOR28*. Since the neuronal cell lines are not available for the OSNs, such a semi-monoclonal expression system of the OR gene will serve as a valuable tool for the studies of the OR gene expression and OSN projection.

Symposium: Receptors Symposium: I

Coding sequence variation in human sweet and umami taste receptor genes

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Sweet and umami (the taste of glutamate) tastes play a major role on the perception of carlorically rich and essential nutrients. In humans, three members of the T1R class of taste-specific G protein-coupled receptors (T1R1, T1R2 and T1R3), which reside on chromosome 1, are known to function in combination as heterodimeric receptors for sweet and umami tastes. We hypothesized single nucleotide polymorphisms (SNPs) or variant haplotypes of the T1R genes in humans may underlie individual differences in the detection and recognition threshold for sweeteners and amino acids. To enable study of genotype/phenotype correlation for these two tastes, we identified coding sequence variation by sequencing these genes in a cohort of unrelated individuals. To achieve maximum genetic diversity in our sample, we sequenced a panel consisting of 30 Europeans, 20 East

Asians, 10 Native Americans, 8 South Asians and 20 sub-Saharan Africans. In the combined sample, we found a total of 47 SNP's in these three genes. Sixty percent of these SNPs cause an amino acid substitution in the encoded receptor protein, and one SNP, in the T1R1 gene, introduces an in-frame stop codon. Although the size of these three genes is much larger than those of the T2R genes that encode bitter taste receptors, the density of cSNPs in the T1R genes is less than that of cSNPs in T2R genes. This suggests that the sequence of the T1R receptor proteins are more conserved than those of T2R receptors, and may explain why individuals do not show broad variation in these taste modalities.

Symposium: Receptors Symposium: I

De-orphaning, functional characterization and camp signalling of five human V1R-like receptors

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The human vomeronasal organ (VNO) is an anatomical entity which is generally considered to be vestigial or non-functional. However, five potentially functional genes have been identified from human genome searches, coding for receptors that are related to the family of V1 receptors of the apical part of the mouse VNO. These five human receptors have been termed hV1RL1–5, one of which has recently been demonstrated to be expressed in the human main olfactory epithelium (MOE). Since nothing was known about their function, all five human V1RL-receptors had to be addressed as true orphan receptors, so far. We found that four out of five V1RL-receptors are expressed in the human MOE. All five receptors have been rho-tagged, and expressed at the plasma membrane level of HeLa/Olf cells. We screened the human V1RL receptors against a collection of 47 odorants from different chemical classes. We de-orphaned all five human V1RL-receptors, and found that they responded to a variety of odorants in a combinatorial way, thus behaving like olfactory receptors from MOE. Testing chemically related odorants with variable sizes and functional groups, specific odorant profiles can be attributed to all human V1RL receptors. Our findings may support the notion that the human MOE has adopted pheromone perception. V1RL-receptors in the human MOE may function as odorant or pheromone receptors.

Symposium: Receptors Symposium: II

Combinatorial coding guides olfactory behavior in *Drosophila* larvae

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Olfactory systems recognize and generate appropriate behavioral responses to a vast number of odors. Odorant receptors (ORs) are thought to act in a combinatorial fashion, such that odor identity is encoded by the activation of distinct sets of ORs. Whether combinatorial coding seen at the level of the OR is maintained at the level of olfactory behavior is not known. We investigated this question in *Drosophila* larvae, and show that these animals express 23 OR genes in 21 olfactory neurons, each of which targets a unique olfactory glomerulus. Larvae with one or two functional olfactory neurons were constructed and tested in chemotaxis assays. Neurons mediating responses to a wide range of odors as well as more odor-restricted neurons were identified. Larvae with two functional neurons respond to more odors than those with either single neuron alone. These data show for the first time that combinatorial activity of multiple ORs is integrated to drive distinct behavioral responses to a range of odors that exceeds the number of OR genes.

Symposium: Receptors Symposium: II

The unique contributions of T1R2 and T1R3 to the detection of sweet stimuli

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Mammals use a heteromeric class C G protein-coupled receptor (GPCR) to detect and transduce sweet taste stimuli, including sugars, high-potency sweeteners and sweet proteins. The native sweet taste receptor contains two subunits, T1R2 and T1R3, both of which are required for normal stimulus sensitivity and selectivity. However, it appears that each subunit could also function as a lower-efficacy homomeric receptor, at least for natural sugars. The use of human:rodent chimeric T1Rs has helped clarify the roles of each subunit in the detection and transduction of some sweet stimuli that display species specificity (e.g. aspartame, cyclamate, brazzein). However, the specific contributions of T1R2 and T1R3 to the detection of sweet stimuli preferred by both humans and rodents (e.g. sugars) remain unclear. We have developed an *in vitro* system for quantifying ligand interactions with T1R proteins. We find that sugars bind to the extracellular N-terminal domains of both T1R2 and T1R3, and do so at physiologically relevant concentrations. Furthermore, each T1R subunit exhibits distinct changes in protein structure upon binding of sugar ligands. We conclude that both T1R2 and T1R3 function to bind sugars, with each subunit contributing unique structural properties that permit the T1R2:T1R3 heteromeric receptor to efficiently transduce natural sugar stimuli.

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Symposium: Receptors Symposium: II

Drosophila OBP lush is required for activity of pheromone-sensitive neurons

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Odorant binding proteins (OBPs) are extracellular proteins localized to the chemosensory systems of most terrestrial species. OBPs are expressed by non-neuronal cells and secreted into the fluid

bathing olfactory neuron dendrites. Several members have been shown to interact directly with odorants, but the significance of this is not clear. We show that the *Drosophila* OBP mutant lush is completely devoid of evoked activity to the pheromone 11-*cis*-vaccenyl acetate (VA), revealing this binding protein is absolutely required for activation of pheromone-sensitive chemosensory neurons. Lush mutants are also defective for pheromone-evoked behavior. Importantly, we identify a genetic interaction between lush and spontaneous activity in VA-sensitive neurons in the absence of pheromone. The defects in spontaneous activity and VA sensitivity are reversed by germline transformation with a lush transgene or by introducing recombinant LUSH protein into mutant sensilla. These studies directly link pheromone-induced behavior with OBP-dependent activation of a subset of olfactory neurons.

Symposium: Receptors Symposium: II

RTP1 and 2 promote functional cell-surface expression of mammalian odorant receptors

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It has been difficult to express mammalian odorant receptors (ORs) on the cell surface of heterologous cells and assay their ligand-binding specificity, because OR proteins are retained in the endoplasmic reticulum and subsequently degraded. We identified RTP1 and RTP2 that promote cell surface expression of ORs. They are expressed specifically by olfactory neurons, interact with OR proteins, and dramatically enhance responses to odorants when coexpressed with ORs in HEK293T cells. Similar, although much weaker, effects were seen with a third novel protein, REEP1. These findings have allowed us to construct a heterologous expression system to identify new ORs that respond to odorants. We are currently testing large number of human and mouse ORs against a panel of odorant chemicals to 'deorphanize' the ORs.

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Symposium: Receptors Symposium: III

A genomic perspective on the evolution of olfaction in primates

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Olfactory receptor (OR) genes constitute the basis of the sense of smell and are encoded by the largest mammalian gene superfamily, with >1000 genes. While the OR gene repertoires of mice, dogs, chimpanzees and humans are roughly the same size, the proportion of putatively functional OR genes is higher in rodents, dogs and new world monkeys than in old world monkeys and apes, and lowest in humans. By comparing the entire human and chimpanzee OR gene repertoires, we estimate that this additional accumulation of pseudogenes in humans started ~3.2 MYA. We further estimate that 133 human intact OR genes are evolving under no evolutionary constraint and may become pseudogenes over time. Our analysis identified one chimpanzee-specific OR subfamily expansion and four human-specific expansions. We also found support for the action of positive selection on a subset of human and chimpanzee OR genes. These observations suggest that while overall humans appear to have a reduced need for the sense of smell compared with chimpanzees, specific-sensory requirements have shaped the functional human OR gene repertoire.

Symposium: Receptors Symposium: III

The molecular response profile of an odorant receptor: *Drosophila* OR22A

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In order to understand olfactory coding we need to know the responses of individual sensory neurons to odorant stimuli. These responses are largely dictated by the binding properties of the olfactory receptor protein, but olfactory binding proteins and auxiliary cellular mechanisms may also contribute to their molecular response profiles. Thus, it is necessary to measure responses to many substances across concentrations *in vivo*. We have therefore developed a semi-automated approach to measuring such odor-response profiles of olfactory receptor cells with all auxiliary mechanisms in place. We genetically expressed a calcium-sensitive fluorescent probe (cameleon2.1) in *Drosophila* sensory cells that express the receptor protein DOR22a using the GAL4-UAS system. We then measured changes in intracellular calcium in the dendrites on the antenna, and in the axonal terminals of these cells in glomerulus DM2 of the antennal lobe, using optical imaging with a CCD camera. Calcium in the dendrites is likely to be linked to sensory transduction, while calcium in axonal terminals is likely to be a function of the firing activity in a cell. We tested a total of 106 odors from a variety of chemical groups across several orders of magnitude of concentration. We found dose-dependent responses to more than one-third of the odors tested, but some odors elicited responses at considerable lower concentrations than others. The best ligands were ethyl and methyl hexanoate, with responses down to a dilution of 10^{-8} saturated vapor pressure.

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Symposium: Receptors Symposium: III

Molecular mechanisms underlying sex-pheromone reception

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The insect chemosensory system has been a subject of great interest because of its remarkable selectivity and because of its association with sexual behavior. The olfactory receptor (OR) family, which comprises ~60 multigenes in insects, is responsible for the first step of chemosensation in the olfactory neurons of antennae. We herein describe two male-specific ORs in the silk moth, *Bombyx mori*, that are mutually exclusively expressed in a pair of adjacent pheromone-sensitive neurons in a long sensillum trichodeum of male antennae: one that is specifically tuned to bombykol, the sex pheromone, and

the other to bombykal, an oxidized form of bombykol. This mutually exclusive expression pattern of two pheromone receptors in single sensilum may provide a paradigm to ensure detection of the specific ratios of a pheromone blend in antenna of various moths. In contrast, social and reproductive behaviors in mammals are modulated by not only volatile pheromones but also non-volatile cues that are likely detected by vomeronasal sensory neurons expressing V2Rs. We identified a male-specific peptide that was encoded by a gene from a previously unrecognized large family in mice. This peptide, named ESP1, is secreted from male mice and transferred to the female vomeronasal organ wherein it elicits an electrical response. Mice appear to send sex-specific information via an accessory olfactory pathway during direct contact. I propose that each animal has acquired a unique strategy to transmit volatile or non-volatile sex-specific cues by adopting their environment during the processes of evolution.

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Symposium: Receptors Symposium: III

Olfactory receptors

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