

ABSTRACTS

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

Symposium: Chemosensory Receptors Satellite

Development and Activity-Dependent Refinement of the Olfactory Intrabulbar Map

L. Belluscio

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In mammals, axons from odorant receptor neurons converge to form glomeruli in the main olfactory bulbs. There are two mirror symmetric glomerular maps in each bulb depicting odorant receptor identity. Isofunctional glomeruli in each bulb are specifically linked through a reciprocal intrabulbar circuit mediated by external tufted cells (ETCs) forming an intrabulbar map. To understand the principles governing the precision of this map, we examined the development of intrabulbar projections (IBPs) using fluorescent tracers injected into the glomerular layer. Previous experiments in adult mice demonstrated that the size of the injected region on one half of the bulb resulted in the labeling of a projection site on the other half of the bulb that was directly proportional in size, producing a 1:1 diameter ratio. Utilizing this ratio as a measure of maturity, we generated a developmental profile of IBPs from birth to 14 wks of age. We showed that a 1:5 injection to projection ratio exists at 1 wk of age and that refinement to a mature 1:1 ratio occurs by 7 wks. To determine the role of activity on this maturation process, we performed injections on anosmic mice. We demonstrated that activity is not required to establish IBPs, but is crucial for their refinement. Naris occlusion experiments further demonstrated activity-dependent maturation of the map. There was a distinct broadening of IBPs when odorant-induced activity was blocked. Interestingly, this ability of the IBPs to reorganize is maintained into adulthood with no apparent critical period. Thus, these data revealed that the intrabulbar map maintains a continuous plasticity that is directly modulated by sensory stimuli.

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Characterizing Human Olfactory Receptor Gene Expression

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Olfactory receptor (OR) genes were discovered more than a decade ago, when Buck and Axel observed that, in rats, certain G-protein coupled receptors are expressed exclusively in the olfactory epithelium. Since, protein sequence similarity has been used to estimate that mammalian genomes contain more than 1,000 putative OR genes, making it the largest known gene family. However, in humans, the functional annotation of almost all predicted OR genes

await demonstration of their expression in the olfactory epithelium. To address this, we examined the expression patterns of OR genes in human olfactory epithelium tissues using a recently developed DNA microarray that contains probes for most predicted human OR loci. We observe the expression of ~80% of human OR genes in olfactory epithelium, thereby confirming their functional annotation as odorant receptors. In addition, we detect the expression of a large number of OR pseudogenes, suggesting that a significant proportion of the OSN cells either express at least two OR genes (one functional and one pseudogene), or that a large number of OSN cells express only a pseudogene and hence probably do not reach maturation. Finally, we characterized the expression of human OR genes in non-olfactory epithelium tissues—an analysis that may shed light on the question of whether OR genes have alternative functions in other tissues.

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Olfactory Receptors Signal Identity

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The large family of ORs are now implicated in at least two processes which effectively determine the phenotypic identity of OSNs. Firstly, they are responsible for selective ligand recognition and therefore determine the molecular receptive range of a neuron. Secondly, they are critical determinants in targeting axons to a particular glomerulus, thereby providing a topographic identity to OSNs. In the ligand binding process there is a well-defined transduction cascade to which the receptor is coupled and which results finally in signal generation and synapse activation. There is no indication that the ORs on OSN cilia are able to have any effect on cell behavior absent in this transduction process. On the other hand, the ORs expressed at axon terminals have been proposed to have a direct effect, possibly through homotypic interactions, on directing axons into the proper glomerular relationships. However, there is evidence that at least the maintenance of the glomerular map requires activity in OSNs, although the source and nature of this activity are not yet defined. Is there a role for activity dependent, receptor mediated, signaling in axon guidance as well? Does glomerular formation and maturation require the activation of a second messenger cascade, as well as expression of the proper OR? Using embryonic retroviral injection technology we have altered gene function in select early developing OSNs. Manipulation of the various molecules known to function in the transduction pathway causes large changes in axonal targeting and glomerular formation, suggesting a critical role for activity generated biochemical processes in OE-OB development.

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Onset of Odorant Receptor Expression

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The olfactory pathway is broadly organized at the periphery and then transformed into a organized spatial map in the olfactory bulb (OB). Olfactory sensory neurons (OSNs) express only 1 of 1,000 odorant receptors (ORs). OSNs expressing the same OR are broadly distributed within restricted regions of the olfactory epithelium (OE). As the OSN axons track from the OE to the OB, they reorganize and project to specific glomeruli based on OR expression. ORs are expressed in OSNs in the absence of the OB, suggesting that OR expression is independent of the target glomerulus. OR expression is believed to contribute to the specificity of axon targeting, including embryonic projections and the rerouting of OSN axons following OR substitutions. Consistent with the notion that ORs are expressed prior to or coincident with axogenesis, earlier reports suggested that OR expression slightly precedes initial axonal contact with the OB and synaptogenesis. Here, we extend these results by clarifying the earliest detectable onset of OR expression using *in situ* hybridization. We also assessed whether the rate of proliferation and the temporal onset of OR expression is uniform. Finally, we studied the spatial organization of OR expression in the embryonic OE. We found that the onset of OR expression is asynchronous and that ORs proliferate at a non-uniform rate. We also determined that ORs are spatially restricted during development in a manner consistent with that seen in the adult. Our results provide compelling evidence that OR choice during axogenesis is an important determinant of glomerular targeting.

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Representation of Natural Stimuli in the Rodent Main Olfactory Bulb

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To understand the organization of natural stimuli in the bulb, we recorded electrophysiological responses of mitral cells and imaged intrinsic signals from glomeruli upon stimulation with volatiles from natural stimuli. All natural stimuli including urine are sparsely represented, activating less than 10% of mitral cells or glomeruli. To further examine the origins of mitral cell and glomerular responses to natural stimuli, we fractionated volatiles in natural stimuli using gas chromatography and delivered them sequentially to the animals while monitoring neural activities continuously. Among hundreds of volatiles present in a natural stimulus, mitral cells and glomeruli are frequently activated by a single component whereas individual components only evoked activation in a small distinct set of glomeruli. The representation of a complete natural stimulus is largely the simple union of representations of its individual components. During experiments using urine volatiles, one cohort of mitral cells were found activated exclusively by male mouse urine. These cells were determined responsive to a previously unknown male specific sulfur containing compound (methylthio)methanethiol. When added to

castrated male mouse urine, synthetic (methylthio)methanethiol significantly enhanced urine attractiveness to female mice. The conclusion from these experiments are as follows: 1) natural stimuli and their individual components are sparsely represented in the MOB; 2) mitral cells and glomeruli encode natural odorant stimuli by acting as feature detectors; 3) individual components are processed in the bulb largely independently.

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Functional and Structural Analysis of Mammalian Odorant Receptors

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Mammalian odorant receptors (ORs) are grouped into two broad classes and numerous subfamilies, which may reflect functional organization. We are using *Xenopus* oocytes to investigate the ligand specificities of members of OR subfamilies. We find that a wide variety of Class I and Class II mouse ORs (MORs) can be functionally expressed. Co-expression of MORs with $G\alpha_{olf}$ and the cystic fibrosis transmembrane regulator allows measurement of odorant responses using electrophysiological methods. All receptor constructs include the N-terminal 20 amino acid residues of human rhodopsin, and for most MORs tested this is sufficient for functional expression. Co-expression of accessory proteins (RTP1, RTP2 and REEP1) allows functional expression of additional MORs. Using this assay, we examined the ligand specificities of the MOR42 subfamily. MOR42-1 responded to dicarboxylic acids (C9–C12). MOR42-2 responded to monocarboxylic acids (C7–C10). MOR42-3 responded to dicarboxylic acids (C8–C10) and monocarboxylic acids (C10–C12). Thus, the receptive range of each receptor is unique. However, overlap between the individual receptive ranges suggests that the members of this subfamily are contributing to one contiguous subfamily receptive range, supporting the idea that OR subfamilies constitute functional units. We are screening a series of mutant MORs to identify residues that confer differences in ligand specificity between MOR42-1 and MOR42-3. This analysis, coupled with computational receptor modeling, provides insight into the structural basis for odorant recognition by ORs.

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Olfactory Receptors in the Septal Organ

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The septal organ is a distinct chemosensory organ in the mammalian nose. We have identified the odorant receptors expressed in this region. The majority of the septal neurons express only a few odorant receptors in varying patterns, with the most prevalent receptor (MOR256-3) present in nearly 50% of the neurons and the eight most prevalent receptors in nearly 95% of the neurons. A single neuron probably expresses only one receptor. Using patch clamp recordings, we have investigated how the mouse septal organ neurons in the

intact epithelium respond to odorants delivered by pressure ejection (puffing). The majority of the septal organ neurons exhibit both odorant and mechanical responses mediated by adenylyl cyclase. These neurons are relatively broadly-tuned by responding to multiple distinct odorants with thresholds at the nanomolar range. In addition, they respond to Ringer puffs (mechanical responses) and the response increases linearly with the pressure of the puff. The septal organ, situated in the air path, may have dual functions in the nose: a sensitive, broadly-tuned odor detector and a mechanical sensor. When the air flows faster in the nose (such as during sniffing), the observed mechanical sensitivity may increase the chance for single neurons to fire upon weak stimulation. Furthermore, the mechanical sensitivity may provide a driving force (besides the episodic exposure of odorants during respiration) for the periodic activities of the olfactory bulb neurons synchronized with breathing cycles.

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Ric-8B, a Putative GEF for G Alpha Olf, Amplifies Signal Transduction through Odorant Receptors

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The canonical pathway for odorant signal transduction in olfactory neurons consists of four major olfactory specific components: odorant receptors, G alpha olf, adenylyl cyclase III and a cyclic nucleotide-gated channel. During the last years, a large number of regulatory mechanisms that lead to odorant signal termination have been described, but so far, there was no evidence for regulatory events that would result in signal amplification. We identified a protein, Ric-8B, which interacts with G alpha olf. Our results demonstrate that Ric-8B, which is a putative guanylyl nucleotide exchange factor (GEF), shows a striking restricted pattern of gene expression, which co-localizes with that of G alpha olf: both genes are specifically expressed in mature sensory neurons in the olfactory epithelium and in a few regions in the brain. In addition, we show that Ric-8B significantly enhances odorant receptor signaling through G alpha olf in HEK293T cells. Our results indicate that Ric-8B can be used to improve high-throughput functional expression of odorant receptors in heterologous cells.

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Symposium: Chemosensory Receptors Satellite

“Deorphanizing” Mammalian Odorant Receptors

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How does mammalian olfactory system use hundreds or more odorant receptors (ORs) to detect and discriminate a vast number of volatile odorants? To tackle this question, it is essential to understand how structurally diverse chemicals activate different ORs. However, it has been difficult to express mammalian ORs on the cell surface of heterologous cells and assay their ligand-binding specificities because OR proteins are typically retained in the endoplasmic reticulum and not transported to the cell surface. We have identified RTP1 and RTP2

that promote functional cell-surface expression of ORs in heterologous cells. Structure-function analysis of RTP1 revealed important domains functioning in trafficking of ORs. We have constructed a heterologous expression system to identify new ORs that respond to various odorants. We have tested ~300 human and ~200 mouse ORs against a panel of ~100 diverse odorant chemicals to determine the odorant-OR interactions. This screening have resulted in identification of ~100 human and mouse ORs that respond to a wide variety of odorant chemicals. Some ORs seem to respond small number of structurally similar chemicals while others seem to respond to many chemicals, suggesting variable tuning specificities of ORs.

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Olfactory Deficits in Mice Deficient for the Transient Receptor Potential Channel M5

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Mice defective for the cyclic nucleotide-gated channel (CNGA2) have a severe olfactory deficit, but respond to putative pheromones implying the presence of other transduction pathways in addition to the canonical cAMP pathway. We studied the responsiveness of individual glomeruli in CNGA2 knockout mice by detecting odor-induced Fos expression in periglomerular cells. While a subset of the glomeruli activated by putative pheromones were necklace glomeruli where the second messenger cGMP is thought to mediate transduction, the majority of active glomeruli in CNGA2 knockout mice were regular glomeruli targeted by olfactory sensory neurons (OSNs) that would normally have expressed CNGA2, and do not express elements of the cGMP pathway. Interestingly, electroolfactogram (EOG) responses elicited by putative pheromones in CNGA2 knockout mice are inhibited by the phospholipase C (PLC) inhibitor U73122 implying an involvement of this pathway in olfactory transduction. Further, we find that the transient receptor potential channel M5, an effector participating in the PLC pathway in taste cells is co-expressed with CNGA2 in a subset of OSNs projecting to glomeruli that respond to putative pheromones and urine. While the olfactory deficits in TRPM5 knockout mice are relatively mild, we find that mice defective for both TRPM5 and CNGA2 have a dramatic phenotype including severely diminished size of the olfactory bulb and missing glomeruli in discrete areas of the bulb. These data imply that, in a subset of OSNs, the PLC/TRPM5 and cAMP pathways are co-expressed and play a role in olfactory transduction.

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Olfaction Targeted

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The sense of smell is mediated by a repertoire of ~1000 odorant receptor genes in mice. Each olfactory sensory neuron is thought to

express just one of these genes. Its axon synapses with second-order neurons within a glomerulus in the olfactory bulb. The axons of all neurons that express the same receptor gene converge to the same glomeruli. The odorant receptor is a critical determinant of which glomerulus is innervated. An olfactory sensory neuron thus faces two tasks during differentiation: to choose one odorant receptor gene for expression and to project its axon to a specific glomerulus.

Symposium: Chemosensory Receptors Satellite

Monitoring Odorant Detection by Olfactory Receptors Expressed in Yeast as a Reporter System

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Breaking down the molecular mechanisms of odorant perception and coding, and screening receptor-odorant couples primarily require the functional expression of olfactory receptors in a cellular system. We have developed techniques to optimize membrane expression of olfactory receptors in engineered *S. cerevisiae* yeast. Receptors functional activity is evaluated both in living cells, where receptor stimulation by its odorant ligand is monitored through the bioluminescence of a luciferase reporter, and in nanosomes membrane fragments, where activation of the receptor upon odorant stimulation can be assessed by monitoring surface plasmon resonance response. We demonstrate that olfactory receptors maintain their activity in membrane fragments. A same bell-shaped concentration-dependence response is obtained, in terms of threshold concentration and optimal concentration, which gives evidence that this receptor functional response in the living cell indeed arises from its own behavior upon odorant stimulation, with no artefactual contribution from the cellular transduction pathway. Olfactory receptors efficiently discriminate between odorant ligands and unrelated odorants. This system can fruitfully serve to evaluate the comparative coupling efficiency of olfactory receptors to various G_α protein subunits, without the interference of cellular contribution. Moreover, nanosomes can be used as sensing elements of bioelectronic sensors, at the basis of potentially powerful electronic noses with a new concept of mimicking *in vivo* odorant specific detection and discrimination.

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Odorant Receptors with Unique Features

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The repertoire of odorant receptors (ORs) in mammals comprises around 1000 different subtypes; despite their enormous sequence

variability, most of them share mainly common features, including membrane topology and expression pattern. However, some of the OR types appear to be unique. Receptors of the OR37 subfamily display quite distinguished features, including an extended third extracellular loop and a clustered expression pattern. These receptors are only found in mammals and in contrast to OR genes in general, the genes encoding OR37 receptors appear to be under negative selection. These outstanding properties suggest that OR37 receptors are specifically tuned to distinct chemical signals. Also the more recently discovered receptor mOR256-17 is characterized by exceptional properties. It is expressed in a larger number of sensory neurons than other ORs, especially during prenatal development. The onset of expression occurs already at embryonic day 10 (E10), thus almost one day prior to other OR types. The axons of olfactory cells expressing mOR256-17 reach the rostral forebrain at E13, whereas the axons of other OR-cells target the presumptive olfactory bulb not before E14.5/15. In addition, mOR256-17 is not only expressed in sensory neurons of the olfactory epithelium, but also in a substantial subpopulation of non-sensory cells which is located in the cribriform mesenchyme during a defined phase of prenatal development. These 'extra-epithelial' mOR256-17 expressing cells are closely associated with olfactory axons and appear to be intimately involved in the initiation and establishment of the wiring patterns in the olfactory system.

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Symposium: Chemosensory Receptors Satellite Symposium

Evolutional and Biological Characterization of the Mouse ESP Family

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We discovered a male-specific 7-kDa secreting peptide, ESP1, that elicited an electrical response in V2R-expressing vomeronasal sensory neurons (VSNs) in mice. ESP1 secreted in tears appears to be transferred to the female vomeronasal organ (VNO) through physical contact upon investigation of the facial areas, which reminisce Larry Katz's finding reported in Science 2003. ESP1 turned out to be a member of a previously unrecognized large family clustered in proximity to the class I MHC region. I herein report comprehensive genomic analysis of the ESP family in rodents and other species and discuss about an evolutionary aspect of the family. The genomic and cDNA analyses suggest that a single peptide is encoded by each gene with a three-exon/two-intron structure. Expression profile revealed that there existed male- and female-specific ESPs. Electro-vomeronasogram was performed for several ESP family peptides, providing insight into function of the family. We previously demonstrated that ESP1-induced c-Fos-positive VSNs expressed a V2R gene recognized by the V2Rp probe that potentially hybridized with several V2R genes. We narrowed down to one V2R, named V2Rp5, expressed in 100% of c-Fos-positive VSNs. The results suggest that ESP1 is recognized by a single type of V2R, and thereby, pheromone recognition in VNO appears to be narrowly tuned and highly specific.

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Complexity and Modularity in the Regulation of Chemoreceptor Gene Expression in *C. elegans*

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Each *C. elegans* chemosensory neuron expresses multiple chemoreceptor (CR) genes. A simple mechanism by which nematodes can rapidly modulate their sensory behaviors in response to changing environmental conditions is via modulation of expression of subsets of CR genes in individual chemosensory neurons. Previously, we and others showed that the expression of CRs can be altered in response to neuronal activity and environmental cues. This provides a simple mechanism by which *C. elegans* can rapidly alter its sensory behaviors in response to changing conditions. What are the molecular mechanisms regulating expression of CR genes? We previously showed that mutations in the salt-inducing kinase related *kin-29* gene result in downregulation of expression of a subset of CR genes. To define the mechanisms of KIN-29 function, we carried out genetic suppressor screens and isolated mutations in the transcription factor *mef-2* and the class II histone deacetylase *hda-4* genes. MEF2 and class II HDACs have been shown to regulate gene expression in response to intracellular signaling and electrical activity. We dissected the *cis*-regulatory sequences of KIN-29-regulated CRs and showed that MEF-2 binds directly to CR gene promoters and that MEF-2 binding sequences are both necessary and sufficient to confer KIN-29-mediated regulation onto CR genes. Modulation of CR gene expression requires phosphorylation of HDA-4, and we show that neuronal activity interacts with the KIN-29-regulated pathway to modulate CR gene expression. Taken together, our findings suggest a role for chromatin remodeling in response to activity and other signals in the regulation of CR gene expression.

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Atypical Membrane Topology and Heteromeric Function of *Drosophila* Odorant Receptors *In Vivo*

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Drosophila olfactory sensory neurons (OSNs) each express two odorant receptors (ORs): a divergent member of the OR family and the highly conserved, broadly expressed receptor OR83b. OR83b is essential for olfaction *in vivo* and enhances OR function *in vitro*, but the molecular mechanism by which it acts is unknown. Here we demonstrate that OR83b heterodimerizes with conventional ORs early in the endomembrane system in OSNs, couples these complexes to the conserved ciliary trafficking pathway, and is essential to maintain the ORs within the sensory cilia, where odor signal transduction occurs. The OR/OR83b complex is necessary and sufficient to promote functional reconstitution of odor-evoked signaling in sensory neurons that normally respond only to carbon dioxide. Unexpectedly, unlike all known vertebrate and nematode

chemosensory receptors, we find that *Drosophila* ORs and OR83b adopt a novel membrane topology with their N-termini and the most conserved loops in the cytoplasm. These loops mediate direct association of ORs with OR83b. Our results reveal that OR83b is a universal and integral part of the functional OR in *Drosophila*. This atypical heteromeric and topological design appears to be an insect-specific solution for odor recognition, making the OR/OR83b complex an attractive target for the development of highly selective insect repellents to disrupt olfactory-mediated host-seeking behaviors of insect disease vectors.

Givaudan Lecture

Fishing for Novel Genes

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Zebrafish are highly visual animals, exhibiting robust light responses after just 4 days of development, making them ideal for the genetic analysis of visual behaviors. They have large eyes and are tetrachromatic, possessing ultraviolet-sensitive cones as well as red-, green- and blue-sensitive cones. Our group has developed behavioral tests that can be used to uncover visual system specific mutations in zebrafish. One test, based on the optokinetic reflex, enables us to isolate recessive visual system mutations in 5- to 7-day-old larval fish that appear normal morphologically. We have isolated totally blind, partially blind, color blind and movement-defective mutant fish. A second test, based on the escape response, permits us to isolate dominant mutations in adult fish that demonstrate late onset retinal defects or degenerations. We also examine young mutagenized fish for developmental eye defects. Eye development is rapid in zebrafish; within 24 h postfertilization (pf), a well formed eye is present. Differentiation of the neural retina occurs between 1 and 3 days pf, so that by 3 days the retina appears functional. In this presentation, I shall describe representative behavioral and developmental mutants out of the more than 150 we have so far isolated. Other non-visual behaviors can also be elicited easily in zebrafish and hold promise for genetic analysis. For example, zebrafish show robust place conditioning to cocaine. After just one exposure, they repeatedly return to that part of the tank where the exposure first took place. So far, we have isolated three mutants that show altered sensitivity to cocaine. Behavioral tests for memory and learning in zebrafish have been developed in our laboratory and will also be discussed.

Slide: Ecology and Social Chemicals

Function of Odors and Chemosignals in Birds

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Bird responses to avian-derived odors challenge the traditional view that birds mediate social behavior primarily through sight and sound. Here I review several new and exciting discoveries regarding the ways in which odors affect bird behavior. The evidence indicates: 1) Odor production is widespread in birds.

2) Odors are linked to a variety of social situations, all of which involve adaptive behavioral responses, such as (a) discrimination between the scent of mates and conspecifics, including aversion to self-odor, (b) courtship and sexual selection, (c) nest building and homing to the odor signature of an individual's specific nest site, and (d) odor learning in chicks. Combined, the data suggest that an individual's odor profile may convey information relevant to complex behavioral responses, such as kin recognition, inbreeding avoidance, the genetic compatibility of a mate, and parasite-mediated sexual selection. The chemical signals of birds represent an entirely new mode of avian communication that has the potential to reveal new and interesting aspects of vertebrate behavioral ecology and sensory perception. Every bird studied thus far has a functional olfactory system, but we have yet to obtain a general understanding of how odors impact the day-to-day social behavior of any avian species. Future, interdisciplinary studies that explore avian odor chemistry, neuroanatomy and social behavior hold great promise, particularly those that compare avian scent to odor-based patterns that are well-documented in other vertebrate systems, such as mammals and fish.

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Slide: Ecology and Social Chemicals

Olfaction, Mechanoreception and Vision Are Used in the Location of a Turbulent Odor Source by a Benthic Shark

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Since turbulent dispersal causes many odor plumes to consist of chemical and mechanical (momentum) discontinuities, aquatic animals may localize the source of these odor plumes by simultaneous chemo- and mechanoreception: Eddy-chemotaxis. We wanted to examine the roles of olfaction, mechanoreception, and vision in food odor localization behaviors of a benthic shark, the smooth dogfish, *Mustelus canis*. Two turbulent plumes were created in a flume: a) squid rinse and b) seawater. The sources of odor and turbulence were physically separated by placing a brick downstream from each oozing odor source. The small odor sources created minor turbulence; each brick caused a major turbulent wake. Sides were alternated to account for side bias. Each shark ($n = 8$) was allowed to acclimate and then confined downstream while the plumes were established. After release, its behavior was monitored for 10 min. The number of strikes on each object was counted under two light conditions: fluorescent and infrared. Strikes on either target on the non-odor side were rare. On the odor side, all animals struck the source of turbulence significantly more than the actual source of odor. More strikes occurred in the light condition but preference for the source of odorous turbulence did not change. These results show that the smooth dogfish is using simultaneous information from its olfactory and turbulence detection systems when searching for food and that they may be using additional visual information. Preliminary experiments have shown that eliminating lateral line input causes serious disruption of localization behavior.

Support: DARPA.

Slide: Ecology and Social Chemicals

Sea Hare (*Aplysia californica*) Defensive Secretions Also Contain Pyrimidine and Other Alarm Cues that Warn Conspecifics of Nearby Predators

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When attacked by predators, such as spiny lobsters and sea anemones, the sea hare *Aplysia californica* releases ink and opaline. These secretions chemically defend *A. californica*, facilitating its 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. 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 four compounds in ink, three of which have been identified as the nucleosides uridine and cytidine, and the base uracil, separately elicit alarm behaviors in juvenile conspecifics. Two components in opaline also function as alarm cues. We are currently in the process of identifying these unknown molecules. In addition, the alarm response by *Aplysia californica* is not species specific, as *A. californica* respond to ink from the octopus *Octopus bimaculoides* and the squid *Loligunculus brevis*. Analyses of these inks demonstrate that they contain uridine and uracil, suggesting that these alarm cues are conserved among ink-producing mollusks.

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Slide: Ecology and Social Chemicals

Chemicals and Ecology: Multitrophic Predator Prey Interactions Mediated by Chemistry

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During development, sensory systems undergo changes in cell receptor machinery. Such modifications may alter the way an animal perceives its olfactory environment. Here we investigate a cannibalistic interaction between two discrete life history stages of the California newt (*Taricha torosa*). The defense compound tetrodotoxin (TTX), in adult newt skin is recognized by conspecific larvae as an avoidance signal. Yet, antipredator behavior is suppressed when TTX is mixed with odors from alternative adult prey. In laboratory assays, newt larvae were exposed to TTX alone, or in binary mixtures with test compounds isolated from invertebrate prey tissues. The larval escape response to TTX (0.1 μM) was significantly reduced when Arg (0.1–0.01 μM) was added. Free-ranging adult newts were exposed to components of prey tissue extracts in the field. Arg was the most attractive compound tested, evoking plume-tracking behavior at concentrations as low as 10 nM. A comparable array

of Arg analogs and TTX/Arg analog mixtures was tested on adults and larvae, respectively. Adult responses were eliminated by even slight alterations to Arg, such as the addition of a single carbon to the side chain or esterification of the α -carboxyl group. In contrast, larval responses to TTX were inhibited by Arg as well as by analogs with the guanidinium group. Thus, adults were more narrowly tuned than larvae to Arg analogs. These results show that Arg has opposing effects (inhibitory/stimulatory) on larval/adult newts, and apparently acts on different suites of olfactory receptors for individuals of the two, distinct, life history stages.

Slide: Ecology and Social Chemicals

Enantiomeric Pheromone Blends in Mammals: Asian Elephants and Bark Beetles Share Chiral Chemistry

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Frontalin (1,5-dimethyl-6,8-dioxabicyclo[3.2.1]octane) a bicyclic ketal of terpene origin can exist in two chiral or mirror image forms. Our recent finding that both enantiomeric forms of frontalin are present in the temporal gland secretions of male Asian elephants during musth mirrors that seen in a number of bark beetle species. Moreover changes in the ratio of the two forms has implications for chemical signalling in both diverse phyletic groups as important behavioral consequences are dependent on this. Our results suggest that stereochemical control of the enantiomeric ratio implemented during biosynthesis involving a putative dihydroxylation step provides modulation of the pheromonal message. This modulation is likely translated into a differential message at the level of pheromone reception based on the inherent chiral selectivity of receptor proteins.

Supported by ISAT.

Slide: Ecology and Social Chemicals

Behavioral Evidence that Goldfish Discern Mosaics of Pheromonal Odorants that Include Both Sex Hormone Derivatives and Bile Acids

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Although behavioral studies have established that many species of teleost fish employ species-specific pheromonal odors to mediate reproduction, all cues identified to date are common hormonal products incapable of imparting species-specific information. A possible explanation for this phenomenon is that fish discern mixtures or 'mosaics' of odorants which include compounds such as bile acids (taxon-specific steroids released via the fish gut). Here, we tested this possibility for the goldfish. Examining bile acid release, we found that while most fish release similar suites of bile acids, their ratios differ. Thus, goldfish release mostly cyprinol sulfate (a bile acid specific to minnows), trout release mostly taurocholic acid, and gour-

amis release mostly cholic acid. Examining the olfactory sensitivity and specificity of the goldfish olfactory system to several dozen bile acids using EOG recording, we next found that cyprinol sulfate is especially potent with a detection threshold of 10–11 M. Cross-adaptation studies found sensitivity to be highly specific. Finally, we found that behavioral responses of male goldfish to 15 keto-prostaglandin F2a (a pheromonal cue released by female goldfish) was strongly suppressed by the addition of bile acids only released by other species. In conclusion, odor mixtures seem important to natural pheromone function in fish.

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Slide: Ecology and Social Chemicals

Pheromonal Recognition Memory Induced by TRPC2-Independent Vomeronasal Sensing

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One of the best known examples of olfactory imprinting in adult vertebrates is the selective pregnancy block (or Bruce effect) in the mouse, which depends on the formation and maintenance of a pheromonal recognition memory by the vomeronasal system. Peptide ligands of major histocompatibility complex (MHC) molecules are the first identified vomeronasal stimuli that can mediate the Bruce effect, but the molecular mechanisms underlying this effect remain to be explored. The cation channel gene TRPC2 plays a critical role in the signal transduction mechanism of vomeronasal sensory neurons (VSNs) and TRPC2^{-/-} mice constitute an important genetic model for investigating the role of the vomeronasal organ (VNO) in mammalian pheromonal sensing. By using mice with a homozygous deficiency in TRPC2, we tested whether TRPC2 is essential for a pheromonal recognition memory. Surprisingly, the loss of the TRPC2 channel gene does not significantly influence the establishment of this memory, whereas, surgical lesions of the VNO do. Furthermore, field potential and single cell patch-clamp recordings show that TRPC2 is dispensable for the transduction of MHC peptide ligands by sensory neurons in the basal zone of the VNO. This indicates that a previously unrecognized TRPC2-independent signal transduction mechanism in the VNO underlies the formation of this pheromonal recognition memory.

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Symposium: Impact of Odorant Metabolism on Scent Perception

Odorant/Pheromone Metabolism in Insects

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Signal termination plays a critical role in all chemically mediated biological processes, and this is no less so in odor detection. The process of insect pheromone degradation has been studied for some years, at least as far back as Kasang (1971) in *Bombyx mori* and Ferkovich *et al.* (1973) in *Trichoplusia ni*. Since then, a few

pheromone and odor degrading enzymes (ODEs) have been identified and characterized in detail and the general principal of odor degradation has become well established. One reason to expand efforts studying ODEs is their potential in insect control. If it is true that pheromones and odors in general are perceived as precise mixtures, then the targeted inhibition of the ODE for a specific component should alter the blend ratio within a sensillum resulting in misperception of the odor. Of the three protein classes with which odors interact (ORs, OBPs and ODEs), ODEs may be the least specific and thus the more generally targetable protein for behavioral inhibition. ODEs characterized include extracellular soluble enzymes localized in the compartmentalized fluid that surrounds the olfactory neurons, enzymes associating with the neuron or support cell membranes, cytosolic enzymes which may serve the dual purpose of inactivating xenobiotics, and body surface enzymes insuring that adsorbed odors do not desorb and become false signals. Enzymes may be sex specific suggesting roles targeting pheromones; others may be sex indifferent suggesting broader roles. Individual species may have multiple but parallel metabolic pathways. The complexity and diversity of odor degrading processes suggests strong evolutionary selection towards noise reduction (rapid removal of accumulated odor signal).

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Symposium: Impact of Odorant Metabolism on Scent Perception

Mammalian Nasal P450 Enzymes and Odorant Metabolism

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The role of mammalian nasal biotransformation enzymes, such as the family of cytochrome P450 (P450) monooxygenases, in olfactory chemoreception has been a subject of much speculation. Nasal metabolism may influence the levels of odorants in the olfactory receptor environment, activate non-odorants to odorants, convert odorants to non-odorants, or change an odorant to a ligand for a differing odorant receptor. Earlier studies have concentrated on the identification and pharmacological characterization of the numerous P450s, as well as other biotransformation enzymes, expressed in the olfactory mucosa. These studies, fueled by findings of tissue-specific, abundant, and relatively early developmental expression of select P450 genes, demonstrated the *in vitro* activity of the olfactory mucosa to transform inhaled chemicals to metabolites that potentially differ from the parent compounds in olfactory potency and odor quality. However, *in vivo* evidence for a chemosensory function of the nasal P450s has been difficult to obtain. Recently, my laboratory has been developing knockout mouse models that can be used to demonstrate the roles of nasal P450 enzymes in odorant metabolism and odor detection. These mice have either a germ-line deletion of one or more P450 genes that are abundantly expressed in the nose, such as the olfactory mucosa-specific Cyp2g1, or a very low expression of the cytochrome P450 reductase (CPR), which is required for the function of all microsomal P450 enzymes. The unique features of these mouse models, and potential confounding factors for application to odorant metabolism and chemosensory studies, will be discussed.

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Symposium: Impact of Odorant Metabolism on Scent Perception

Odorant Metabolism in the Human Nose

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The initial events taking place in odor recognition have been studied extensively in recent years primarily at the olfactory receptor and olfactory bulb level. Much less attention has been paid to peri-receptor events that may influence the responsiveness of receptors to olfactory stimuli. Occurrence of enzymatic reactions in nasal tissue has been described for rodents in connection with xenobiotic metabolism and toxicity. A human P450 enzyme (CYP2A13) that is predominantly expressed in nasal tissue has been characterized, and additional biotransformation enzyme genes that are expressed in human olfactory epithelium have been described. Several studies were conducted to address the question whether or not such metabolism is changing the odorant quality and if such peri-receptor events are increasing the chemical variability of potential receptor ligands in the nose. *In vitro* enzymatic studies were used to identify substrates and inhibitors of nasal P450 enzymes which exhibit broad substrate specificity. *In vivo* studies were designed to monitor metabolite formation in real time by detecting metabolites in exhaled air using mass spectrometry (APCI-MS). Ultimately, sensory tests using substrates and inhibitors of P450 enzymes showed that enzymatic metabolism can influence the quality of a provided odorant. The latter studies also showed that there seems to be variability in the extent of metabolism among individuals. The results indicate that in-nose biotransformation of odorants can modify the quality and quantity of compounds reaching the olfactory mucosa, and those events may have to be taken into consideration when interpreting SAR, SOR and OB-imaging results.

Symposium: Impact of Odorant Metabolism on Scent Perception

Flavor Metabolism in the Oral Cavity

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Prolonged retronasal aroma perception, called aftertaste or better "aftersmell," is relevant for food consumption, but also for medical or cosmetic purposes, such as usage of mouthwashes or toothpastes. Also, undesired aftersmell impressions as from cigarettes or onions are part of this phenomenon. Various factors influence the dwell time of odorants within the oral cavity. This study highlights the influence of odorant adsorption to oral mucosa, and that of odorant degradation by saliva. It was shown that odorants can be effectively metabolized, depending on the odorant concentrations and the food composition. The turn-over rates were highly dependent on the chemical structures of the odorants, with significant differences in metabolization for diverse substance classes, e.g., esters, thiols, aldehydes, etc. These results were correlated with sensory experiments, as well as with quantitations by means of spit-off odorant measurement (SOOM) and buccal odor screening system (BOSS)

techniques. Both methodologies monitor trace key odorant adsorption to oral mucosa and subsequent release under *in vivo* conditions. Using this analytical concept, salivary odorant metabolization together with adsorption to mucosal tissue were found to play a decisive role in aftersmell.

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Poster: Peripheral Olfaction and Peripheral Taste

Lipid Rafts Organize Chemosensory Signal Transduction Molecules in the Chemoattraction of Paramecium

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Lipid rafts are membrane microdomains that are enriched in cholesterol, glycosphingolipids and GPI-anchored proteins. They can serve as platforms of protein-protein interactions which organize signal transduction components. According to biochemical criteria, lipid rafts are in the cell body membrane of Paramecium. Cold Triton X-100 insoluble rafts and cytoskeleton bound membrane proteins can be separated in sucrose gradients into fractions by the density. These membrane proteins include the GPI-anchored surface antigens, the GPI-anchored folate chemoreceptor and plasma membrane calcium pump isoform 2 (PMCA2). In immunofluorescent microscopy studies, all these proteins are co-localized to the base of cilia. Methyl β cyclodextrin (M β CD) treatment of Paramecium can sequester the cholesterol molecules from plasma membrane and consequently affects the composition in lipid rafts. This reduction of cholesterol disrupts the localization of the surface antigens and PMCA2 to the base of cilia, but not the folate chemoreceptor. M β CD affects the distribution of PMCA2 and the surface antigens in the rafts in the sucrose gradient fractions, but not the folate chemoreceptors. M β CD treatment also perturbs chemoattraction of Paramecium to glutamate and cyclic AMP, but not folate. There are large differences in the cholesterol dependence of the chemoreceptors and PMCA2. PMCA2 but not PMCA3 is in the rafts. PMCA2 disperses across the cell surface and is no longer in the rafts fractions when cholesterol is reduced. However, not all chemoresponses are disrupted with cholesterol reduction leading us to a model of 3 kinds of membrane domains and signaling organization.

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Poster: Peripheral Olfaction and Peripheral Taste

The CNGB1 Subunit Is Required for Normal Olfaction and Localization of the CNG Channel to Olfactory Cilia

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When exposed to odorants olfactory receptor neurons (ORNs) respond with an increase in cAMP, which leads to the opening of cyclic nucleotide-gated (CNG) channels, a channel consisting of a principal and two modulatory subunits (CNGB1, CNGB2, and CNGB3, respectively). Here, we analyzed the functional relevance of CNGB1 by gene targeting in mice. Compared to wild-type mice, CNGB1^{-/-} mice exhibited profoundly decreased olfactory behavioral performance and reduced electro-olfactogram responses, the latter also showing slow onset as well as recovery kinetics. ORNs of CNGB1^{-/-} mice weakly expressed a CNGB1/CNGB2 channel with decreased cAMP sensitivity, very rapid flicker-gating behavior and no fast inhibition by Ca²⁺-calmodulin. Interestingly, CNG channels lacking either CNGB1 or CNGB2 failed to be trafficked to olfactory cilia, but were normally targeted to the plasma membrane of olfactory knobs. Inhibition of the proteasome *in vivo* caused accumulation of mutant CNG channels at the subciliary region but did not increase expression levels in the cilia. Together, this suggests that ORNs have developed an efficient control mechanism ensuring that only correctly assembled CNG channels are targeted to the cilia.

Poster: Peripheral Olfaction and Peripheral Taste

Ciliary Targeting of Olfactory CNG Channels Requires the CNGB1B Subunit and the Kinesin Motor Protein, KIF17

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Cilia are complex, microtubule-rich, organelles uniquely adapted for diverse cellular functions, and defects in cilia structure and function have been shown to underlie a number of human diseases. Cilia from olfactory sensory neurons (OSNs) compartmentalize signaling molecules, including odorant receptors and cyclic nucleotide-gated channels that are the primary targets of odorant-induced signaling. Despite a wealth of knowledge about CNG channel structure and function, little is known about the mechanisms of their subcellular targeting. Using MDCK cells as a model system, we show that heteromeric assembly with the CNGB1b subunit is required for trafficking of olfactory CNG channels to primary cilia. CNGB1b contains a carboxy-terminal motif that is necessary, but not sufficient, for ciliary enrichment. CNG channel localization is also dependent on the kinesin motor protein, KIF-17, which is endogenous to both MDCK cells and OSNs. The dynamics of CNG channel movement in cilia show a substantial mobile fraction with bidirectional movement. These results define the role of subunit composition and specific targeting motifs in olfactory CNG channel trafficking and provide novel insights into mechanisms of mammalian ciliary transport.

Poster: Peripheral Olfaction and Peripheral Taste**Clustering of Cyclic-Nucleotide-Gated Channels in Olfactory Cilia**R.J. Flannery¹, D.A. French² and S.J. Kleene¹¹Cell Biology, Neurobiology, and Anatomy, University of Cincinnati, Cincinnati, OH, USA and ²Mathematical Sciences, University of Cincinnati, Cincinnati, OH, USA

Olfactory cilia contain the known components of olfactory signal transduction, including a high density of cyclic-nucleotide-gated (CNG) channels. CNG channels play an important role in mediating odor detection. The channels are activated by cAMP, which is formed by a G-protein-coupled transduction cascade. Frog olfactory cilia are 25–200 μm in length, so the spatial distribution of CNG channels along the length should be important in determining the sensitivity of odor detection. We have recorded from excised cilia and modeled diffusion of cAMP into a cilium in order to determine the spatial distribution of the CNG channels along the ciliary length. The proximal segment, which in frog is the first 20% of the cilium, appears to express a small fraction of the CNG channels, while the distal segment contains the majority, mostly clustered in one region. This is consistent with a previous electron micrographic study in rat that found CNG channels to be primarily located in distal segments.

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Poster: Peripheral Olfaction and Peripheral Taste**Mechanism of Signal Amplification in the Newt Olfactory Sensory Cilia**

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Molecular mechanisms underlying olfactory signal amplification were investigated by monitoring cAMP dynamics in the intact sensory cilia. We saw that [cAMP]_i increased superlinearly with time during odorant stimulation for >1 s. This course was remarkably different from that obtained with the rapid quench method previously applied to the *in vitro* preparation, in which [cAMP]_i change has been reported to be transient. The superlinear increase of [cAMP]_i was attributable to a gradual increase of cAMP production rate that was consistent with the thermodynamical interaction model between elemental molecules, as has been revealed on the rod photoreceptor cell. It seems likely that the fundamental mechanism for molecular interactions between olfactory transduction elements is similar to that of the rod. In olfaction, however, cAMP production was extremely small (an order of 2×10^4 molecules/s/cilium at the maximum), in contrast to the cGMP hydrolysis in the rod (10^4 – 10^5 molecules/photon). The observed numbers indicate that the olfactory receptor cell (ORC) has lower amplification at the enzymatic cascade. Seemingly, such low amplification is a disadvantage for the signal transduction, but this unique mechanism would be essential to reduce the loss of ATP. Apparently, transduction by a smaller number of second messenger formations would be achieved by the fine ciliary structure that has surface-volume ratio.

It is speculated that this low amplification at their enzymatic processes may be the reason why that ORC has acquired high amplification at the final stage of transduction channels, using Ca²⁺ as a third messenger.

Poster: Peripheral Olfaction and Peripheral Taste**Sensitivity of Lobster Olfactory Receptor Neurons to Amiloride Derivatives**

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Amiloride and some amiloride derivatives (ADs) inhibit a variety of sensory transduction processes in vertebrates. Here we report that odorant-evoked activity in lobster olfactory receptor neurons (ORNs) *in situ* can be suppressed by ADs. ADs reversibly inhibit the response of both the tonically- and rhythmically-active subpopulations of ORNs to odorants in a concentration-dependent manner with a potency sequence of (K_{1/2}, mM): Methylisobutylamiloride (MIA, 0.02) > Ethyliso-propylamiloride (EIPA, 0.03) > Phenamil methanesulfonate (>0.5) > Amiloride (>1) ~ Diltiazem (>1). The TRP-related sodium-gated nonselective cation (SGC) channel, the likely downstream target of phosphoinositide signaling in these cells, is also blocked by ADs. ADs reversibly block both the Ca²⁺-sensitive and Ca²⁺-insensitive forms of the SGC channel in a voltage- and calcium-independent manner. While ADs nonspecifically block other potential components of sensory signaling cascades, e.g., Na⁺/H⁺-, Na⁺/Ca²⁺- exchangers, our results suggest the 5-amino substitutes (pyrazine derivatives) of amiloride could be a useful tool to study olfactory transduction in this system and possibly in other systems with TRP channel effectors. We are currently identifying the mechanism of action of ADs on the SGC channel and other potential targets of ADs in these cells.

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Poster: Peripheral Olfaction and Peripheral Taste**Plasma Membrane Calcium Pumps' Role in Calcium Removal in Mouse Olfactory Neurons**

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Intracellular free calcium rises in olfactory sensory neurons (OSNs) as they respond to odorants and depolarizing stimuli as cyclic nucleotide gated channels and voltage dependent calcium channels are activated. Calcium, in turn, affects important signal transduction components in the cells, such as chloride channels. The removal of calcium following stimulation depends in part upon the calcium-sodium exchangers, as several labs have demonstrated. However, plasma membrane calcium pumps (PMCA) also contribute to calcium removal. We have demonstrated the presence of all 4 isoforms of mouse PMCA in mouse OSNs through deconvolution microscopy and RT-PCR of sorted GFP-OMP labeled cells. We have since identified the splice variants of the isoforms that are expressed. These include PMCA2b, the isoform variant with the highest affinity for calcium. Here we also describe the changes in

free calcium in response to depolarizing stimuli in cell bodies and dendritic knobs of the OSNs of wild type mice and those with no functional gene for PMCA2, one of the four isoforms. The cells from the heterozygote and wild type show very similar kinetics of calcium removal, but the knock out mice show a significantly reduced rate of calcium removal. Also, wild type OSNs treated with carboxyeosin, a general PMCA inhibitor, the rate of removal is reduced. The behavior of the knock out mice in response to odorants is under investigation.

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Poster: Peripheral Olfaction and Peripheral Taste

Calcium ATPase in Olfactory Cilia

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Olfactory sensory neurons (OSNs) respond to odorants increasing intraciliary Ca^{2+} concentration. Calcium enters the olfactory cilia through cyclic nucleotide-gated channels and activates Cl^- or K^+ channels. The removal of Ca^{2+} gained during the odor response is thought to be attained by a $\text{Na}^{2+}/\text{Ca}^{2+}$ exchanger (NCX). Immunoblots of purified rat olfactory cilia membranes revealed that they are enriched in plasma membrane Ca^{2+} -ATPase (PMCA), besides NCX. Immunocytochemistry of toad OSNs confirmed the ciliary presence of PMCA, where it co-localizes with NCX. We determined an ATP-dependent Ca^{2+} transport in inside-out ciliary membrane vesicles preloaded with Fluo 5N. Pump activity vs. $[\text{Ca}^{2+}]$ curve exhibits a bell shape, which peaks at $\sim 5 \mu\text{M}$. Carboxyeosin ($50 \mu\text{M}$), specific PMCA blocker, reduced Ca^{2+} transport. Calmidazolium ($10 \mu\text{M}$), a calmodulin (CaM) blocker, decreased PMCA activity, whereas the addition of CaM increased it ($K_{0.5} = 31 \text{ nM}$). The results indicate the ciliary presence of a CaM-dependent PMCA. We propose that both Ca^{2+} transport proteins, PMCA and NCX, work in a complementary fashion to extrude Ca^{2+} from the cilia during the odorant response.

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Poster: Peripheral Olfaction and Peripheral Taste

Effect of External Na^+ on Na-Ca Exchange-Mediated Current Recovery in Frog ORNs

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During the olfactory response, Ca^{2+} enters through CNG channels, opening Ca^{2+} activated Cl^- channels which augment the depolarising current. The dependence of Ca^{2+} extrusion upon external $[\text{Na}^+]$ was studied using the decay of CaCl current to monitor the recovery of $[\text{Ca}^{2+}]$ following a brief exposure to the PDE inhibitor IBMX, which elevates ciliary $[\text{cAMP}]$. The solution bathing the cilia was rapidly exchanged by translating the suction pipette, which recorded receptor current, between solution streams. Cilia were exposed for 1 s to $100 \mu\text{M}$ IBMX, and returned to IBMX-free solution, allowing the receptor current to decay with monoexponential

kinetics. The decay time constant (τ_c) was greatly prolonged by a factor of 34.6 ± 8.2 if $[\text{Na}^+]_o$ was reduced to 10% (11 mM) of its value in Ringer by substitution with guanidinium, an ion which permeates the CNG channel but does not support Na-Ca exchange. When the cilia were returned to Ringer after 3 s in low- Na^+ solution, the τ_c was similar to that when returning immediately to IBMX-free Ringer, suggesting that Ca^{2+} extrusion via Na-Ca exchange dominates current decay, since $[\text{cAMP}]$ falls rapidly after IBMX removal. The τ_c was remarkably insensitive to $[\text{Na}^+]_o$, being substantially retarded only after reduction to third or less of that in Ringer. Rate constants at different $[\text{Na}^+]_o$ were fitted by a Hill equation with Hill coefficient of 3.1, suggesting a stoichiometry of $3\text{Na}^+ - 1\text{Ca}^{2+}$ for the exchanger. A K_d of 58.7 mM indicates that Na-Ca exchange in frog ORN's is surprisingly insensitive to $[\text{Na}^+]_o$, in contrast to the photoreceptor and cardiac exchangers. The high affinity of the olfactory exchanger for external Na^+ allows normal response termination even following mucus dilution.

Poster: Peripheral Olfaction and Peripheral Taste

Investigating the Role of Sodium Calcium Exchangers in Cultured Human Olfactory Cells

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Olfactory receptor neurons (ORNs) respond to odorants with changes in intracellular calcium concentrations ($[\text{Ca}^{2+}]_i$). In human ORNs, about one-fourth of these responses are decreases in $[\text{Ca}^{2+}]_i$; thus this type of response represents a large portion of the input into the olfactory bulb, yet the mechanism behind this type of response is unknown. We therefore investigated the involvement of $\text{Na}^+/\text{Ca}^{2+}$ exchangers (NCX) in this system, and used human olfactory cultured cells due to their availability, ease of use, and because they demonstrate structural and functional characteristics that are similar to those found in acutely isolated human ORNs. Cells were grown *in vitro* on 6-well plates and tested with odorants in the presence or absence of extracellular Na^+ ; responses were measured using calcium imaging techniques. Immediately following imaging, cells were localized on the culture plate, fixed, and tested with immunocytochemistry using antibodies against NCX. Our results have shown that NCX is present and functional in these cells, suggesting that this may be the primary mechanism for mediating $[\text{Ca}^{2+}]_i$ decreases. An understanding of the mechanism responsible for generating $[\text{Ca}^{2+}]_i$ changes may have important implications for the study of the physiology of olfaction.

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Poster: Peripheral Olfaction and Peripheral Taste

Mechanisms of Chloride Accumulation in Intact Mouse Olfactory Epithelium

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A depolarizing chloride current is a substantial part of the olfactory response to odors; this requires a mechanism for accumulation of

chloride against an electrochemical gradient. The sodium-potassium-chloride exchanger NKCC1 has been shown to play an important role in this process. However, two other classes of transporter that might play a role in chloride accumulation are common. We used the electroolfactogram (EOG) to investigate the mechanisms of chloride accumulation in intact mouse olfactory epithelium. In mice lacking the NKCC1 gene, an EOG was present that was 60% of the amplitude of the EOG in wild-type mice. Niflumic acid, a chloride channel blocker, reduced this response by 80% in both wild-type and knockout mice. Bumetanide, a blocker of NKCC, reduced the amplitude of the EOG in wild-type epithelium by 53%. There was no effect of bumetanide on the EOG in mice lacking NKCC1. These results strongly suggest that NKCC1 is a major part of a more complex system of chloride accumulation. Hence we are testing other blockers of chloride transport. DIDS (1 mM), a blocker of chloride-bicarbonate exchangers, reduced the EOG amplitude by about 60% in both wild-type and NKCC1 knock-out mice. In frog, DIDS also blocks the ciliary chloride channels by 23%, but this is not sufficient to account for the reduction of the EOG by DIDS. Thus, it is probable that both NKCC and chloride-bicarbonate transporters contribute to maintenance of the chloride gradient in olfactory neurons, but further experiments are necessary to fully understand the system.

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Poster: Peripheral Olfaction and Peripheral Taste

Dual Effect of ATP in the Olfactory Epithelium of *Xenopus laevis* Tadpoles: Activation of Both Receptor and Sustentacular Supporting Cells

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Nucleotides and amino acids are acknowledged categories of waterborne olfactory stimuli. In previous studies it has been shown that larvae of *Xenopus laevis* are able to sense amino acids. Here we report on the effect of ATP in the olfactory epithelium (OE) of *Xenopus laevis* tadpoles. First, ATP activates a subpopulation of cells in the OE. The ATP-sensitive subset of cells is almost perfectly disjoint from the subset of amino acid-activated cells. Both responses are not mediated by the well-described cAMP transduction pathway as the two subpopulations of cells do not overlap with a third, forskolin-activated subpopulation. We further show that in contrast to amino acids, which act exclusively as olfactory stimuli, ATP appears to feature a second role. Surprisingly it activated a large number of sustentacular supporting cells (SCs), and to a much lower extent olfactory receptor neurons. The cells of the amino acid- and ATP-responding subsets featured differences in shape, size and position in the OE. The latencies to activation upon stimulus application differed markedly in these subsets. To obtain these results two technical points were important. We used a novel dextran-tetramethylrhodamine backfilled slice preparation of the OE and we found out that an antibody to calnexin, a known molecular chaperone, also labels SCs. Our findings thus show a strong effect of ATP in the OE and we discuss some of the possible physiological functions of nucleotides in the OE.

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Poster: Peripheral Olfaction and Peripheral Taste

Expression of the GABA Pi Subunit in the Olfactory Epithelium

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Objective. A recent microarray analysis of enriched preparations of mouse ORNs indicated an increased expression of the peripheral GABA A receptor subunit, GABA pi (GABRP), suggesting that GABA A receptors may function in the olfactory epithelium of mammals. The present study was undertaken to confirm these microarray results by examining the location of expression of the GABA pi subunit at the level of mRNA and protein. **Methods.** The expression of the GABA pi subunit was determined in the olfactory epithelium of 21-24 day old C57/BL6 mice by *in situ* hybridization using sense (control) and antisense dioxygenin-labeled riboprobes. mRNA transcripts of other GABA A subunits was determined on cDNA prepared from olfactory epithelium total RNA and oligonucleotide primers designed to amplify cDNA fragments of alpha(1-6), beta(1-3), and gamma(1-3) subunits. The GABA pi protein was determined in Western blots on homogenates of olfactory epithelium. **Results.** A hybridization signal for GABA pi subunit was detected uniformly over the four zones of the olfactory epithelium in presumably mature ORNs and in a subpopulation of cells in the respiratory epithelium. Sense probes were negative, as were sustentacular cells, Bowman's glands, and basal cells. GABA pi protein was also detected as a single band in Western blots. The presence of other GABA A subunits was indicated by generation of fragments of the appropriate size for alpha 1, alpha 2, alpha 4, beta 2, beta 3, gamma 2, and gamma 3 subunit by RT-PCR. We conclude that GABA pi is one subunit of a GABA A receptor that may function other than in presynaptic inhibition of ORNs.

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Poster: Peripheral Olfaction and Peripheral Taste

Acetylcholine Modulates Activity in the Olfactory Epithelium in Axolotls, *Ambystoma mexicanum*

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Research on peripheral odorant processing tends to focus on individual cells; the role of multicellular interactions, such as those involved in modulation, has received less attention. The terminal nerve, which extends between the nasal cavity and preoptic area, contains modulatory peptides, including gonadotropin releasing hormone and neuropeptide Y. Acetylcholinesterase histochemistry in a variety of vertebrates suggests that the terminal nerve may also contain acetylcholine (ACh). We are using immunocytochemistry to verify that the terminal nerve contains ACh and that the olfactory epithelium contains ACh receptors. Preliminary results with axolotls ($n = 2$) indicate that the terminal nerve can be labeled with an antiserum directed against vesicular ACh transporter. We have obtained robust labeling in the olfactory epithelium using a nonspecific antiserum directed against all muscarinic ACh receptor subtypes, but not with one directed against neuronal nicotinic ACh receptors. Whole-cell voltage clamp recordings from olfactory receptor neurons in epithelial slices indicate that bath-applied ACh (1–10 μ M) alters the magnitude of

voltage-activated inward and outward currents and that the magnitude returns toward baseline within 10 min of washing off the ACh. In addition, we recently began using electro-olfactogram recordings to examine the effects of ACh on odorant responses evoked by L-glutamic acid (100 μ l at 1 mM). Our preliminary data suggest that, like other terminal nerve-derived compounds, ACh modulates odorant responses in the olfactory epithelium.

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Poster: Peripheral Olfaction and Peripheral Taste

Investigations on Presence and Function of Nitric Oxide in the Murine Olfactory System

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The small gaseous signalling molecule nitric oxide (NO) is involved in various physiological processes including regulation of blood pressure, immunocytotoxicity and neurotransmission. In the mammalian olfactory bulb, NO seems to play a role in formation of olfactory memory related to pheromones as well as to conventional odorants. In the peripheral olfactory system, NO generated by the neuronal isoform of NO synthase (nNOS) and expressed solely during development and regeneration, seems to regulate neurogenesis in the olfactory epithelium as well as axonal outgrowth of the olfactory receptor neurons. However, an implication of NO in olfactory signal transduction has not been demonstrated yet. Here we show for the first time the expression of the endothelial isoform of NO synthase (eNOS) in mature olfactory sensory neurons (OSNs) of adult mice. We report that in these cells eNOS is able to produce NO in a stimulus and Ca²⁺-dependent way, thereby affecting the desensitization of odor responses. Immunocytochemistry using eNOS-specific antibodies revealed developmentally regulated expression of the endothelial NOS isoform in adult OSNs. We found that NO was liberated from OSNs in response to odor or depolarization in wild type, but not in eNOS deficient mice, pointing to eNOS being the enzyme responsible for activity dependent NO production in OSN. Analyzing EOG recordings from wild type and eNOS^{-/-} mice revealed a significant role for NO in modulation of temporal aspects of olfactory signal processing and desensitization of odorant-induced signals. In summary, we found evidence for presence and function of eNOS in mammalian olfactory sensory neurons and propose NO as a novel player in olfactory signal transduction.

Poster: Peripheral Olfaction and Peripheral Taste

Anatomical and Functional Evidences for a Neuromodulatory Role of Endothelium on the Olfactory Mucosa Cells

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Besides its sensory function, the olfactory mucosa (OM) undergoes regulatory and homeostatic controls, finely tuned by the physiological status of the animal. In search of regulatory factors, we identified several peptides and their receptors in the OM, including orexine, leptine, AVP and endothelium. To characterize their modulatory roles, trans-

duction pathways and possible interactions with the olfactory signal, we developed an *in vitro* primary culture of rat OM cells, and used molecular and cellular approaches, including immunocytochemistry, RT-PCR and real-time measurement of intracellular Ca²⁺ concentrations. Among others, endothelium (ET) shows major effects on the different OM cell types. Both ET-receptors (A and B), endothelium converting enzyme, and their mRNAs are present in OM cells. Moreover, ET triggers a robust, dose-dependent, intracellular Ca²⁺ response in primary cultured OM cells: a transient peak, which can extend in a sustained plateau-phase. Olfactory neurons display only transient Ca²⁺ responses, mediated by ETB receptors, whereas sustentacular cells display both transient and plateau-type Ca²⁺ responses, generated by lower ET concentrations through ETA receptors. Both types of response are triggered by a PLC/InsP3-dependent intracellular calcium release; moreover the plateau-phase also significantly depends upon calcium influx, suggesting an additional transduction pathway. Our results show that endothelium acts on different OM cells, triggering different intracellular Ca²⁺ responses, suggesting possible roles either as a neuromodulator of the olfactory signal or as a differentiation/survival factor, which remain to be explored.

Poster: Peripheral Olfaction and Peripheral Taste

Bursting with Odor: Intrinsically Oscillating Olfactory Receptor Neurons

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Pacemaker and/or intrinsically oscillating neurons are fundamental to neuronal network function but generally have not been considered in the context of primary sensory signaling. Here, we report a novel subpopulation of lobster primary olfactory receptor neurons (ORNs) that exhibit spontaneous, rhythmic bursts of action potentials between 0.02 to 0.9 bursts/s. The bursting is intrinsic: the structure of the bursts and the inherent frequency of bursting are consistent for any given cell, bursting is sensitive to membrane potential and Ca²⁺, and pharmacological treatment presumably targeting HCN channels that frequently underlie neural oscillations (ZD7288, 200M) disrupts bursting. The ORNs can be entrained by odorants. Odorants transiently applied to the cells evoke bursts similar to the spontaneous bursts in phase-dependent manner. The efficacy of entrainment is concentration dependent: more intense stimuli cause the cell to discharge earlier in the cycle. Synchronization of the bursting ORN ensemble by periodic stimulus acquisition such as sniffing would effectively enhance the detection and amplification of weak signals, generally assumed to be one of the hallmarks of olfaction.

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Poster: Peripheral Olfaction and Peripheral Taste

Use of Ciliary Beat Frequency for Measuring Chemoresponse in *Paramecium*

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Swimming behavior in *Paramecium* is dependant on the direction and frequency of ciliary beating. When *Paramecium* encounter a chemoattractant, the membrane hyperpolarizes and ciliary beat frequency increases, while the frequency of action potentials decreases. Chemorepellants cause a depolarization and can increase the frequency and/or duration of action potentials that cause a reversal of ciliary beat. Chemoresponse in *Paramecium* is measured by population-based assays such as the T-Maze or the capillary tube assay or by scoring the behavior of individual cells in backward-swimming or avoidance reaction tests. Analog videotape has been analyzed by various means to quantify swimming speed and turning or to measure ciliary beat frequency (CBF). We have utilized a novel digital analysis system to accurately quantify CBF in immobilized *Paramecium*. This system yields data similar to analog systems at beat frequencies less than 15 Hz, but is significantly more accurate at faster beat rates. This digital system also reduces data analysis time from hours to minutes. Our immobilization method, adherence to coverslips using a biological adhesive, resulted in low mortality and CBF in unstimulated cells similar to that collected by analog video analysis. When cells were exposed to the chemoattractant molecules acetate and ammonia, CBF increased significantly. Hyperpolarizing the membrane by reducing external potassium ion concentration also increased CBF, as expected. Acetate concentrations that caused measurable changes in CBF are similar to the minimal concentrations required to detect population-based attraction in T-Maze assays.

Poster: Peripheral Olfaction and Peripheral Taste

Implantable Neural Interfaces for Characterizing Population Responses to Odorants and Electrical Stimuli in the Nurse Shark, *Ginglymostoma cirratum*

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The objective of this study is to develop short- and long-term implantable neural interfaces within the central nervous system of sharks. Nurse sharks were wild-caught in the Florida Keys and kept in holding facilities at Albion College. Animals were placed in a custom stereotaxic tank and anesthetized with MS-222 (Sigma, 100 mg/kg) in artificial seawater (Instant Ocean, Aquarium Systems, Inc.), the gills continuously perfused. A surgical access of ~5 cm by ~2.5 cm was made to expose the right olfactory rosette, bulb, tract, and lobe. Following surgical access, animals were paralyzed (pancuronium bromide, 0.9 mg/kg, IM in elasmobranch ringer's), anesthesia removed, and vital signs monitored. Two arrays of 16 microelectrodes were placed in the olfactory bulb and lobe, and two Ag|AgCl electrodes placed within the olfactory rosette through the inlet naris. Single-unit and local field potential (LFP) activity was recorded in the olfactory lobe. The median single-unit spontaneous activity in the lobe was 0.4 imp/s in the awake animal. Electrical stimulation (monophasic, bipolar, 300 μ A) of the seawater space within the rosette produced electrically-driven LFP and single-unit spike activity in the olfactory lobe with a latency of 300–450 ms.

A five channel odorant delivery system will allow us to characterize odorant responses simultaneously in the olfactory bulb and lobe.

Support provided by DARPA Bio-inspired undersea sensors program HR0011-05-C-0018.

Poster: Peripheral Olfaction and Peripheral Taste

Responses of Spontaneously Inactive Olfactory Receptor Neurons Correlate with EOG in Black Bullhead Catfish (*Ameiurus melas*)

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We investigated electrophysiological responses of the olfactory organ of black bullhead catfish to amino acids in water with low ion concentrations ($R > 10^6 \Omega\text{cm}$). Prior to stimulation, olfactory receptor neurons (ORNs) were either spontaneously active or inactive. In previous studies, single spontaneously active ORNs' responses to amino acids were unpredictable over successive tests and did not correlate with the relative magnitude of the EOG response. In the present study, the number of spontaneously inactive ORNs responding to amino acids correlated highly with the amplitude of EOG (Pearson $R = 0.9$, $P < 0.001$; 10 amino acids). Both the EOG and single unit responses indicated that L-Met and L-nVal are the most stimulatory amino acids. The spontaneously inactive ORNs responded to increasing L-nVal concentrations with an increasing duration of action potential activity; the maximum frequency of action potentials occurred at concentrations that were ~10 times higher than thresholds (10^{-7} – 10^{-4} M for different ORNs). The number of responding ORNs correlated highly with the relative peak amplitude of the EOG at the same concentrations (Pearson $R = 0.97$, $P < 0.05$). Mean ratios (~0.5) for ORNs' response durations at lower and 10 times higher L-nVal concentrations indicated the durations' dose-dependence. Repeatable responses of spontaneously inactive ORNs to amino acids, their dose-dependence and high correlations with the EOG suggest that these units are important in the coding of amino acid quality and quantity.

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Poster: Peripheral Olfaction and Peripheral Taste

Cultured Olfactory Receptor Neurons Show Summation, Adaptation, and Age-Related Differences in EOG Response Kinetics

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We investigated EOG responses of olfactory receptor neurons in organotypic olfactory epithelium (OE)-olfactory bulb (OB) cultures. We exposed cultures to charcoal-filtered air, individual odorants, and a mixture of (+) and (-) carvone, eugenol, and ethyl butyrate and recorded EOG potentials. We characterized OMP

and β -tubulin expression in the same cultures with fluorescence immunocytochemistry. EOG responses of cultured OE were similar to those of acutely dissected OE. Cultures 3–4 days old ($n = 8$) showed rapid rise times but prolonged decay times. Cultures 13–15 days old ($n = 8$) showed both rapid rise and decay times. All cultures responded to the four odorants when presented individually, but their responses were variable; ethyl butyrate elicited the strongest response on average. At high frequency stimulation with an interstimulus interval (ISI) of 200 ms, the EOG responses summated. With a longer ISI of 800 ms, adaptation occurred with the second and succeeding responses of lower amplitude than the first. The response amplitude only fully recovered when the duration of the ISI reached 20 s. The cultures contained cells positive for OMP, a marker for mature olfactory receptor neurons *in situ*, and other cells positive for β -tubulin, a protein found in maturing neurons. These results suggest that OE passes through an immature stage in the week following explantation. This likely reflects development of olfactory receptor neurons immature at the time of culturing.

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Poster: Peripheral Olfaction and Peripheral Taste

Structure and Function of a Trace Detector System: Canine Olfactory System

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Studies of the canine olfactory system are important for dogs, known to be one of the best odorant trace detection systems, are utilized by humans in tracking, rescue, explosive and drug detection missions. Canine detection offers the most powerful and cost effective programs available to law enforcement agencies. In the present study we utilized immunocytochemistry, electrophysiology and computer modeling to examine the canine olfactory system. Our results show that the main and accessory olfactory system was positive for several specific neural markers, NCAM, BT and OMP. Olfactory receptor neurons were labeled for GAP-43 suggesting neurogenic activity is persistent in the adult canine olfactory system. In addition G protein subunits Gi and Go were expressed in the sensory epithelium. Electrophysiological results show that endogenous Gi protein negatively regulates odorant evoked intracellular signaling. This suggests a mechanism for controlling the activities of adenylyl-cyclases which could contribute to the ability of olfactory neurons to discriminate odors. The complex canine nasal cavity was CT serially examined and combined with EOG data to produce a one dimensional linear response model. Our results showed that a sniff frequency of 7 Hz yields an optimal response. This corresponded well with the normal 8 Hz level recorded from working detector dog sniff rates. Currently, three dimensional reconstruction of human and canine olfactory nasal cavity regions and steady airflow dynamics are underway.

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Poster: Peripheral Olfaction and Peripheral Taste

Responses of the Rat Olfactory Epithelium to Reversed Air Flow

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We tested electroolfactogram (EOG) during inspiration (odorized air drawn through the external nares by a vacuum applied to the trachea) or expiration (odorized air pushed through the nose from the trachea) in five rats. Two micropipettes were placed in the dorsomedial and lateral recesses of the nasal cavity. Responses were tested to methyl benzoate, phenyl acetate, anisole, isoamyl acetate, limonene, vinyl cyclohexane, and heptane (listed in order of calculated water solubility) with a stimulus duration of 2 s. Concentrations were selected to roughly match the sizes of the response at the better of the two sites for each odorant. The same concentrations were used in all animals and the electrodes remained in place throughout the experiment. Dorsomedial responses were greatest to the soluble odorants, while lateral responses were greatest to the insoluble odorants. These odorants were then tested during expiration at the same concentrations using a longer stimulus (5 s) to provide maximal opportunity for diffusion into the olfactory area. Responses to the insoluble odorants were roughly the size of responses during inspiration. Responses in the dorsomedial region were very small during the stimulus, but for odorants in the mid-range of solubility (e.g., anisole and isoamyl acetate) there was usually (in 4 of the 5 rats) a strong response when the flow was turned off, suggesting a backflow into the dorsomedial recess after expiration ceased. Retest with inspiratory stimuli showed the original responses. These data support the concept that the physical chemical properties of odorants are important in determining response. They suggest that these properties may be important in retronasal olfaction.

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Poster: Peripheral Olfaction and Peripheral Taste

Olfactory and Other Chemosensory Receptor Cells in the Nasal Cavity of the American Alligator

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Crocodylians including alligators possess the chemosensory systems of olfaction and taste but unlike other reptiles, do not have a vomeronasal organ. Alligators lead a semi-aquatic life and detect both water- and airborne chemical cues. Little is known about the morphology of the olfactory system in this group of reptiles. The present study examined the epithelium of the nasal cavity of the American alligator (*Alligator mississippiensis*). Light and electron microscopic techniques were utilized to establish the types of olfactory receptor neurons (ORNs) and their distribution in the nasal cavity. Furthermore, the possible presence of solitary chemosensory cells (SCCs), a chemosensory cell found in various epithelia of fish and in the respiratory epithelium of rodents, was investigated. The results indicate a scenario different from that in other vertebrates. Almost the entire nasal cavity is lined with olfactory sensory epithelium.

However, the ORNs are more widely spaced than in other groups of vertebrates and the density of ORNs varies from rostral to caudal. Additionally, scattered cells occur in the olfactory epithelium, which are morphologically similar to SCCs as described for fishes and rodents. The reason for the different distribution of ORNs and SCCs needs further investigation.

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Poster: Peripheral Olfaction and Peripheral Taste

Differential Expression of Neuronal Markers in Olfactory Epithelia

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All three olfactory epithelia [olfactory epithelium proper (OE), septal organ of Masera (SO), vomeronasal organ of Jacobson (VNO)] originate from the olfactory placode. Nevertheless they exhibit some diversities. In order to characterize their neurochemical phenotypes we analyzed their expression pattern of different neuronal marker proteins using immunohistochemical techniques. Olfactory bulb (OB) served as neuronal control. Neuronal Nuclei Marker (NeuN) is neither expressed in any olfactory sensory neuron, nor in relais neurons (mitral/tufted cells) of OB. However, OB interneurons (periglomerular/granule cells) label as do supranuclear structures of VNO sustentacular cells and VNO glands. PGP9.5 (=UCHL1) expression is exact the opposite: all olfactory sensory neurons express PGP9.5 as well as OB relais neurons but not interneurons. Neuron specific enolase (NSE) is highest expressed in the most apically located OE and SO neurons and patchy in VNO. In contrast, the most basally located neurons of OE and SO express GAP-43 cytoplasmically. In VNO neurons GAP-43 labeling is also nuclear. OMP is cytoplasmically most intense in SO, followed by OE and least in VNO neurons; in basally located VNO neurons OMP is nuclearly localized. OB mitral cells express OMP at low levels. The overall epithelial expression pattern of neuronal markers reveals that OE and SO are more similar to each other than to VNO. Within the VNO the neurons show a clear apical-basal expression diversity for neuronal markers, as they do for factors of the signal transduction cascade suggesting an involvement of those markers in the neuronal function.

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Poster: Peripheral Olfaction and Peripheral Taste

Sea Lamprey (*Petromyzon marinus*) Olfactory Sensory Neurons Display Polymorphisms

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Fish lack a vomeronasal organ—a spatially distinct nasal chemosensory system with microvillous sensory neurons. Rather, their olfactory epithelium (OE) contains three types of olfactory sensory neurons (OSNs): ciliated, microvillous and crypt. In teleost fish, ciliated OSNs express G_{olf} , as is seen in mammalian ciliated OSNs.

Less is known about the OSNs of the sea lamprey, an ancestral jawless fish, phylogenetically removed from both mammals and teleosts. Though all lamprey OSNs are ciliated, previous work in our lab leads us to believe that they are polymorphous. In larval lamprey G_{olf} positive OSNs were widely distributed in the OE, while G_{olf} negative OSNs were spatially confined and projected to a distinct area of the olfactory bulb. In the current study, we sought to identify OSN polymorphisms in the OE of the larval, transformer and adult stages of lamprey through retrograde labelling of OSNs with biocytin and DiI, as well as G_{olf} immunoreactivity. We found three types of OSNs differing in G-protein expression and resembling those seen in teleosts, with somata located in different positions within the OE. These were 1) ciliated, with a thin dendrite and soma in the bottom region, 2) ciliated with a thick axon and soma in the middle and 3) crypt-like, with soma near the top. The crypt-like cells are mainly confined to the ventral hemisphere of the OE. More complete spatial analysis of polymorphisms in all lamprey life stages is on-going.

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Poster: Peripheral Olfaction and Peripheral Taste

G-Proteins in the Squid Olfactory Epithelium

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Olfactory signals from various invertebrate species are processed by at least two different G-protein mediated transduction cascades, the cAMP and IP3 pathway. In squid ORNs, physiological studies indicate that both pathways may be present, however characterization of the transduction molecules at the protein level is absent. Both an adenylate cyclase-like enzyme, crucial to the cAMP pathway, and two different structural and functional forms of the G-protein $G\alpha_q$ have been shown to be present in cephalopods. Here we provide evidence that the G-proteins involved in both cAMP and IP3 pathways are present in squid ORNs (*Lolliguncula brevis*). We used immunoblotting to show that $G\alpha_s/olf$ and $G\alpha_q$ are present in the squid olfactory organ and have the same molecular weight as the mouse OE G-proteins. We also showed that both structural forms of $G\alpha_q$ found by Narita *et al.* (1999) are present in the olfactory organ. Fixed, frozen olfactory organs were cut into 10 μ m sections, double labeled for $G\alpha_q$ and $G\alpha_s/olf$, and imaged. Three dimensional reconstructions of entire olfactory organs were analyzed for epithelial area and patterns of immunofluorescence. Analysis of the individual images show conserved patterns of expression across the olfactory organ, and low co-expression in single cells. These data suggest that the G-proteins important for cAMP and IP3 production are present in squid ORNs.

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Poster: Peripheral Olfaction and Peripheral Taste

Immunocytochemical Localization of Serotonin in the Central and Peripheral Chemosensory System of Mosquitoes

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Serotonin is a biogenic amine, which plays a crucial role in vertebrate and invertebrate nervous systems. In insects, serotonin has been shown to act as a neurotransmitter and a neuromodulator. Mosquitoes, being blood feeding insects, mainly depend on their chemosensory system for host-seeking and blood feeding activities. Despite the socio-economical importance of mosquitoes as vector species, there is a dearth of information on the morphology and distribution of neurotransmitters and neuromodulators in their chemosensory system. Here, for the first time, we have been able to show a detailed map of serotonin-like immunoreactivity in the central and peripheral chemosensory system of mosquitoes by means of immunocytochemistry. In central chemosensory system, serotonin-like immunoreactivity was detected in the antennal lobe (AL) as well as in the suboesophageal ganglion (SOG) and the tritocerebrum. A single centrifugal neuron was found innervating all AL glomeruli as well as the Johnston's organ centre. The axon of this neuron projected through the inner antennocerebral tract with dendritic arborizations found in the calyces of mushroom body and lateral horn. In the SOG and tritocerebrum, serotonin-like immunoreactive fibers were found in most of the previously identified neuropil. In the peripheral chemosensory system, serotonin-like immunoreactive fibers were found in Johnston's organ, the antennal flagellum, the maxillary palp, as well as the labium. Detection of these fibers in peripheral system suggests the presence of a neurohemal release of serotonin in the periphery, which may play an important role in the control of host-seeking and blood feeding behavior in these insects.

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Poster: Peripheral Olfaction and Peripheral Taste

Regional Differences in Cytoarchitecture in the Anterior Olfactory Nucleus

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The anterior olfactory nucleus (AON) is the first bilaterally innervated structure in the olfactory system. It is typically divided into 5 subregions (*pars medialis, dorsalis, lateralis, and ventro-posterior*), however boundary definitions vary widely in published work. We examined the cytoarchitecture of the central region to determine if it is homogeneous. Brains from male Long-Evans rats were embedded in glycol methacrylate, 2- μ m thick sections were cut and stained with 1% toluidine blue O. Image analysis revealed a greater number of larger cells (>2 SD above mean) in the lateral and dorso-lateral regions. Small cells (<1 SD below mean) were more numerous in medial and ventral areas. Further evidence for regional differences in the organization of the area were obtained with immunohistochemistry for calbindin (Calb), parvalbumin (Parv), GAD+ fibers and choline transporter (CHT). Calb+ cells were denser in the deep portion of layer II ($P < 0.01$) though homogeneously dispersed throughout the circumference of the AON ($P > 0.05$). Parv+ cells were located in the superficial half of layer II ($P < 0.01$) and were sparse in ventral and medial regions ($P < 0.01$). CHT+ and GAD+ fibers were denser in lateral vs. medial regions ($P < 0.05$). No significant differences were found in GAD⁺ somata, or in norepinephrine transporter or serotonin transporter immunoreactivity. The AON displays substantial regional differ-

ences in cyto- and chemoarchitectural features, perhaps reflecting functional heterogeneity.

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Poster: Peripheral Olfaction and Peripheral Taste

Can Dielectric Antenna Theory Help Explain Insect Olfaction?

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Current mainstream theory of insect olfaction describes diffusion as the key mechanism by which insect olfaction is reported to occur. However, a viscous wax layer followed by attachment to a much larger pheromone binding protein, both contribute to slower diffusion rates for a traveling pheromone. Furthermore, an insect pheromone receptor has not yet been found on a dendrite within a sensilla. The inability of the current mainstream theory to satisfactorily explain insect olfaction has led to a search for alternate theories. Robert Wright's Vibrational Theory of Odor was put forward in the 1950's and a mechanism for this was put forward by Philip Callahan in the 1960's. Wright suggested that insects detect the vibrational energies from odorants. Callahan put forward a mechanism whereby the insect sensilla act as dielectric antennae. Using antennas as a model, it is possible to explain how olfaction operates as reported in the scientific literature (1–3 ms). Electromagnetics works instantaneously, while diffusion can be a slow process and wholly unreliable when dealing with different shaped sensilla and different pore densities. An antenna model also explains how some antenna can work as lossless, or perfect antennas by utilizing the tiny pores to create, electrically speaking, an infinitely thin antenna wall. This antennal property is deemed useful for detecting low energy. Finally, the polarizing ability of all antennas helps to explain the differential detection of enantiomers as well as their ability to cancel one another out.

Poster: Peripheral Olfaction and Peripheral Taste

Basolateral Monosodium Glutamate Induce a Closure of Channels in Some Rat Taste Bud Cells

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Fungiform taste bud cells were recorded in an isolated rat tongue epithelium. Using the whole cell configuration, voltage-clamp recordings indicated a diversity of voltage-gated currents upon depolarizing pulses or ramps. Some cells presented a large TTX-sensitive voltage-gated inward current. These cells were able to generate action potentials either spontaneously or upon depolarization. The duration of the action potential varied from one cell to another one, according to the kinetics of activation of the outward K⁺ voltage-gated current. Other cells presented only voltage-gated outward current largely suppressed by barium-TEA. The effects of monosodium glutamate (MSG, 100 μ M) were examined when applied on the basolateral side of the recorded taste cells. Voltage-clamp recordings indicated no modification of voltage-gated currents. In some cells, depolarizing ionic channels (which

were opened at rest) were transiently closed and increased the cell input resistance. These observations suggest a resulting increase of the electrical sensitivity of the taste cells which might be related to the "taste enhancer effect" of glutamate. This effect of MSG was observed in cells with various amplitudes of voltage-gated currents.

Poster: Peripheral Olfaction and Peripheral Taste

Chorda Tympani Taste Responses Modified by Agonists and Antagonists of Brain Glutamate Receptors

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Various umami tasting compounds including monosodium glutamate (MSG) were applied as stimuli using a continuous flow protocol while recording the chorda tympani (CT) nerve in hamsters. Antagonists of brain glutamate receptors were applied in mixture with these stimuli. In some cases, a partial inhibition (5–30%) of umami responses was observed. However, a paradoxical potentialisation was sometimes observed. These observations suggest some pharmacological analogies between taste and brain glutamate receptors, but also discrepancies. When applied at the basal part of the taste bud using systemic injection in the common carotid 20 s before the lingual stimulation, agonists and antagonists of brain glutamate receptors also decreased the response to MSG. Some of these pharmacological agents also decreased the basal activity of the CT nerve. No interaction with NaCl-induced CT responses was observed, suggesting some specificity of the effect. Results are discussed in terms of multiple receptor processes for umami taste and a possible neuromodulation at the basal part of the taste buds.

Poster: Peripheral Olfaction and Peripheral Taste

Expression of a Voltage-Gated Potassium Channel KCNQ1 in Taste Bud Cells

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Bitter, sweet and umami tastes are mediated by G protein coupled receptor T1Rs and T2Rs while sour and salty stimuli are received by channel receptors. Activation of T1R and T2R receptors results in an elevation of cytosolic Ca²⁺ in taste receptor cells. The increase in Ca²⁺ stimulates a non-selective monovalent cation channel TRPM5, triggering influx of cations and consequently depolarizing receptor cell membrane potential. Interestingly, activation or inhibition of channel receptors by salty and sour stimuli also leads to membrane potential depolarization. To understand how these gustatory signals of depolarization are converted into the release of transmitters such as ATP from taste bud cells onto afferent gustatory nerves, we reason that voltage-gated ion channels may respond to the depolarization and regulate the

output of ATP. We set out to isolate voltage-gated ion channels using various molecular methods. Here we report the occurrence of a voltage-gated K⁺ channel KCNQ1 (Kv7.1) in taste papillae. Immunohistochemistry showed that KCNQ1 is expressed in a large number of taste bud cells in mouse circumvallate and foliate papillae including TRPM5-expressing cells. However, in fungiform papillae, KCNQ1 expression is in a near-total overlap with T1R3. Previous studies have shown that in fungiform, T1R3 is largely coexpressed with T1R1, forming T1R1/T1R3 receptors for umami substances, suggesting that KCNQ1 may play a role in umami taste. Preliminary two-bottle tests in fact showed that KCNQ1-null young mice exhibited reduced preferences for some umami compounds. The results from this study may help understand the taste perception of patients with long QT or Jervell and Lange-Nielsen (JLN) syndromes.

Poster: Peripheral Olfaction and Peripheral Taste

Calibration of a Lingual Electric Stimulator, Laterality of Response and Metallic Taste

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Metallic tastes can arise from electrical stimulation of the tongue. Two studies compared responses to fabricated electrical stimulators (a 1.5 V, anode side exposed) and to a clinical electrogustometer (RION TR6). The first study compared responses to the battery to the electrogustometer and found equal intensities to be at 1.61 V and 16.94 db, respectively. Verbal responses showed a frequency of metallic (36%), bitter (5.3%), sour (13.6%), and other (45%) reports. A list of over 20 responses was shown as a guide, but was not limiting. A second study examined responses on three areas of the tongue on each side, to follow up on some differences in laterality observed in the first study. Results showed no clear laterality that persists through multiple testing sessions. Responses were in no way cued (no choice suggested) in the second study. Frequency of reports of different qualities showed metallic (33.1%), bitter (7.5%), sour (8.3%), and other (51%) reports. The lack of a large difference in verbal reports suggests that a list of responses with sufficient choices, even though some are novel, does not strongly cue towards those novel responses. This allows for use of such a list when procedure dictates. The lack of strong laterality over multiple sessions supports findings by others suggesting that there is not a persistent laterality in the tongue. These results still leave open the possibility of a less persistent laterality, changing over a time interval shorter than the weeks tested in this study.

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Poster: Peripheral Olfaction and Peripheral Taste

Aquaporin Expression in Mice

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Recent studies have begun to characterize responses to hypoosmotic stimuli in the peripheral gustatory system in mouse. Aquaporin channels (AQP) have been implicated in the initial rapid

movement of water during this response. Cellular and behavioral responses to changes in osmolarity have been examined in 2 inbred mouse strains (C57/6ByJ and 129X/SvJ) that differentially express AQP5, an apically located water channel. To examine the role of AQP5 more directly, we have characterized behavioral responses to taste stimuli in AQP5 null mice. However, we have not observed the magnitude of differences in these responses we would predict based on differences in AQP5 expression in B6 and 129 mice. We are exploring the possibility that there is compensatory expression of one or more of the other AQP channels in AQP5 null mice. As a precursor to testing this hypothesis, we have begun identifying which additional AQP channels are present in mouse taste buds. Using RT-PCR, we have found expression of other AQP channels including AQP2 and 3, but not AQP6 in B6 and 129 mouse taste receptor cells (TRCs). Therefore, it is plausible that expression of these or other AQP channels may be altered in AQP5 null mice and that these AQP channels may also play a role in the water transduction pathway. Studies are in progress to determine whether AQP1, 4, 7, 8, and 9 are expressed in mouse and we plan to use real time qPCR and immunocytochemistry to quantify and localize expression of AQP channels in mouse TRCs.

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Poster: Peripheral Olfaction and Peripheral Taste

Dietary Fat Induced Obesity Alters K⁺ Channel Expression and Reduces Fatty Acid Responsiveness in Rats

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Recently, using heterologous expression and patch clamp recording to determine the fatty acid sensitivity of delayed rectifying K⁺ (DRK) channel subtypes and quantitative real time PCR (qPCR) to measure DRK expression levels, we have shown that obesity-resistant rats (S5B) express a much higher ratio of fatty acid-sensitive (fa-s) to fatty acid-insensitive (fa-i) DRK channels (5.2:1) than those from obesity-prone rats (O-M; 1.7:1). We hypothesize that the fa-s:fa-i ratio may be important in peripheral fat chemosensitivity and ultimately contribute to dietary fat intake. To test this idea directly, we induced obesity in a normally obesity-resistant S5B rats by placing them on a diet containing 70% fat for 2 months. S5B rats exhibit pronounced hyperphagia and developed obesity similar to that seen in obesity-prone rats on this diet. After 63 days on the high-fat diet, we analyzed expression of DRK channels and found that the fa-s:fa-i DRK ratio dropped from 5.2:1 to 0.65:1. We next recorded from taste receptor cells (TRCs) from these rats using patch clamp recording to determine if these changes in expression correlated with reduced responsiveness to fatty acids. On normal diets, fatty acids (10 μM) inhibit about 90–95% of the total DRK current in TRCs from S5B rats, however, after developing obesity TRCs were inhibited only ~30%. Thus, the fa-s:fa-i DRK ratio is sensitive to dietary fat intake and a reduced sensitivity to fatty acids in TRCs is correlated with higher dietary fat intake and may contribute to fat-induced obesity.

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Poster: Peripheral Olfaction and Peripheral Taste

Chorda Tympani Nerve Electrophysiological Responses to Lingual Coapplication of MSG and Linoleic Acid in Male and Female Rats

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We reported that female rats have a lower taste threshold for linoleic acid (LA), a free fatty acid that is the main component of dietary fat, than do male rats. Furthermore, bilateral transection of the gustatory chorda tympani nerve (CTX) significantly impaired the ability of both male and female rats to detect LA. Surprisingly, lingual application of LA alone did not produce whole nerve electrophysiological responses from the CT. Electrophysiological studies of isolated taste receptors have shown that LA inhibits delayed rectifying potassium channels, presumably broadening action potentials, and may thereby augment responses to other taste stimuli. Therefore, the goal of this study was to determine whether LA facilitates CT responses to other taste stimuli. As an initial approach, we used monosodium glutamate (MSG), a compound that activates multiple gustatory receptors, including receptors for sweet and sodium. We recorded whole nerve electrophysiological activity from the CT in response to lingual application of MSG (40, 100, and 300 mM) and to combined application of 88 μM LA and MSG in male and female rats. Preliminary data indicate that CT responses to co-application of LA and MSG were greater than those to MSG alone, especially in male rats. Thus, it appears that LA enhancement of CT responses to MSG is greater in male rats than in female rats, suggesting that fat may enhance taste stimuli to a greater degree in male rats than female rats.

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Poster: Peripheral Olfaction and Peripheral Taste

Near Threshold Concentrations of Linoleic or Oleic Acid Specifically Inhibit Bitterness of Quinine in Humans but Do Not Modulate Perception of Other Tastants

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Studies conducted in rodents suggest that free fatty acids can affect taste receptor cells either through modulation of K⁺ channels or via putative fat receptors/transporters such as CD36. Only few studies have been reported so far in humans. We first assessed the potential taste sensation elicited in subject by solutions of linoleic acid (LA) and oleic acid (OA) at 10, 50, and 50 mM. We then tested a putative modulation by these fatty acids of the taste of other compounds varying in their detection and transduction by taste cells (NaCl, sucrose, citric acid, MSG, caffeine, phenyl alanine, quinine). Solutions were all made in 0.5% ethanol demineralised water. Perceivable differences between samples with and without fatty acid were assessed by triangle and pair tests conducted with 24 panellists wearing nose-clip to minimize odour cues. We found that 50 μM LA alone could be differentiated from the solvent, but not 50 μM OA. However, both fatty acid at 50 μM modulated the taste of selected tastants similarly: a significant decrease of the bitterness of quinine whose bitterness is thought to be mediated by direct activation of

G proteins and/or inhibition of some K⁺ channels, but no modulation of GPCR mediated tastes (sucrose, MSG, phenyl alanine), no modulation of intracellular PDE mediated taste (caffeine), and no modulation of neither Na⁺ nor H⁺ channels mediated taste (NaCl, citric acid). These results raise the hypothesis of a competition between fatty acids and some bitter compounds leading to a decrease of bitterness in food product.

Poster: Peripheral Olfaction and Peripheral Taste

Expression of Arachidonic Acid Signaling-Related Molecules in Rat Circumvallate Taste Buds

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Arachidonic acid and its metabolites are known as modulators for various ion channels. Reportedly, some taste responses in chorda tympani nerves change when a gerbil tongue is pretreated with an inhibitor of phospholipase A₂ (PLA₂), an arachidonic acid-releasing enzyme, or arachidonic acid itself. However, no information is available on the potential role of arachidonic acid in the taste signal transduction. There are multiple pathways to generate or metabolize of arachidonic acid. Responsible enzymes for the generation include PLA₂, diacylglycerol lipase (DAG lipase), and monoglyceride lipase (MGL). On the other hand, generated arachidonic acid is metabolized to prostaglandins by cyclooxygenases (COX) and to leukotrienes by lipoxygenases (LOX). We investigated these enzymes in rat circumvallate taste buds by *in situ* hybridization and found that, in a subset of taste bud cells, MGL was expressed in addition to PLA₂-IIA we reported previously. Also, COX-2 was found to be expressed in a subset of taste bud cells. These results suggest that arachidonic acid is enzymatically released from glycerides and metabolized actively in taste buds. Double-labeling *in situ* hybridization and immunohistochemical analysis revealed that all these enzymes, MGL, PLA₂-IIA, and COX-2, were actually expressed in taste receptor cells. Arachidonic acid should thus play some crucial role in taste signaling.

Poster: Peripheral Olfaction and Peripheral Taste

Transgenic Mice Expressing GFP in PLC2 Taste Cells Demonstrate Functional Classes of Cells

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Tissue-specific promoters represent a powerful tool to monitor specific cell-types within various tissues. In this experiment, we adopted the promoter of the phospholipase C type β2 (PLCβ2) gene that is expressed in a subset of cells within mammalian taste buds. PLCβ2 is involved in the signal transduction of sweet, bitter, and umami stimuli. We have developed transgenic mice expressing GFP under the control of either 2.9 or 8.0 kb of the PLCβ2 promoter. Across four taste fields, the expression of GFP was easily detected in living tissue with epifluorescence microscopy. Using immunostaining in several lines of transgenic mice, we confirmed that cells expressing GFP also express endogenous PLCβ2. In addition, the temporal regulation of the GFP transgene seems to be coordinate with the endogenous

PLCβ2 gene. When loaded with a calcium indicator dye, GFP-positive taste cells produced typical Ca²⁺ responses to the bitter compound cycloheximide. In these same cells, KCl-depolarization did not result in Ca²⁺ responses, confirming the absence of voltage-gated Ca²⁺ channels in taste receptor cells. Conversely, only GFP-negative cells generated Ca²⁺ responses to depolarization. These findings confirm the presence of at least two independent functional classes of cells within taste buds. These PLCβ2 promoter-GFP transgenic mice could be useful for further studies involving taste transduction, sensory signal processing, and taste bud development.

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Poster: Peripheral Olfaction and Peripheral Taste

Inhibition of the IP₃ Pathway Permits Fly Sugar Receptor Cell Responses to Na-Saccharin

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Since flies give no behavioral, and little “sugar cell” responses to “artificial” sweeteners, it has been thought that they lack excitatory receptor mechanisms for these compounds. Yet the fly “deterrent cell” responds to Na-saccharin (Na-S), as well as bitter stimuli, and there is reciprocal inhibition for Na-S and sugar stimuli. We studied sugar cell responses to Na-S during inhibition of the IP₃ pathway with 1-(5-isoquinolinesulfonyl)-2-methylpiperazine 200 μM and Na-deoxycholate 0.03% w/v (H7). Receptor cell action potentials were tip-recorded from single sensilla in isolated *Phormia regina* proboscises. Addition of H7 to Na-S 4.87 mM increased sugar cell firing rates from medians of 1.5 to 5 action potentials/first 100 ms ($P < 0.002$, Mann-Whitney). There was a typical concentration-dependent response curve for firing to Na-S ($P = 0.05$, Kramer), while the adaptation rate increased with Na-S concentration, in the presence of H7. These data show that the fly sugar cell has an excitatory receptor mechanism for Na-S. An IP₃ bitter transduction pathway in the deterrent cell, leading to inhibition of sugar cell firing, could be responsible for the lack of fly responses to Na-S. But an IP₃ pathway is known to mediate adaptation of sugar cell responses to sugars. Given the positive relationship for adaptation rate and Na-S concentration, and the lack of synapses between sensillum receptor cells, activation of the sugar cell IP₃-mediated adaptation process so as to cut off an excitatory response seems a more likely mechanism.

Poster: Peripheral Olfaction and Peripheral Taste

Investigating Cyclic AMP in Taste Transduction Using Real Time Imaging

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There is unequivocal biochemical and physiological evidence that the diffusible second messenger 3′–5′ cyclic adenosine monophosphate (cAMP) is involved in taste transduction. Yet, recent reports

have emphasized the role of Ca^{2+} signaling in taste transduction (i.e., taste receptor \rightarrow G protein \rightarrow PLC β 2 \rightarrow IP $_3$ \rightarrow $\Delta[\text{Ca}^{2+}]_i$) and attention has been shifted from cAMP signaling in taste. The significance of the original cAMP data is presently unresolved. The goal of our research is to re-investigate cAMP in taste transduction in the light of current understanding. We have developed techniques to image real-time changes in intracellular cAMP in taste cells using genetically-encoded cAMP reporters. These FRET-based reporters are modifications of PKA subunits and permit one to measure single-cell cAMP levels with excellent spatial and temporal resolution. Using a biolistic approach we have transfected rat fungiform taste buds with cAMP reporter plasmids. Focal application of the bitter tastant cycloheximide (100 μM) to living fungiform tastebuds *in situ* produced a decrease in $[\text{cAMP}]_i$ within individual taste receptor cells. These results are qualitatively similar to previous biochemical measurements from homogenized taste tissue but are now allowing us to examine the cAMP response in individual, identified cells.

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Poster: Peripheral Olfaction and Peripheral Taste

Identification of Two Putative Taste Signal Transduction Components

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To identify genes important for taste receptor cell function, we analyzed the sequences and expression patterns of clones isolated from a mouse taste receptor cell-enriched cDNA library. Here, we report the analyses of two of these genes. One, *Gpr113*, encodes a G-protein-coupled receptor belonging to family 2B, members of which are characterized by having long N-terminal, extracellular domains. The predicted N-terminal extracellular domain of GPR113 contains 696 amino acids with two functional domains, a peptide hormone-binding domain and a G-protein-coupled receptor proteolytic site. The second encodes a novel member of the TRP family of ion channels, many members of which have been implicated in sensory signal transduction. Expression analyses with both of these genes indicate that their expression is highly restricted to subsets of taste receptor cells. Furthermore, co-localization studies with various taste receptor cell markers suggest that GPR113 plays a role in sweet taste, whereas the TRP channel plays a role in either salty or sour taste transduction. Knock out mouse models are currently being developed to test these hypotheses.

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Poster: Peripheral Olfaction and Peripheral Taste

Drosophila norpA Expression in Taste Neurons: Role in Trehalose Detection

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Drosophila norpA (no receptor potential A) gene encodes phosphatidylinositol (PI)-specific phospholipase C (PLC- β) and yields two products: subtype I and II. PLC hydrolyses phosphatidylinositol 4,5-bisphosphate (PIP $_2$) into second messengers diacylglycerol (DAG) and inositol trisphosphate (InsP $_3$), which ultimately leads to Ca^{2+} release from the intracellular stores. The best studied example of a transduction pathway involving *norpA* product is the *Drosophila* photoreception; flies with strong alleles of *norpA* are blind due to a dramatic decrease in the photoreceptor PLC levels. Subsequently, the *norpA*-encoded PLC has been shown to be required for *Drosophila* olfaction. Here, we report that *norpA* may also be involved in *Drosophila* gustation. Firstly, RT-PCR results indicate that major taste organs, labella and tarsi, contain detectable levels of subtype II *norpA* transcript; in contrast compound eyes show high levels of subtype I. Secondly, using a GAL4/UAS approach with the minimal *norpA* promoter we show that *norpA* is expressed in a relatively large subset of gustatory neurons. Finally, genetic ablation of *norpA*-expressing taste neurons leads to a marked decrease in *Gr5a* transcript levels and to significantly reduced feeding responses to trehalose. Our findings suggest that both *norpA*-encoded PLC may function in trehalose detection pathway.

Poster: Peripheral Olfaction and Peripheral Taste

Recombinant Neoculin Produced by *Aspergillus oryzae* has the Native Taste-Modifying Activity, Recognizable by Human Sweet Taste Receptor

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Neoculin (NCL), a protein tasting as approximately 500-fold sweet as sugar, can be utilized as a non-glycemic sweetener. It also has a taste-modifying activity to convert sourness to sweetness. Structurally, NCL is a heterodimer composed of an *N*-glycosylated acidic subunit (NAS) and a basic subunit (NBS), which are conjugated by disulfide bonds. For production of recombinant NCL (rNCL) by *Aspergillus oryzae* as a host, NAS and NBS were simultaneously expressed as independent fusion proteins. We used α -amylase as a carrier and also inserted the dibasic cleavage site, Lys-Arg, that can be recognized by a Kexin2 (KEX2)-like protease belonging to the subtilisin subfamily. For accurate and efficient cleavage of the fusion construct by KEX2, a triglycine motif was added to the C-terminus of the KEX2 cleavage site. As a result, rNCL was secreted as an NAS-NBS heterodimer into the culture medium. rNCL was purified by chromatographies, and investigated for its biochemical and sensory properties. Recombinant NAS was found to be *N*-glycosylated like the native NAS. CD spectroscopy suggested that the overall conformation of rNCL resembled that of native NCL. Calcium imaging analysis using HEK cells made to express the human sweet taste receptor, hT1R2/T1R3, demonstrated that the sweet-tasting activity of rNCL was comparable to that of native NCL. Human sensory test to evaluate the taste-modifying activity showed that purified rNCL elicited the same activity as native NCL did.

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Poster: Peripheral Olfaction and Peripheral Taste**Expression of Sweet Taste Receptors and Signaling Molecules in the Enteroendocrine STC-1 Cells**

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The content of the intestinal luminal substance varies significantly with diet. It is essential hence that the intestinal epithelium senses and responds to the considerable changes and regulate its functions consequently. Though it is becoming evident that the gut epithelium senses and responds to luminal nutrients, little is known about the nature of the nutrient sensing molecules and the downstream signal transduction. Here we report the expression, as revealed by reverse transcriptase-PCR and immunohistochemistry, of members of the T1R sweet taste receptors, the $G\gamma 13$, gustducin, PLC $\beta 2$, TRPM5, SNAP 25 and Synaptobrevin-2 in the enteroendocrine cell line, STC-1. Cellular responses of STC-1 cells to sweet tastant SC45647 and bitter tastant were investigated using a calcium-imaging technique. Furthermore, the signaling pathway was blocked by a G protein inhibitor, demonstrating the essential involvement of G protein in cellular responses to study the regulatory system of G protein signaling in STC-1 cells. Thus, we showed that STC-1 cells emerge as a cell model for studying the molecular mechanism of sweet taste signaling. In the small intestine, there is a highly coordinated expression of sweet taste receptors and gustducin, a G-protein implicated in intracellular taste signal transduction, throughout the gut. The feasible involvement of these sweet sensing receptors in the intestine will broaden our understanding of intestinal nutrient sensing, with implications for better nutrition and health maintenance.

Poster: Peripheral Olfaction and Peripheral Taste**Expression of the G Protein Subunit Gustducin in Mammalian Spermatozoa**D. Meyer¹, J. Fehr¹, H. Borth¹, P. Widmayer², B. Wilhelm³, T. Gudermann¹ and I. Boekhoff¹¹*Pharmacology, University of Marburg, Marburg, Germany,*²*Physiology, University of Hohenheim, Stuttgart, Germany and*³*Anatomy, University of Marburg, Marburg, Germany*

The G protein subunit α -gustducin is generally accepted as a marker of chemosensitive cells. Since chemosensation is especially important for the navigation of sperm towards the egg, attempts were made to explore whether α -gustducin might also be expressed in spermatozoa. RT-PCR experiments revealed that a gustducin PCR product with the predicted size could be amplified from mouse as well as from rat testis. To identify the testicular cell type in which α -gustducin is expressed, immunohistochemical experiments were performed with an anti-gustducin-specific antibody. The most intense immunoreactivity was visible in differentiating spermatids in the lumen of the seminiferous tubules whereas no staining was detectable in spermatogonia. To verify whether α -gustducin is also expressed in mature spermatozoa, mouse and rat sperm were subjected to immunocytochemistry as well as electron microscopy. A strong staining of the innerdense fibres was obtained within the midpiece of the flagellum whereas no labeling was detectable in

the principal and end piece as well as in the head of the sperm. Analyzing human sperm for α -gustducin staining also revealed a strong labeling of the midpiece of the flagellum leaving the principle piece almost completely unstained. In bovine spermatozoa, the midpiece of the flagellum did not show pronounced labeling; in contrast, maximal signal intensity was restricted to the cytoplasmic droplet, the residual cytoplasm of the condensing spermatid. The observation that gustducin is expressed in the tail of mammalian spermatozoa may now allow to identify the linked signaling cascade which subsequently may define the functional role of α -gustducin in spermatozoa.

Poster: Peripheral Olfaction and Peripheral Taste**Further Characterization of Neuropeptides in Rat Taste Receptor Cells**

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Peptide-expressing taste bud cells (TBCs) have been reported in several species though their function is not yet known. In these studies we further characterize the roles of peptides within rat TBCs using electrophysiological and immunocytochemical methods. Previous studies demonstrated that CCK and NPY have opposite actions on an inwardly rectifying-potassium current (K_{ir}), inhibiting or enhancing it, respectively. Here we report that a third peptide, somatostatin (SST), is without effect on this current. Instead SST appears to produce a steady outward current at the resting potential that would be hyperpolarizing. Additionally SST inhibits a calcium-activated potassium current (a current also inhibited by 5HT). Immunocytochemical studies have previously demonstrated that CCK, VIP, and NPY cells are mostly co-expressed in the same cells. In separate experiments, CCK, VIP, and NPY were examined with the taste cell markers glutamic acid decarboxylase (GAD), NCAM, PGP 9.5, or SNAP-25. For all three peptides, there was about a 40% overlap of expression with GAD, a marker of GABAergic TBCs. Little overlap was observed with either NCAM or PGP 9.5 whereas overlapping expression was observed with SNAP-25. These data strengthen notions of peptidergic co-transmission since peptides may be expressed in cells equipped for vesicular exocytosis. Additionally they suggest GABA as one possibility of a co-transmitter whereas serotonin, since it typically co-expresses with NCAM, may be unlikely.

Poster: Peripheral Olfaction and Peripheral Taste**Expression of Genes Involved in Synthesis and Secretion of Biogenic Amine Neurotransmitters in Mouse Taste Buds**

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Cells within mammalian taste buds are known to express a number of neurotransmitters. These may play roles in communication between cells of a taste bud and/or for signaling to afferent nerve fibers. One of the biogenic amines, serotonin, is found in a subset of taste cells in many species, and is released following taste stimulation.

We explored the expression of genes involved in the synthesis of biogenic amine neurotransmitters in mouse taste buds. Aromatic L-amino acid decarboxylase (AADC) is common to the pathways for serotonin, dopamine, norepinephrine and epinephrine. We find this enzyme is expressed at high concentration in taste buds, and is lacking from surrounding non-taste epithelium. Further, mRNA for tryptophan hydroxylase isoforms (Tph1 and 2) and the serotonin transporter all key for serotonin synthesis or packaging are also detected, albeit at lower levels. Enzymes involved in catecholamine synthesis, packaging or release, including tyrosine hydroxylase (TH), vesicular monoamine transporter (VMaT2), dopamine transporter, and α -synuclein also are expressed. Single-cell RT-PCR and immunocytochemical analyses suggest that some of these enzymes are found in a common subset of cells in mouse taste buds. To date, we have been unable to detect either dopamine β -hydroxylase (DBH) or phenylethanolamine-N-methyltransferase (PNMT), enzymes necessary for the synthesis of norepinephrine and epinephrine.

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Poster: Peripheral Olfaction and Peripheral Taste

Cell-To-Cell Communication in Taste Buds: The Role of ATP and 5HT

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We previously have shown that sweet, sour, and bitter stimuli, and K^+ -induced depolarization all evoke 5HT release from mouse vallate taste buds *in vitro*. Furthermore, applying ATP also stimulates 5HT release. Procedures that interfere with ATP stimulation, including applying suramin (a purinoceptor antagonist) or apyrase (an ATPase), block taste-evoked 5HT release. These data suggest that ATP acts as an intermediary in the release of 5HT from taste buds. Here we present direct evidence for ATP release from taste cells. Using CHO cells expressing highly sensitive P_{2X} receptors as biosensors, we are able to detect ATP release from isolated taste buds. ATP release was evoked by saccharin (2 mM), SC45647 (100 μ M), cycloheximide (10 μ M), or denatonium (1 mM). We next isolated single vallate taste cells, loaded them with Fura-2 for Ca^{2+} imaging, and identified 2 separate cell populations—cells stimulated by tastants (taste receptor cells) and cells stimulated by depolarization (synaptic cells). Isolated taste receptor cells released ATP and isolated synaptic cells released 5HT in biosensor studies. There was zero overlap in these two populations. Our data emphasize the existence of two functional classes of taste cells and indicate that mouse vallate taste receptor cells communicate with nearby serotonergic synaptic taste cells via ATP. Specifically, ATP secreted from receptor cells triggers 5HT release.

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Poster: Peripheral Olfaction and Peripheral Taste

Ecto-ATPase in Taste Buds of Fishes

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ATP is claimed to be a crucial neurotransmitter in mammalian taste buds and taste buds in mammals display robust ectoATPase activity, suggesting that this enzyme plays a role in inactivation of the neurotransmitter. We utilized histochemical techniques to assess whether ectoATPase activity is present in taste buds of fishes. Taste bud-bearing tissue was obtained from 3 species of fish: 2 catfish: *Plotosus lineatus* (barbels), *Ictalurus punctatus* (barbels), and goldfish, *Carassius auratus* (lips and palatal organ). Tissue was fixed 1 h in mixed aldehydes in Tris-maleate buffer, then washed and cryoprotected overnight. Free-floating sections were then placed in reaction solution (2 mM $Pb(NO_3)_2$ containing inhibitors of non-specific phosphatases (levamisole, ouabain, α, β -methylene ADP) along with either ATP or ADP as substrates. Following a reaction period of 30 min, the tissue was washed and exposed to 1% ammonium sulfide for 1 min. Reaction product outlined elongate cells of taste buds in all tissues treated with ATP but not ADP. This high specificity for ATP is similar to the ectoATPase in rodent taste buds. Reaction product also was evident in the nerve bundles coursing through the submucosa and reaching the base of the taste bud. This neural ATPase, however, was present in tissue reacted with either ADP and ATP indicating the presence of a different ATPase than is present in taste buds. Further studies will determine the cell type or types that exhibit this ectoATPase reactivity.

Supported by NIH Grant R01 DC007495 (T.E.F.)

Poster: Peripheral Olfaction and Peripheral Taste

Numerical Density of Taste Cells in Rat and Mouse Circumvallate Taste Buds

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We have previously shown that significant differences exist between mouse and rat taste buds with regard to the expression of markers for taste signaling and synaptic proteins. In the present study we wished to determine if there are differences in the numerical density of taste cells and taste bud volume between rat and mouse taste buds. For this study we sliced serial transverse sections (1 μ m thickness) from mouse and rat circumvallate papillae to count the numbers of taste cells in the taste buds and to calculate taste bud volume. Forty-one taste buds from 3 mice and 42 taste buds from 3 rats were analyzed. Our results indicate that mouse taste buds contain an average of 85.8 taste cells vs. 68.4 taste cells in rat taste buds. The numbers of taste cells/bud ranged from 32 to 152 in the mouse and 34 to 126 in the rat. Although the average mouse taste bud contains more taste cells than a rat taste bud, the average volume of a mouse taste bud (42,000 μ m³) is smaller than a rat taste bud (64,200 μ m³). The numerical density of taste cells in mouse circumvallate taste buds (2.1 cells/1000 μ m³) is significantly higher than that in the rat (1.2 cells/1000 μ m³). The results of the present study, taken together with our previous work, demonstrate that mouse and rat taste buds are both quantitatively and qualitatively different.

This work is funded by NIH grant DC00285.

Poster: Peripheral Olfaction and Peripheral Taste**Synaptotagmin-1-like Immunoreactivity in Circumvallate Taste Buds of the Rat**

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Synaptotagmin (synt) is a vesicle associated glycoprotein of neurosecretory granules and synaptic vesicles. Synaptotagmin has two C2 domains that interact with Ca^{2+} and is thought to be the primary sensor for synaptic vesicle fusion and neurotransmitter exocytosis. We used immunocytochemistry with synt-1 and other known functional markers in taste cells to learn if synaptotagmin is associated with taste cells thought to possess synapses. Preliminary results reveal that synt-1 is present in a small subset of taste cells and nerve processes in rat circumvallate taste buds. Approximately 13% of taste cells display synt-1-like immunoreactivity (LIR). The immunoreactive taste cells are slender in shape and extend the entire height of the taste bud from basal lamina to taste pore. Synt-1-LIR colocalizes with synaptobrevin-2-, protein gene product 9.5 (PGP 9.5)-, and serotonin-LIR in a subset of taste cells, however, synt-1-LIR taste cells do not contain α -gustducin or PLC β 2 which are believed to be present in type II taste cells. In addition, we used DAB immunoelectron microscopy and found that synt-1-LIR taste cells possess synapses onto nerve processes in rat circumvallate taste buds. These data suggest that synt-1 is present in type III taste cells and is associated with synapses onto nerve processes.

Supported in part by NIH grant DC00285.

Poster: Peripheral Olfaction and Peripheral Taste**Characteristics of Gustatory Responses from the Soft Palate in C57BL Mice**

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Gustatory responses from the soft palate in C57BL mice were studied by electrophysiological and behavioral experiments. Integrated taste responses from the greater superficial petrosal nerve (GSP), innervating the soft palate, to NaCl, HCl, sucrose (Suc), quinine HCl (QHCl), and other taste substances at various concentrations were recorded. Threshold for QHCl was 0.00001 M and QHCl produced robust phasic and tonic responses at 0.01 M. Threshold for HCl was 0.0003 M and robust phasic and tonic responses were observed at 0.01 M. Threshold for Suc response was rather high at 0.03 M and the response magnitude increased abruptly with increasing concentration until 1 M. Artificial sweeteners tested (0.1 M acesulfame-K, 0.1 M sucralose, 0.02 M saccharin) also produced large responses similar to 1 M Suc. Stimulatory effectiveness for NaCl was smaller than that in the chorda tympani (CT). Although inhibitory effects of amiloride on responses to Na salts was weak in the rat GSP, 50 μM amiloride strongly inhibited tonic responses to NaCl and Na-acetate in mice GSP similar to that in the CT. To determine the relative importance of the three major nerves [the GSP, the glossopharyngeal (GL), and the CT] for mediating taste information, effects on licking behavior by bilateral transection of each nerve or combination of the nerves in mice were studied. As for the QHCl, the effect of GSP + GL transection was large by $\sim 75\%$.

These results suggest functional differences of soft palate taste buds among rodents.

Poster: Peripheral Olfaction and Peripheral Taste**Mixing Sweet and Sour Stimuli: Effects on the Hamster Chorda Tympani**

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Chorda tympani (CT) and central hamster taste responses to sucrose are inhibited by quinine-HCl when presented together in a mixture; in addition, central sucrose responses are also inhibited by mixtures with citric acid (HCit). Thus, we hypothesized that mixtures of sucrose with acids would reduce sucrose CT responses. We recorded CT responses in a total of 11 golden hamsters (*Mesocricetus auratus*) to the following stimuli: 3 mM HCl, 10 mM HCit, 10 mM acetic acid (HAc), 100 mM sucrose and the binary combinations of sucrose with each acid. Responses to 500 mM NH_4Cl were used to normalize response measurements. Responses to sucrose mixtures with HCit equaled responses to HCit alone and were smaller ($P < 0.001$) than responses predicted by an additive response model, implying sucrose response suppression by HCit. In contrast, responses to sucrose mixtures with HAc or HCl were larger than responses to either mixture component alone ($P < 0.001$) and equaled responses predicted by an additive response model, implying response independence. Solution pH did not account for these effects. We also recorded CT responses to a concentration series of HCit (1, 3, and 10 mM) alone and mixed with 100 mM sucrose in 4 animals. As with HAc and HCl, responses to mixtures with 1 mM HCit were larger than responses to either mixture component presented alone ($P < 0.001$); however, the mixture response was still smaller ($P < 0.05$) than that predicted by an independent response model. Thus, HCit may modulate the sucrose receptor complex making it less effective. Alternatively, cross-talk may occur between the sweet and sour stimulus modalities in the hamster gustatory periphery.

Supported by NIH grant DC 040499.

Poster: Peripheral Olfaction and Peripheral Taste**Amiloride Inhibition of the Na-Evoked Lingual Surface Potentials (LSP) Varies in Humans**G. Feldman¹ and G. Heck²*¹Internal Medicine, Virginia Commonwealth University, Richmond, VA, USA and ²Physiology, Virginia Commonwealth University, Richmond, VA, USA*

As we previously reported, Na affects the LSP in humans, and in some people the Na-evoked LSP is partly inhibited by amiloride, a blocker of the epithelial Na channel. In this study we examined whether the amiloride effect is constant in given individuals or whether it varies from one day to the next. Using a voltage sensing gustometer adhering to the anterior surface of the tongue, the amiloride effect was measured as 150 mM NaCl superfused the lingual surface. The amiloride effect was quantified as the induced rate of change of the LSP (mV/s); larger positive numbers signify greater effects. In 10 subjects, 100 μM amiloride inhibited the Na-evoked

LSP an average of 0.0280 (± 0.049 SD). When the same subject was tested repeatedly on different days the amiloride effect varied considerably. For example, in one subject amiloride's effects were determined on 13 days; the average effect was 0.155, the minimum was 0.020 and the maximum was 0.357. In another subject amiloride's effects were determined on 5 days; the average was -0.003 , the minimum was -0.025 , and the maximum was 0.041. These data demonstrate that the amiloride effect on the Na-evoked LSP varies among subjects and that in individual subjects the amiloride effect varies from one day to the next. Daily variation of the amiloride effect could suggest that the Na-evoked LSP is responding to physiological regulatory events, such as hormones. If this speculation is correct, then it is possible that in humans the perception of salt taste may also vary in response to physiological events.

This work is funded by a Merit Review Grant from the Department of Veterans Affairs.

Poster: Peripheral Olfaction and Peripheral Taste

Time Course of Altered Chorda Tympani Nerve Response After Contralateral Nerve Section in Sodium-Restricted Rats

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Unilateral section combined with dietary sodium restriction results in decreased sodium responses from the intact chorda tympani (CT) nerve by day 4 post-section; no other taste modalities are affected. We recorded CT responses from sodium-restricted rats during the early period following contralateral nerve section to provide insight into the mechanisms underlying this functional plasticity. SPF Sprague-Dawley rats received unilateral CT section and a low sodium diet (0.03% vs. 1.0%) on day 0. Recordings from the intact CT were performed at days 2, 3, and 4 post-section. From day 2 to 4, there was a gradual decrease in the CT response to NaCl. Other taste modalities were unaffected. Therefore, the mechanism by which this alteration occurs is not sudden, but progresses over several days. In the presence of the epithelial sodium channel (ENaC) blocker, amiloride, sodium responses were reduced to equal levels in control and experimental rats. We propose that there is a concomitant, gradual decrease in ENaC expression and/or function. The gradual decrease in sodium sensitivity suggests that the biosynthesis of ENaCs within intact taste receptor cells, rather than ENaC stability in the membrane, may be affected by contralateral CT section and sodium restriction. Within this period, sodium-restricted rats also exhibit a deficient immune response to CT sectioning. Perhaps a decrease in the presence of beneficial cytokines ultimately results in decreased ENaC expression.

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Poster: Peripheral Olfaction and Peripheral Taste

Expression and Regulation of Lingual Vascular Adhesion Molecules Following Unilateral Chorda Tympani Nerve Section

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Activated macrophages increase in number on both sides of the tongue following unilateral chorda tympani nerve (CT) sectioning. The mechanisms responsible for recruiting these macrophages to the tongue are unknown. We hypothesize that adhesion molecules, specifically intracellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1, are upregulated following CT sectioning and allow macrophage entry. SPF Sprague-Dawley rats received unilateral CT or sham section. Rats were euthanized at several time points ranging from 6 h to 7 days post-sectioning, and frozen sections were processed for immunohistochemical staining for ICAM-1 and VCAM-1. In separate groups of rats, ELISAs (R & D Systems) and Western blot analyses were used to quantify relative changes in adhesion molecule expression in tongue homogenates. The endothelial layer of blood vessels robustly expresses ICAM-1 (1:1000; Accurate Chemical and Scientific Corporation) on both sides of the tongue following CT section. The peak increase in ICAM-1 expression occurs on the sectioned side of the tongue at 24 h post-sectioning compared to sham animals ($P < 0.001$). However, VCAM-1 (1:250; Covance) expression remains minimal after CT section. We propose that ICAM-1 is important for macrophage recruitment following CT section. Ultimately, we propose that leukocytes modulate taste function after neural injury by secreting growth factors and cytokines that act on taste receptor cells.

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Poster: Peripheral Olfaction and Peripheral Taste

Macrophage Activation Patterns Following Chorda Tympani Nerve Section

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Within days following unilateral chorda tympani (CT) nerve section, there is a bilateral increase in activated lingual macrophages. This macrophage response to injury does not occur in sodium-restricted rats, which also have deficits in sodium taste function in the intact CT. Therefore, activated macrophages are associated with normal taste function after injury, and may communicate with taste receptor cells via cytokines. We investigated the effects of CT section on cytokines that are stereotypical of classical (IL-1 β) or innate (TGF- β) macrophage activation. Macrophage activation markers were also examined. SPF female adult Sprague Dawley rats received unilateral CT or sham sectioning and a control or low-sodium diet (1.0% vs. 0.03%). At 6 h to 4 days post-section, rats were sacrificed, tongues dissected, and protein lysates collected for ELISAs. Cryosections were obtained from separate groups, and immunohistochemistry was performed. At day 2 post-section, levels of the proinflammatory cytokine, IL-1 β , were elevated in control-fed but not sodium-restricted rats as measured by ELISA. IL-1 β was expressed by activated macrophages. A subset of taste receptor cells, epithelial cells, neurons, and endothelial cells were also IL-1 β + regardless of surgical or dietary treatment. Levels of TGF- β , a hallmark of the innate activation pathway, were low in all treatment groups. Likewise, macrophages did not express arginase or mannose receptors, which are typical of alternative

activation. These data suggest that macrophages that respond to neural and taste receptor cell injury are classically activated, and may modulate taste function either directly or indirectly through IL-1 and other proinflammatory cytokines.

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Poster: Peripheral Olfaction and Peripheral Taste

Brief and Prolonged Dietary Sodium Deprivation Reduce Chorda Tympani Nerve Responses to NaCl

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Eight to ten days of dietary Na⁺ deprivation are necessary to increase 24 h intake of a concentrated NaCl solution. Na⁺ deprivation of similar duration also decreases the sensitivity of the chorda tympani nerve (CT) to NaCl, suggesting that changes in CT responses are necessary for increased NaCl intake. However, our studies indicate that behavioral taste responses change following as little as 2 days of dietary Na⁺ deprivation. Specifically, short-term lickometer tests and microstructural analysis showed that after 2 days of Na⁺ deprivation, rats increased licking to concentrated NaCl solutions. Accordingly, the goal of the current study was to determine whether brief dietary Na⁺ deprivation decreases CT responses to NaCl, and to assess CT amiloride-sensitivity after brief (2 days) or prolonged (10 days) dietary Na⁺ deprivation. We recorded whole nerve electrophysiological activity from the CT in response to lingual application of NaCl (75, 150, 300, 450, and 600 mM) and to NaCl mixed with 100 μM amiloride, an epithelial Na⁺ transport blocker. CT responses to NaCl were reduced at all concentrations after both brief and prolonged Na⁺ deprivation compared to Na⁺-replete controls. Moreover, amiloride, which suppressed CT responses to NaCl by 35% in controls, had virtually no effect on CT responses in Na⁺-deprived rats. These results suggest that both brief and prolonged Na⁺ deprivation lead to changes in CT responses to NaCl that may selectively involve the amiloride-sensitive component of NaCl taste.

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Symposium: Taste & Smell in Translation: Applications from Basic Research

Taste and Smell in Translation: Applications from Basic Research

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The symposium is an exploration of recent key advances in the chemical senses of interest to industry scientists and basic scientists. The speakers are internationally known experts with a clear view of

the cutting edge and a broad perspective. Each will focus on two or a few recent advances in basic research that have potential applications. For example, recent characterization of taste and olfactory receptor molecules provides opportunities for high throughput screening and for de-novo design of commercially important tastants and olfactants; rigorous examination of the effects of fragrances on human mood and behavior point to applications with a firmer scientific basis; and, genetic differences in taste perception are now seen to affect food preferences. The speakers will take the time to explain the basic science background behind their examples for an audience that cannot be expert in all relevant areas. The audience will include industry scientists and policy makers as well as basic scientists and students new to the field, interested in applications of basic research. The symposium will conclude with a roundtable discussion with audience participation. Our goal is to explore how collaborations between industry and academic scientists can benefit both, but particularly how basic-science expertise can contribute. The speakers' broad areas of interest are: taste molecular biology (Margolskee); olfaction molecular biology (Reed); olfaction perception/psycho-physics (Herz); taste perception/psychogenomics (Breslin). The symposium will be followed by a reception with buffet and cash bar: an opportunity for industry participants to network and to interact one-on-one with the symposium speakers and other interested basic scientists.

Symposium: Structure/Function and Pharmacology of GPCR

The Organisation and Molecular Relevance of GPCR Quaternary Structure

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It is now widely accepted that rhodopsin-like G protein coupled receptors (GPCRs) exist as dimers or higher-order oligomers. However, apart from rhodopsin in rod outer segments the organisational structure of other GPCRs remains unclear. We mapped sites of interaction between monomers of the α1β-adrenoceptor using fragments from the receptor comprising the N-terminal domain linked to various transmembrane domains and intracellular loop connections. Symmetrical interactions were shown for TM1 and TMIV. This resulted in a model in which a "daisy chain" of monomers generates an oligomeric structure. Because two protein resonance energy transfer techniques are poorly suited to distinguish dimers from oligomers we employed "three colour" fluorescence resonance energy transfer imaging in single cells. Co-expression of forms of the α1β-adrenoceptor C-terminally tagged with CFP, YFP and a FRP produced sequential 3 protein FRET that was absent when α1β-adrenoceptor-YFP was replaced with the non-fluorescent construct α1β-adrenoceptor-Y67C-YFP. Mutation of key hydrophobic residues in TM1 and TMIV resulted in a marked reduction in sequential 3 colour FRET suggesting an alteration in oligomeric organisation. The mutated α1β-adrenoceptor was expressed as well as the wild type but only a fraction was able to bind the ligand [3H]prazosin and the mutant was unable to reach the cell surface because it did not become core-glycosylated. Not surprisingly, the mutant was completely unable to signal in response to addition of α1β-adrenoceptor agonists.

Symposium: Structure/Function and Pharmacology of GPCR

Pharmacology of Mouse Olfactory Receptors

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An olfactory receptor (OR) possesses a broad but selective ligand spectrum. Functional experimental analysis and computational docking simulation allowed for identification of the odorant-binding site of a mouse OR, suggesting the molecular basis of the structure-activity. We also identified amino acids that were involved in receptor dynamics from an inactive to an active conformation and in coupling to G proteins. These studies revealed molecular mechanisms underlying odorant binding and subsequent G protein activation of an OR. The pharmacology of an OR in the olfactory epithelium is thought to reflect the pharmacological property of the corresponding glomerulus in the olfactory bulb. We generated mOR-EG-ires-gapEGFP transgenic mice and performed calcium imaging to examine the odorant receptive range of the glomerulus innervated by mOR-EG-expressing neurons. The relative thresholds for various ligands in the mOR-EG-glomerulus were different from those observed in isolated mOR-EG-expressing neurons, suggesting that an odorant-induced activity pattern created in the olfactory bulb *in vivo* is not exactly reflected by peripheral OR pharmacology. Studies on OR function in the aspect of pharmacology, biophysics, and physiology will be presented.

Supported by PROBRAIN, Japan.

Poster: Chemosensory Coding and Clinical

Olfactory Event-Related Potentials: How Many Stimuli Do We Really Need?

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Objective: Measurement of chemosensory event-related potentials (CSERPs) is useful to quantify olfactory function in a relatively unbiased manner. We investigated the influence of the number of delivered stimuli on amplitude and signal-to-noise (S/N) ratio of CSERPs. Methods: CSERPs from 20 normosmic subjects were obtained in response to stimulation by two olfactory (H₂S and phenylethyl alcohol), and one trigeminal (CO₂) stimulant. For each of these odours, a series of 160 stimuli was delivered into the right nostril (stimulus duration 200 ms, mean ISI 30 s) using a constant-flow, air-dilution olfactometer. Only artefact-free trials were used for analysis. For each EEG recording site (Fz, Cz, Pz, C3, C4), peak-to-peak amplitudes PIN1 and NIP2 were determined, as well as noise amplitude levels. Subsequently, S/N ratios were calculated. The S/N ratio for olfactory ERPs significantly improves up to 80 delivered stimuli. The optimal number of stimuli for trigeminal ERPs is slightly lower, i.e., 60 stimuli. This result is mainly due to a reduction of the noise-level with increasing numbers of responses averaged. Applying more stimuli has little additional effect on the S/N

ratio due to a concomitant decrease in the amplitude of the signal. Conclusion: S/N ratio in olfactory ERPs is optimal when using 80 consecutive stimuli, while 60 stimuli appear to be optimal for CO₂.

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Poster: Chemosensory Coding and Clinical

Olfactory Event-Related Functional Magnetic Resonance Imaging Study in Young Adults

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Objectives: To explore brain activation mapping following odor presentation with event-related functional magnetic resonance imaging. Methods: Experiments were performed on 10 healthy young volunteers aged from 18 to 28 (5 men and 5 women). Odorant isoamyl acetate was delivered by olfactometer synchronously with inspiration birhinally for 10 times with interstimulus interval 60 s. fMRI method based on the blood-oxygen-level dependent effect were carried out on a 3.0 T scanner, using gradient-echo EPI technique. Results: Bilateral activations of the orbitofrontal cortex, anterior cingulate cortex, piriform cortex, insular cortex, amygdala, thalamus, the basal nuclei, temple cortex, frontal cortex seen. A greater extent of activation was evident in the right frontal cortex and left orbitofrontal cortex. Conclusions: Olfactory event-related fMRI is an objective measurement of olfaction, and has potential clinical significance.

Poster: Chemosensory Coding and Clinical

Hedonic-Specific Temporal Pattern of Response in Primary Olfactory Cortex of Humans

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Evidence suggests that the primary perceptual axis of odor is valence, or how pleasant or unpleasant an odor is. Although human imaging studies have consistently found that this primary perceptual axis is reflected in orbitofrontal cortex (secondary olfactory cortex), it is not known whether or how it is reflected in primary olfactory cortex. We set out to probe this using fMRI. Subjects were presented with two pleasant odorants (strawberry and citral) and two unpleasant odorants (hydrogensulfide and propionic acid). Odorants were chosen such that the pleasant and unpleasant groups contained trigeminal and non-trigeminal odorants. In data from a single subject, we find that trigeminal odorants elicited significantly lower amplitude BOLD response. We also found that unpleasant odorants had significantly greater full-width-at-half-height, and therefore a much lower slope. This result implies that the hedonic value of odors might be temporally encoded at the level of primary olfactory cortex.

Funding: cz funded by NSFGRFP.

Poster: Chemosensory Coding and Clinical**The Association between Olfactory Recognition Memory Performance and Brain Activation in Older Males and Females: An fMRI Study**M. Wang¹, B. Cerf-Ducastel¹, E. Pirogovsky¹, E. Sundermann¹, K. Rattner¹, T. Allmon¹, M. Miller¹, J. Hackbarth¹ and C. Murphy²¹San Diego State University, San Diego, CA, USA and ²San Diego State University and UCSD Medical School, San Diego, CA, USA

The present study investigated associations between olfactory recognition memory performance during fMRI scanning sessions and brain activation among healthy older adults (10 males, 10 females). Each subject was presented 16 odors immediately prior to entering the scanner. During two functional runs at 3T, target and foil names of odors were presented and each subject responded via button box whether or not each name corresponded with an odor presented to them. Older females demonstrated significantly higher performance on the discriminability index (d') than older males, consonant with a higher false positive rate of males during functional run 1. The d' for females was negatively correlated with activation in left parahippocampal gyrus and superior frontal gyrus and positively correlated with bilateral activation in fusiform gyrus. The d' for males was positively correlated with activation in left parahippocampal gyrus and negatively correlated with activation in right superior frontal gyrus. The false positive rate of males was positively correlated with activation in bilateral superior frontal gyrus and right parahippocampal gyrus and was negatively correlated with right medial frontal gyrus. For females, there were no significant correlations between false positive rate and brain activation. This study suggests greater impairment in odor recognition memory in older males that is associated with patterns of cortical activity in frontal and temporal areas.

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Poster: Chemosensory Coding and Clinical**Olfactory Perceptual Learning in Human Piriform and Orbitofrontal Cortex**J.A. Gottfried¹, W. Li¹, E. Luxenberg² and J. Howard¹¹Neurology, Northwestern University, Chicago, IL, USA, and ²Linguistics, Duke University, Durham, NC, USA

Enhancement in sensory acuity as a result of experience is known as perceptual learning. In humans, olfactory perceptual learning is critical to the development of odor discrimination and identification, but the underlying neural correlates remain poorly defined. Using a cross-habituation paradigm and functional magnetic resonance imaging, we examined the neural substrates in human piriform and orbitofrontal cortex (OFC) for perceptual learning to odorants qualitatively (QR) or structurally (SR) related to an odorant destined for habituation (TG). Sixteen subjects smelled TG, QR, SR odorants, and an unrelated control odorant (CT), before and after 3.5-min continuous exposure to TG. Behavioral and neural markers of habituation to the TG odorant were observed in the form of reduced intensity ratings and progressive signal decline in piriform cortex. Perceptual learning, indexed as a decrease in similarity ratings from pre- to post-habituation, was evident for the

TG/QR and TG/SR pairs, but not for the TG/CT pair. In parallel to these behavioral effects, we observed increased neural activity (from pre- to post-habituation) in piriform cortex for QR and in OFC for both QR and SR, in comparison to CT. Importantly, the increased activity to QR and SR in OFC significantly predicted the subsequent enhancement of odor discrimination. The data provide robust evidence for olfactory perceptual learning in human piriform cortex and OFC. These findings imply that sensory-specific representations of odor quality or structure in piriform and OFC are flexible and can be rapidly updated by mere perceptual experience.

Funding: Northwestern University.

Poster: Chemosensory Coding and Clinical**The Effects of Sleep Quality on Olfactory Event-Related Potentials in Healthy Adults**J.K. Essoe, E. Ramage, A.M. Parks, K. Lloyd, K. Hunt and M.W. Geisler
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Past research established that the amount of sleep (quantity) affects many indices of cognitive performance, including event-related brain potentials (ERP's); however, there is a paucity of research on how the characteristics of sleep (quality) may affect sensory perception and cognition. This study measured amplitude and latency in response to olfactory ERP's and odor threshold scores using the odorant amyl acetate, and subjective ratings of odor intensity in 26 (9 male, 17 female) good sleepers (GS) and 13 (7 male, 6 female) poor sleepers (PS) as measured by the Pittsburgh Sleep Quality Index (PSQI). Greater amplitudes for early stimulus detection (N1 component) were found in GS as compared to PS, while greater amplitudes for early sensory processing (P2), and later cognitive olfactory information processing (P3) were found in PS as compared to GS, with no significant differences in latencies found. Left nostril olfactory threshold sensitivity was significantly impaired in PS as compared to GS, and subjective odor intensity ratings revealed no significant differences between sleep quality groups. These results suggest that greater neuronal resources are allocated toward early sensory detection and may be a benefit of good sleep quality. However, there appears to be a hyper-arousal in early sensory processing, and the later cognitive processing as a result of poorer sleep quality. Thus, sleep quality may play an important role in enabling the brain to detect olfactory stimuli more accurately, and to process and interpret the stimuli more efficiently.

Poster: Chemosensory Coding and Clinical**Echo Time Dependence of Bold FMRI Studies of the Piriform Cortex**R. Kopietz¹, J. Albrecht¹, J. Linn¹, V. Sakar¹, O. Pollatos¹, A. Anzinger¹, J. May¹, T. Wesemann¹, G. Fesl¹, G. Kobal² and M. Wiesmann¹¹Department of Neuroradiology, University of Munich, Munich, Germany and ²Sensory Research R&T, Philip Morris USA Inc., Richmond, VA, USA

Objectives: In FMRI studies brain areas commonly associated with the processing of olfactory stimuli are often obscured by

susceptibility-induced signal loss. We hypothesized that decreasing the echo time (TE) should not only reduce the susceptibility artifacts but also increase the overall signal-to-noise ratio and allow us to retrieve a BOLD signal in regions normally affected by these artifacts. Methods: We compared two gradient echo sequences with echo times of 60 and 32 ms within two experimental paradigms: a standard motor paradigm ($n = 12$) and an olfactory stimulation paradigm ($n = 11$). Comparisons were done by measuring signal intensity changes in defined regions of interest and counting local activation maxima. Results and Conclusions: Reducing the TE decreased geometrical distortions and signal drop-out at orbitofrontal and temporomesial brain areas. At TE = 32 ms signal intensity changes were reduced by 51% in the motor cortex (motor paradigm), but also by 48% in the piriform cortex (olfactory paradigm). Moreover, piriform activations were detected in less subjects at TE = 32 ms than at TE = 60 ms. We conclude that although shortening TE reduces signal drop-outs, it is not sufficient to recover the BOLD signal from regions affected by susceptibility artifacts such as the piriform cortex and can not be recommended for olfactory fMRI studies.

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Poster: Chemosensory Coding and Clinical

Contribution of the Lateral Orbitofrontal Cortex to Processing of Binary Odor Mixture

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We recently demonstrated that secondary olfactory regions, contrary to primary regions, are preferentially activated during the processing of binary odor mixtures in comparison to single compounds. Particularly, the lateral orbitofrontal cortex (IOFC) appears to have a key role in this process. Our current aim is to establish whether the IOFC is activated proportionally to increasing impurity of odorants, defined as a deviation from pure odorant, in binary mixtures. Twelve subjects underwent PET, and the data from 5 scans of pyridine and citral mixtures, in varying physical proportions (from 10/90 to 90/10, with 50/50 being the most impure), were analyzed. We calculated the mean regional cerebral blood flow (rCBF) in 4 regions of interest (ROI) for all 5 conditions: left and right IOFC and left and right piriform cortex (PIR). For ROIs in the IOFC, we predicted maximum rCBF in response to the 50/50 mixture and minimum activation to the 10/90 and the 90/10 mixtures. For both ROIs in the PIR we expected no difference in rCBF across these conditions. In both hemispheres, rCBF in the IOFC increased with odorant impurity, as indicated by an inverted U-shaped function. There was no significant difference in rCBF in the PIR across conditions. The results support the notion that activation in the IOFC increases in conjunction with odorant impurity in binary mixtures and add credence to the vital role of the lateral orbitofrontal cortex in the processing of binary odor mixtures.

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Poster: Chemosensory Coding and Clinical

Brain Activations to Chemical Signals

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It has been demonstrated in animals across every phylum that affective and motivational states can be communicated through chemical signals. Moreover, the mechanism and neural substrates involved in processing social and nonsocial chemical information can be different. Humans have also been shown to distinguish among chemical signals from different emotional states. Brain imaging studies on human responses to social chemosignals have been limited, primarily using positron emission tomography and mostly focusing on brain activations to one or two steroids. In this present study, we use functional magnetic resonance imaging to investigate the neural basis of social chemosignals. We hypothesize that social chemosignals will likely activate common areas of the brain involved in processing social information from other modalities. We use emotional as well as other types of social and nonsocial olfactory stimuli, and compare the activations in the amygdala, hypothalamus, cingulate, insular, and prefrontal cortex, areas previously associated with processing socio-emotional information. Emotional chemosignals consist of sweat collected from donors while they were experiencing a particular emotion. All olfactory stimuli are delivered through a computer-controlled fMRI-compatible odor delivery device (olfactometer). Preliminary analysis suggests different neural substrates in processing social vs. nonsocial chemosignals. We are currently determining whether the pattern applies to a greater number of subjects.

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Poster: Chemosensory Coding and Clinical

Olfactory Event-Related Brain Potentials to Near-Threshold Stimuli in Healthy Adults

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Increased amplitudes and shortened latencies of olfactory event-related potentials (OERP's) in response to supra-threshold stimuli have been correlated to behavioral odor threshold scores, however, the physiological processing of near-threshold olfactory stimuli and the relationship to behavioral measures have not been examined. This study used a modified single-stimulus paradigm measuring OERP's at midline and 10 global sites in response to odor stimuli presented in block randomization at slightly below-threshold (SBT), near-threshold (NT), and supra-threshold (ST) concentrations. Reaction time, odor intensity ratings, behavioral odor thresholds and magnitude matchings of the odor stimuli to a series of weights, were collected in 16 male and 23 female college students. Results indicate that: 1) the amplitudes for early sensory olfactory information processing of the N1 and P2 ERP components are similar for SBT and NT stimuli and greater for ST, while the timing, or latency, of the P2 and N2 ERP components are more similar for SBT and NT stimuli with longer P2 and shorter N2 latencies for ST, 2) the amplitudes for later cognitive olfactory information

processing of the P3 ERP component significantly increased as odor stimuli concentration increased, and 3) amplitudes were greatest in the frontal electrode sites for both N1 and N2 components and greatest in the parietal electrode sites for both the P2 and P3 components. Results suggest that the greatest difference between the OERP's of NT and SBT stimuli are found during cognitive information processing.

Poster: Chemosensory Coding and Clinical

Central Olfactory Activity to Different Odor Intensity in Older People

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During aging, human ability to evaluate odor intensity declines. The purpose of this study was using fMRI to examine the aging effect on central olfactory activity in response to different odor intensity. The odor lavender (intensities 0.10%, 0.32%, and 1%) was presented to the nose with an olfactometer. The odor intensities used were determined psychophysically by another group of normal volunteers. The subjects were asked to rate odor intensities using a scale of 0–10. Twelve subjects (66.0 ± 10.9 years, 8 m, UPSIT score 33.3 ± 2.8) received fMRI at 3T. Ten of them provided complete pre-scan and after-scan evaluations of the odor intensities. While the subjective rating did not provide significant difference between odor intensities ($P > 0.11$), fMRI showed significant activation difference induced by different odor intensities (except between intensities 0.10% and 0.32%). Compared to two weaker intensities, 1% lavender introduced significantly stronger activation in the bilateral primary olfactory cortex, hippocampus, and entorhinal cortex ($P < 0.001$). These findings suggest that the decline of the ability to evaluate different odor intensities during aging is not caused by the functional decay of the brain structures mentioned above.

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Poster: Chemosensory Coding and Clinical

Reduction of Magnetic Susceptibility Artifacts in Olfactory fMRI with GESEPI-SENSE-EPI Method

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The severe magnetic susceptibility artifacts (signal loss and geometric distortion) in the images of inferior brain areas pose a difficult challenge in conducting olfactory fMRI in these brain regions. To remove the artifacts for consistent olfactory fMRI mapping, we developed the Gradient-Echo Slice Excitation Profile Imaging (GESEPI) technique. Combining the GESEPI with the state of art SENSE technology yields an effective method (GESEPI-SENSE-EPI) for olfactory fMRI with magnetic susceptibility artifact reduc-

tion. The effectiveness of GESEPI-SENSE-EPI in detection of olfactory fMRI in the brain areas with severe artifacts was tested on nine human subjects with GESEPI-SENSE-EPI and conventional EPI. The activations detected by the GESEPI-SENSE-EPI method in the base of the brain are in the areas of anterior olfactory nucleus and olfactory tubercles, which are impossible to see with conventional EPI. The success in detection of olfactory activation in these brain areas demonstrates that the GESEPI-SENSE-EPI is highly effective in artifact reduction and fMRI data acquisition.

Supported by NIH RO1 EB00454.

Poster: Chemosensory Coding and Clinical

Olfactory Tests in the Diagnosis of Essential Tremor

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Most patients with tremor-dominant Parkinson's disease (PD) have impaired smell function but it is unclear whether this is true for subjects with essential tremor (ET). If ET patients do not exhibit meaningful smell loss, then olfactory testing may help to distinguish PD from ET. We assessed olfactory function of 59 ET and 64 PD patients using the University of Pennsylvania Smell Identification Test (UPSIT) and the olfactory event-related potential (OERP). UPSIT scores were compared to those from 245 healthy controls, and OERPs were compared to those from 74 controls. Unlike the PD test scores, those of ET patients were indistinguishable from controls when the effects of age, age of onset, gender, and smoking were taken into account. ET patients with a family history of tremor scored significantly better than controls on the UPSIT, and their rate of decline with age was slower. The effect was not observed on the OERP. This suggests that smell testing may help to distinguish between ET and tremor-predominant PD, and that patients with a positive family history of tremor may represent a subgroup whose olfactory function is enhanced by some unknown mechanism.

Poster: Chemosensory Coding and Clinical

Chemosensory Measurement in Essential Tremor

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Objective: Tremulous Parkinson's disease (PD) may be confused with Essential Tremor (ET). Smell and taste are abnormal in early stage PD but probably not at any stage in ET. We wished to determine whether chemosensory testing is normal in ET and if so this might help distinguish it from PD. Methods: Three procedures were used: (1) University of Pennsylvania Smell Identification test (UPSIT); (2) Smell Threshold Test (Senonics Inc.) using phenylethylalcohol in (i) 45 healthy controls, mean age 49 y (17–93 y), and (ii) 50 ET patients mean age 62 y (17–82 y); (3) taste threshold measurement with Rion TR-06 electrogustometer applied to (a) tip of tongue (chorda tympani; CT) or (b) base of tongue over the most lateral vallate papilla (VP; IX). All participants scored at least 27/30 on the Mini-Mental Status Test. Results: There were no significant differences in any control vs. ET comparisons as follows (t -test, $P > 0.05$). (1) Mean UPSIT scores between controls and ET (32.8/40 vs.

32.3/40). (2) Mean control smell thresholds: -6.7 vol/vol compared to -6.3 vol/vol for ET. (3) Mean taste threshold: controls CT: 11.2 dB; VP: 13.5 dB. ET CT: 13.5 dB and VP 14.6 dB. Conclusions: Smell sense and taste threshold are normal in ET compared to healthy controls. This information may be of value in distinguishing ET from tremulous PD patients.

Poster: Chemosensory Coding and Clinical

Nasal Mucosa in Patients with Parkinson's Disease

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Idiopathic Parkinson's disease (PD) is a neurodegenerative disorder involving several neuronal systems. The pathognomonic formation of neuronal inclusion bodies (Lewis bodies) usually starts in the medulla oblongata and the anterior olfactory nucleus, before motor symptoms become evident. Thus, an impaired olfactory function, when tested, may constitute one of the earliest symptoms of PD. However, it is still unknown to what degree eventual changes of the olfactory epithelium may contribute to dysosmia in these patients. The aim of this pilot study was to investigate the morphology of the olfactory epithelium in subjects diagnosed with PD since several years. Biopsies of seven individuals diagnosed with PD (mean age: 76 years) as well as four anosmic controls (mean age: 53 years) were taken from different sites of the nasal mucosa. For immunohistochemistry, antibodies against olfactory marker protein (OMP), neurotubulin, protein gene product 9.5 (PGP 9.5) were applied to paraffin embedded tissue sections. Further, mRNA for OMP was isolated from olfactory and respiratory mucosa using RT-PCR. Preliminary immunohistochemical findings showed irregular areas of olfactory epithelium positive for PGP 9.5 and neurotubulin, but mostly negative for OMP. However, mRNA for OMP was found in mucosa of the olfactory cleft as well as in the respiratory mucosa. There were no apparent differences to the olfactory epithelium of patients with idiopathic anosmia. With due caution in this small number of individuals tested, we conclude from these data that PD-related olfactory impairment is not immediately associated with specific changes in the olfactory epithelium.

Poster: Chemosensory Coding and Clinical

Idiopathic Parkinson's Disease Is a Primary Olfactory Disorder

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In 1999, I proposed that idiopathic parkinson's disease (IPD) was a primary olfactory disorder and that the pathogen gained access to the brain through the nose. Since then there have been further supportive developments: (1) confirmation of alpha-synuclein deposits in the olfactory bulb in nearly all cases, (2) observations by Braak *et al.* (2003) that the earliest changes are in the olfactory bulb and dorsal medullary nuclei of IX and X, (3) virtual absence of mitral cells—the first relay in the olfactory path, (4) demonstration of hyposmia in at least 80% patients measured by identification score

or olfactory event related potentials, (5) correlation in some studies of smell tests and disability, (6) association of impaired dopamine transporter uptake or abnormal s. nigra transcranial Doppler in about one third cases with idiopathic anosmia, (7) prospective community-based studies from the Honolulu-Asian Aging study show predictive value of olfactory testing for IPD on the basis of clinical and pathological examination. It is proposed that in IPD, olfactory impairment is the earliest change along with damage in cranial nuclei IX and X; that these alterations predate the motor component by several years and that the likely agent is a neurotropic virus which accesses the olfactory nerves and medulla through the nasopharynx.

Poster: Chemosensory Coding and Clinical

Clarifying the Nature of the Olfactory Impairment Found in Parkinson's Disease

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Over the last 30 years investigators have shown that patients with Parkinson's disease (PD) perform poorly on odor identification tests. However, the relative contributions of sensory and cognitive impairment to this decline in performance are not well understood. This study investigated the possible role of a decrease in olfactory signal strength on odor identification in PD patients. Patients were assessed using a nine-odor olfactory identification test (OIT) and the Sniff Magnitude Test (SMT). The SMT is a reliable measure of olfactory function that examines sniffing behavior to quantify olfactory abilities. The tests were modified to incorporate odors that could be administered in increasing concentrations under the hypothesis that patient performance on both measures would improve as odors are intensified. Repeated measures ANOVA revealed that patients with PD correctly identified more high concentration ($M = 5.36$, $SD = 2.28$) than low concentration odors ($M = 4.09$, $SD = 2.20$) on the OIT, $F(1,21) = 13.77$, $P < 0.001$. Similarly, higher odor intensities produced more sniff suppression when taking the SMT, $F(1,21) = 13.69$, $P < 0.001$. The results suggest that a portion of the olfactory identification deficit in patients with PD is the result of decreased olfactory signal strength. The findings are discussed in terms of the theoretical understanding of olfactory functioning in PD and stimulus selection in olfactory tests.

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Poster: Chemosensory Coding and Clinical

Cerebral Activation in PD during Olfactory Stimulation—An fMRI Study

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Objectives: Olfactory dysfunction is common in patients with Parkinson's disease (PD). The cerebral systems however being involved in the olfactory disorder in PD are still largely unknown. **Methods:** In 12 PD patients and 16 age-matched healthy controls olfactory function was evaluated using psychophysical testing (Sniffin'Sticks test battery) and functional (f)MRI. In both experiments, olfactory stimuli (phenylethylalcohol, PEA) were separately applied to the left and right nostril using a computer-controlled dynamic olfactometer (OM2S; Burghart, Germany). Statistical analysis of functional images was performed using SPM2. **Results:** In contrast to controls, in PD patients, activation was almost exclusively observed during left-sided stimulation. In PD left-lateralized activation was observed in the amygdala, the hippocampal formation, and in region of the inferior parietal lobe. Direct contrasts between both groups revealed reduced activity in the inferior parietal lobe in PD patients. **Conclusions:** Olfactory stimulation produced distinct patterns of activation in PD with a prevalence for left-sided activation. Olfactory stimuli in PD patients seem not to be sufficient to gain bilateral access to olfactory processing even in the amygdala, a primary olfactory relay. A reduced interhemispheric exchange could explain the lateralized activity in regions of the inferior parietal lobe in PD patients.

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Poster: Chemosensory Coding and Clinical

Impairments in Source Memory for Olfactory Stimuli in Preclinical Gene Carriers of Huntington's Disease

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Source and item memory for olfactory and visual stimuli were examined in 10 pre-symptomatic Huntington's disease (HD) gene carriers and 10 controls. During the study phase, a male and a female experimenter (sources) presented odors and objects to the participant in an alternating sequence. To assess item memory, the participant chose between a stimulus from the study phase and a novel stimulus. To assess source memory, the participant identified whether the male or female experimenter had previously presented the stimulus. The present study also assessed odor detection in gene carriers and controls. No significant differences were detected between gene carriers and controls in source memory for visual stimuli. However, source memory for olfactory stimuli was impaired in gene carriers compared to controls. Gene carriers and controls did not differ in item memory for olfactory or visual stimuli. In odor detection, gene carriers were significantly impaired compared to controls. The effect size for the olfactory source memory result was notably higher than the effect size for the odor threshold finding. This suggests an important cognitive component in addition to the olfactory sensory impairment in gene carriers. Overall, these results suggest that source memory for olfactory stimuli may be particularly sensitive to neuropathological changes in pre-clinical stages of HD.

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Poster: Chemosensory Coding and Clinical

Olfactory Identification as a Function of ApoE-Status in Nondemented Adults: Evidence from a Population-Based Sample

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The ϵ -4 allele of the ApoE gene is a well-established risk factor for Alzheimer's disease (AD). Having one or two ϵ -4 alleles is associated with impairment in cued odor identification, a test that therefore might indicate AD at a preclinical stage. The present study investigates the ApoE gene's influence on odor identification ability in a large population-based sample ($N = 1572$) of individuals screened for dementia at 5 years post-test. The sample was divided into a middle aged (45–60) and an elderly (65–80) group. Results show that ApoE ϵ -4 is associated with poor odor identification ability which is more pronounced in elderly males. We also show that homozygotic ϵ -4 carriers (two ϵ -4 alleles) display poorer odor identification ability than heterozygotic ϵ -4 carriers (one ϵ -4 allele) at older age, but not at middle age. The present findings provide evidence that elderly individuals with high and low genetic risk of developing AD can be differentiated on the basis of olfactory identification ability.

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Poster: Chemosensory Coding and Clinical

Longitudinal Evaluation of Smell Identification Deficits in Patients with Mild Cognitive Impairment

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Patients with Alzheimer's disease (AD) and mild cognitive impairment (MCI) consistently exhibit smell identification deficits relative to healthy control subjects in cross sectional studies. Few longitudinal studies have directly examined the evolution of these deficits over time in AD or MCI patients relative to elderly controls. In a prospective study of putative predictors of conversion to AD in MCI patients, we administered the University of Pennsylvania Smell Identification test (UPSIT) to 150 MCI patients (40 converters to AD on follow-up evaluation and 110 non-converters) and 63 group-matched healthy controls at baseline (BL), 2-year follow-up, and 4-year follow-up evaluations. Two- and four-year test-retest reliability of UPSIT scores for the entire sample of controls and patients was high (BL to 2 year, $r = 0.86$; 2–4 year, $r = 0.87$; and BL to 4-year, $r = 0.74$). All groups demonstrated significant

($P < 0.05$) declines in mean UPSIT scores across successive test intervals. Using ANOVA, a two-way group by test interval interaction revealed that patients who converted to AD showed significantly greater decline across test intervals than did healthy controls or non-converting patients ($P < 0.001$), even after adjusting for group differences in age ($P = 0.001$). These findings demonstrate that the UPSIT is sensitive to the evolution of smell identification deficits over time as a function of normal aging and early Alzheimer's disease.

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Poster: Chemosensory Coding and Clinical

Specific Effects of Chlorhexidine on Taste Identification

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Chlorhexidine, a bitter bis-biguanide, depresses the salty and bitter tastes. We tested 13 human subjects to see how pretreatment with two different concentrations of chlorhexidine gluconate, 1 and 3 mM, affects the ability to identify an ensemble of 10 taste stimuli, each presented 10 times. Stimuli were equal-perceived-intensity solutions of NaCl, quinine-HCl, sucrose, citric acid, mixtures of all possible pairs except sucrose + citric acid, and water. Two information theoretic measures, transmitted information in the entire 10×10 taste confusion matrix (T_{10}) and transmitted information for each pair of stimuli (T_2), served to quantify overall performance and pairwise stimulus discriminability, respectively. Both concentrations of chlorhexidine depressed discriminability relative to pretreatment with water in 12 of the 45 pairs compared ($P \leq 0.0001$). 3 mM chlorhexidine had a greater effect than did 1 mM chlorhexidine ($P = 0.03$). As expected, pretreatment with chlorhexidine affected performance with NaCl and quinine. In each case in which chlorhexidine depressed pairwise discriminability, one or both of the stimuli contained NaCl and/or quinine. Moreover, chlorhexidine depressed discriminations of NaCl, quinine, and NaCl + quinine from water, but not discriminations of sucrose or citric acid from water. Thus, concentration did not have a differential effect: 3 mM chlorhexidine interfered with taste identification more than did 1 mM chlorhexidine but the effect remained specific to NaCl and quinine.

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Poster: Chemosensory Coding and Clinical

Olfactory Function in Patients with Post-infectious and Post-traumatic Smell Disorders Before and After Treatment with Vitamin A: A Double-Blind, Placebo-Controlled, Randomized Clinical Trial

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Introduction: The presented data is based on a double-blind, randomized, placebo-controlled clinical trial for investigation on effectiveness of vitamin A in post-infectious and post-traumatic smell disorders. The effect is probably based on the stimulation of regeneration and repair of the peripheral olfactory system. Material and methods: A total of 60 patients (age: 20–70 years, mean age: 52 years) participated 26 of whom received placebo (7 male, 19 female) and another 26 verum (6 male, 20 female). A standardized history was obtained in each patient. Olfactory function was measured by means of the “Sniffin’Sticks” test kit, a validated technique to investigate odor thresholds, odor discrimination, and odor identification. Vitamin A was prescribed at a dose of 10.000 I.U./d for 3 months. Follow-up testing was performed on average 5 months after the first investigation. Results: Forty-four percent of all patients reported subjective recovery of their sense of smell; 27% of the participants exhibited significant improvement in measured olfactory function. However, there was no significant difference between the outcome of patients receiving verum or placebo. Conclusion: The application of vitamin A at a dose of 10.000 I.U./d for 3 months does not appear to be useful in the therapy of olfactory loss.

Poster: Chemosensory Coding and Clinical

Serotonin and Noradrenaline Differentially Modulate Taste Sensitivity in Humans

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To determine the effect of altering serotonin and noradrenaline levels on taste sensitivity in humans a series of quinine, sucrose and sodium chloride and hydrochloric acid solutions were presented to the tip of the tongue in 20 healthy human subjects. The subjects indicated whether or not they could recognise the taste stimuli at each concentration. Each volunteer was then given a single acute dose of either paroxetine (serotonin selective reuptake inhibitor), reboxetine (noradrenaline reuptake inhibitor) or placebo (lactose), in a double blind cross-over design. Taste recognition tests were performed again 2 h after drug administration. Psychophysical taste function curves were constructed for the group for each taste modality before and after drug intervention. Paroxetine significantly increased quinine [threshold 30.4 μ M (24 μ M to 38 μ M 95% CI) before, 14.4 μ M (11 μ M to 19 μ M 95% CI) after; $P < 0.0001$] and sucrose [threshold 23.4 mM (21 mM to 27 mM 95% CI) before, 16.6 mM (14 mM to 19 mM 95% CI) after; $P < 0.001$] taste sensitivity. Reboxetine significantly increased quinine taste sensitivity [threshold 30.8 μ M (24 μ M to 39 μ M 95% CI) before, 19.4 μ M (15 μ M to 26 μ M 95% CI) after; $P < 0.02$] but had no effect on sucrose acuity. NaCl and HCl taste responses were not affected by either drug. Placebo had no significant effects on any taste modality. Our findings support a differential, modality specific neuromodulatory role for serotonin and noradrenaline in human taste perception.

Experiments complied with UK legislation and funded by the Department of Physiology and AWP NHS Trust.

Poster: Chemosensory Coding and Clinical**Olfactory Deficits in Alcoholism: Association with Impaired Executive Function**

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Olfactory dysfunctions are common in patients with chronic alcoholism. Brain regions implicated in the neuropathology of alcoholism overlap with those involved in olfaction. The goal of the present study was to investigate whether deficits in olfactory functions are related to cognitive deficits in neuropsychological tasks, which are suggested to represent sensitive measures of frontal and medial-temporal lobe functioning. We assessed olfactory functions (Sniffin' Sticks), executive function and memory in patients with a diagnosis of alcohol dependence and healthy control subjects, comparable in age, gender and smoking. Patients performed more poorly than controls in their ability to detect an odor in low concentrations, to discriminate between qualitatively different odors, and to identify odors. Compared to controls, patients were also impaired in both neuropsychological domains (executive function and memory). In patients, olfactory discrimination ability was positively correlated with executive function performance. Regression analyses indicated that group was the only significant predictor of olfactory detection threshold and identification, and both, group and executive function were found to be the significant predictors of olfactory discrimination. Results support the assumption that olfactory deficits in alcohol-dependent patients may not be explained with cognitive deficits. Our findings suggest that deficits in olfactory discrimination ability may be associated with frontal lobe dysfunction in chronic alcoholism.

Poster: Chemosensory Coding and Clinical**Early Postnatal Alcohol Exposure Reduced the Size of the Nucleus of the Solitary Tract (NST) in Neonatal Rat Pups**C.X. Li¹, S.E. Maier², S.M. Brassler¹ and R.S. Waters¹*¹Anatomy and Neurobiology, University of Tennessee Health Science Center, Memphis, TN, USA and ²NIAAA, NIH, Rockville, MD, USA*

Introduction: A strong association exists between alcohol ingestion and sucrose responsive neurons in rat NST. Here, we examined the effect of postnatal alcohol exposure on the size of NST in rat pups examined on postnatal day 10 (P10). **Methods:** Pups obtained from untreated and normally conceived Sprague-Dawley dams were randomly assigned to alcohol (EtOH), pair-fed (PF), and untreated (UC) groups. EtOH pups received 6 g/kg dosage of alcohol while PF pups received isocaloric maltose-dextrin solution from P4–P9. EtOH and PF pups were treated daily (binge model) through a gastrostomy tube implanted for artificial rearing. On P10, pups were sacrificed, brains removed and weighed, brainstem blocked, cut along a sagittal plane, and sections stained with cytochrome oxidase. The NST was digi-

tized, reconstructed in Photoshop 7.0, and NST size measured using ImageJ. Body weight was also examined over postnatal days. **Results:** The total area of NST in EtOH pups (mean area = 0.32 mm²) was significantly smaller ($P < 0.5$) compared to PF (mean area = 0.39 mm²) controls. Normalization of the data (EtOH vs. PF) revealed a 16% reduction of total NST in EtOH pups. Similarly, normalized body and brain weights of EtOH pups were 12% and 25% lower compared to PF pups, respectively. **Conclusion:** The present results suggest that early alcohol exposure results in detrimental effects on developing NST.

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Poster: Chemosensory Coding and Clinical**Lactisole Greatly Decreases Differential Thresholds for Sucrose: A Case for Increased Cooperativity**V. Galindo-Cuspinera¹, A.A. Tharp¹, M. Winnig², B. Bufe², W. Meyerhof² and P.A. Breslin¹*¹Monell Chemical Senses Center, Philadelphia, PA, USA and ²German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany*

We have previously demonstrated that adaptation to a taste stimulus results in increased sensitivity, despite decreasing the overall perceived intensity. Here, we demonstrate that the addition of a taste inhibitor can also lead to increased differential sensitivity. Lactisole, a broad-acting sweetness inhibitor, suppresses the sweet taste of sugars, protein sweeteners, and artificial sweeteners. Lactisole's inhibitory effect is specific to primates and does not affect responses to sweet compounds in rodents. Here we show by using two different psychophysical methods, Weber fractions and psychometric functions, that lactisole added in small amounts greatly increases differential sensitivity to sucrose, reducing Weber fractions to as low as 0.25%. Some subjects were able to distinguish 400 from 401 mM sucrose. We observed that lactisole acts as an inverse agonist by studying, *in vitro*, its interaction within the intrinsically active hTAS1R2-hTAS1R3 sweetener receptor heteromer. The presence of an inverse agonist, lactisole, could enable an increased cooperativity of sucrose molecules when binding to the hTAS1R2-hTAS1R3 receptor, which would explain the greatly increased differential sensitivity to sucrose observed in the presence of lactisole.

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Poster: Chemosensory Coding and Clinical**Effects of Pregnancy on Olfaction**

E.L. Cameron

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Reports of changes in olfactory sensitivity during pregnancy have been inconsistent, but hedonic ratings do appear to change. Odor identification is unaffected by pregnancy, but the range of odors tested has been limited. The current study tested 30–18 to 45-year-old non-smoking women (15 pregnant, equally divided across trimester, and 15 age-matched controls) on the 40-item UPSIT.

Intensity ratings and number of scratches and sniffs were collected as indicators of sensitivity, and participants rated the pleasantness of each odor. Participants also rated their own sense of smell. Mean UPSIT scores were 35.4 (pregnant) and 35.5 (controls), indicating no difference in odor identification for a large range of odors. Pregnant women rated their sense of smell significantly higher than controls (Wilcoxon test), and trends suggest that they used fewer scratches and sniffs per odor, potentially confirming their self-reports. Between groups, mean intensity and pleasantness ratings approached significance; 87% of odors were rated as slightly more intense and 67% as less pleasant by pregnant women. Consistent with previous reports, over 90% of pregnant women reported that specific odors smell less pleasant (e.g., meat and smoke) and about 75% reported that some odors smell more pleasant (e.g., fruit). In conclusion, pregnant women report that their odor sensitivity is enhanced during pregnancy and appear to need to scratch and sniff less often in order to identify odors on the UPSIT. Moreover, pregnant women show enhanced intensity and hedonic ratings. Odor identification, however, is unchanged. These data are part of an on-going study that will examine trimesters and postpartum phases separately.

Poster: Chemosensory Coding and Clinical

Nicotine Suppression of Taste and Gustatory Responses of NTS Neurons

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Smokers weigh less than nonsmokers and gain weight disproportionately upon quitting. We investigated the hypothesis that the anorectic effect of nicotine in tobacco is mediated partly by a reduction in the intensity of and preference for certain tastes via depression of central gustatory transmission. In psychophysical experiments using a sensitive two-alternative forced choice method coupled with magnitude ratings of taste on each side of the tongue, nicotine reduced the perceived intensity of sweet and bitter taste qualities and reduced hedonic ratings of a naturally sweetened custard. The effect was transient and no longer present 5 min later after nicotine irritation had waned. In single-unit recordings from anesthetized rats, nicotine directly excited gustatory neurons in the nucleus of the solitary tract (NTS) and significantly attenuated their responses to the preferred tastant (sucrose, NaCl, citric acid or MSG) in a dose-dependent manner. Nicotinic excitation of NTS units, and depression of tastant-evoked responses, were significantly attenuated by the nicotinic antagonist mecamylamine. Nicotine excited NTS units in rats in which trigeminal afferent input was blocked by ganglionectomy, but no longer depressed tastant-evoked responses, indicating that nicotine excites NTS units directly via taste nerves and inhibits their tastant-evoked responses by a central trigeminally-mediated mechanism. The results are consistent with the hypothesis that nicotine reduces food intake in part by a physiologically mediated reduction in taste intensity and palatability.

Poster: Chemosensory Coding and Clinical

Multimodal Sensory Stimulation of the Nasal Mucosa with Nicotine—fMRI Study

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Objectives: No fMRI data are available on cortical activations induced by the effects of nicotine on the olfactory system. If applied to the nasal mucosa in low concentration, nicotine evokes odorous sensations by activation of olfactory receptors. At higher concentrations, trigeminal fibers are activated as well leading to burning or even stinging sensations. The aim of this study was to investigate brain areas activated by nasal stimulation with nicotine at low suprathreshold olfactory concentrations. Methods: Nicotine was applied at suprathreshold concentration in 19 healthy subjects using a constant-flow olfactometer (block-design) Functional images were acquired using a 1.5T MRI scanner. Results and Conclusions: We found activation of brain areas known to be involved following olfactory stimulation of the nasal mucosa (piriform cortex, orbitofrontal cortex, insula), as well as areas specific to the processing of emotions (amygdala, cingulum) and areas related to attention and memory (middle frontal gyri, superior parietal lobule). We also found activations in areas specific to the processing of painful and aversive stimuli (ventroposterolateral thalamus, S2) indicating that nicotine is a multimodal stimulus which affects both olfactory and somatosensory areas. In summary, even at low concentrations nicotine activates both the olfactory and the trigeminal system.

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Poster: Chemosensory Coding and Clinical

TOKI-Shakuyaku-San in the Treatment of Sensorineural Smell Dysfunction

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Steroid therapy is used in the treatment of sensorineural smell dysfunction such as viral infection of upper respiratory tract, however its efficacy is still considered controversial. Toki-shakuyaku-san (TSS) (Tsumura & Co., Tokyo, Japan), a Chinese herbal medicine, has recently been reported as effect in the treatment of Alzheimer's dementia. The purpose of this study was to compare the clinical efficacy of TSS with local steroid therapy for sensorineural smell dysfunction. TSS was prescribed for 40 patients (28 with post viral infection and 12 with head trauma) from 1998 to 2004. A group of 60 patients (46 with post viral infection and 14 with head trauma) who received local steroid therapy from 1993 to 1997 served as controls. For assessment of olfactory function, T&T olfactometry was employed before and after treatment. Of the 28 post viral infections treated by TSS, 19 cases (67.9%) recovered completely or partially. This was significantly better than the efficacy for local steroid therapy (43.5%). For the head trauma group, there was no significant

difference between the efficacy of TSS (41.7%) and local steroid therapy (28.6%). Since local steroid therapy produces side effects such as ACTH suppression and glucose intolerance and TSS did not show any serious side effects, TSS may prove to be a preferred therapy for smell dysfunction following viral infection.

Poster: Chemosensory Coding and Clinical

The Potential Effect of Ambient Arsenic in Drinking Water on Odor Identification in an Agricultural Sample in Inner Mongolia

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There is evidence that exposure to arsenic (As) can have neuropathic and neurosensory effects in humans. It is unknown if As affects the sense of smell. To determine if odor identification is impaired by As exposure in drinking water, 308 persons in Inner Mongolia were given the Brief Smell Identification Test (BSIT). The mean score was 7.398. The age range was 10–61. The subjects were primarily farmers who drink well water. The mean As concentration in drinking water was 126.0 $\mu\text{g/L}$ (range 0.34–825.7). The drinking water standard in the USA is 10 $\mu\text{g/L}$. This resulted in mean urinary As_2O_3 , As_2O_5 , dimethylarsinic acid V (DMAV), and monomethylarsonic acid V (MMAV) levels of 53.75, 5.8, 201, and 53.4 $\mu\text{g/L}$, respectively. Regression analyses of the BSIT score vs. arsenic species indicates a significant relationship between urinary As species and odor identification. The *P*-values for the species are as: As_2O_3 *P* = 0.007; As_2O_5 *P* = 0.139; MMAV *P* = 0.016; DMAV *P* = 0.017. The regression with water As was not significant (*P* = 0.237). Thus, body burden is a better predictor of effect than water concentration. It is possible that the variability in quantity of water consumed which would alter the total dose or that some persons metabolize inorganic As into more toxic methylated species. The mechanism of the toxicity may reside in its chemotherapeutic usage. As_2O_3 is used to treat acute myeloid leukemia and its efficacy is thought act by causing incomplete cytodifferentiation and subsequently inducing apoptosis. Neurogenesis of olfactory receptor cells may be similarly impaired by As compounds. This is an abstract of a proposed presentation and does not reflect EPA policy.

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Poster: Chemosensory Coding and Clinical

Heterosexual Females, but Not Lesbians, Sensitize to Low Levels of Odorant

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Sexual orientation influences human olfaction. Here we report that lesbians, like heterosexual males, do not demonstrate a gender-typical process of olfactory sensitization to low level odorants previously reported for women, but not men. This finding suggests

that within-gender variation in sensitization among women may not be fully explained by changes in levels of activational hormones.

Poster: Chemosensory Coding and Clinical

Can Heterosexual Men and Women Discriminate Each Other from Their Axillary Secretions? If So, Do They Exhibit a Preference?

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Heterosexual participants (16 male; 24 female) identified the gender of an axillary sample (Study 1) and selected their preferred sample (Study 2) in a 2AFC task. Each study consisted of 25 intensity-matched pairs (male and female samples). Prior to the study, fabric swatches were worn in the under-arm for a 24-h period by 89 volunteers (40 male; 49 female). Samples were coded and double-blind rated by 8 odour assessors trained and experienced in evaluating axillary intensity. For Study 1 planned one sample *t*-tests (two-tail) were performed on mean accuracy for each gender separately, revealing that males (57.3% accuracy) and females (55.2%) identified the gender of sample significantly above chance. Study 2 (preference) revealed that females significantly preferred the swatches from female derived samples (56.7%). In contrast, males showed no preference for male or female derived samples. Results from the gender identification study challenge observations of near-universal superiority of females in olfactory function and suggest that males can be equally sensitive to biologically relevant odours. The preference study builds on findings by Martins, Preti, Crabtree and Wysocki (2005) who revealed patterns of preference that differed across groups of individuals having different sexual orientations. Our results now show a significant heterosexual female preference for heterosexual female odours. Although contradicting a sexual orientation based explanation hormonal fluctuations during the menstrual cycle, familiarity with own-gender relevant odours, and mate search during the ovulatory period only may offer alternative interpretations of these results.

Poster: Chemosensory Coding and Clinical

Leptin, Insulin and Sweet Taste in Gestational Diabetes Mellitus

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Leptin is the protein product of the LEP gene and is associated with adiposity, satiety and regulation of energy intake. The *db/db* mouse, a genetic model of diabetes that is hyperleptinemic but lacking a functional leptin receptor, shows elevated behavioral responses to sweet taste. Thus, leptin may exert effects on taste cells to modulate sweet taste. This relationship has not been studied in diabetic humans. This study assessed relationships between plasma leptin and insulin, and sweet taste in women with gestational diabetes mellitus (GDM). We measured fasting plasma leptin and insulin in 12

women with GDM at 24–28 weeks gestation (at diagnosis), 68 pregnant women without GDM and 12 non-pregnant controls. Subjects also rated sweetness intensity and liking of glucose solutions (0.01–0.16 M) and commercial fruit cocktail, using a 15-cm line scale. There were no group differences in sweetness intensity or liking for any of the stimuli. In women with GDM, leptin was correlated with sweetness intensity of fruit cocktail ($r = 0.58$, $P = 0.05$) and insulin was correlated with sweetness liking of both the fruit cocktail ($r = 0.61$; $P = 0.03$) and the glucose solutions (averaged across concentrations) ($r = 0.65$, $P = 0.02$). These associations were not observed in the other study groups. These preliminary findings are novel and suggest that leptin and insulin play a role in sweet taste disruptions in diabetic humans.

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Poster: Chemosensory Coding and Clinical

Revisiting the Sweet Tooth: Relationships between Sweetness Perception, Sweet Food Preference, and BMI

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As Pangborn noted in 1958, an obese individual is commonly thought to have a sweet tooth, but an experiment in her lab with sweet foods failed to show any association between sweet liking and body size. A variety of studies followed supporting the same conclusion. In addition, sensory studies suggest that perceived sweetness does not change with body mass index (BMI). We have argued that across-group scaling comparisons (e.g., obese vs. non-obese) are invalid unless investigators show that scale labels denote the same experiences to all. We solve this dilemma by asking subjects to rate sensory and hedonic experiences in larger contexts (i.e., all sensation, all hedonic experience). Data collected in this manner produced conclusions that differ from prior findings. In particular, sucrose liking (measured with the hedonic gLMS) rises with rising BMI, but the perceived sweetness of a candy (measured with the gLMS) declines. Thus, to make valid comparisons of sweet liking across BMI, sweet liking must be corrected for variation in perceived sweetness with BMI. Following Moskowitz's lead, we compared sucrose sweetness/liking functions for underweight, normal, overweight, and obese individuals. As BMI increases, the slope of the function increases; that is, for the same perceived sweetness, sweet liking increases with BMI.

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Poster: Chemosensory Coding and Clinical

PROP Bitterness and Cardiovascular Disease (CVD) Risk Factors in Adult Women

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Previous research from our group has found relationships between measures of taste genetics and CVD risk factors in middle-aged men, elderly women, and preliminary findings among middle-age women. Here we examined this relationship in 86 females (mean age = 45 ± 9 yrs) with multivariate analysis, controlling for non-taste factors that affect CVD risk ($P \leq 0.05$). Using the general labeled magnitude scale, Ss rated the bitterness of 6-n-propylthiouracil (PROP) and preference for surveyed high fat foods as well as reported frequency of consuming core high-fat foods on a validated instrument. For CVD risk assessment, total cholesterol, HDL and LDL subfractions were analyzed from fasting venous bloods and adiposity calculated from measured height/weight and waist circumferences. Resting blood pressure was measured on women who were 35 years and older ($n = 75$). Those who tasted less bitterness from 3.2 mM PROP averaged greater preference across 14 high-fat foods and more frequent intake of a 40-item high-fat group. As reported previously, PROP bitterness was negatively correlated with central adiposity, but only in those without excessive obesity (circumference ≤ 40 inches; $n = 76$). Those tasting less PROP bitterness showed greater CVD risk factors—higher systolic blood pressures, higher LDL and lower HDL cholesterol subfractions. These data support that those who taste PROP as least bitter may have greatest CVD risk and that the relationship may be mediated in part through dietary behaviors toward high-fat foods.

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Poster: Chemosensory Coding and Clinical

Inflammatory Pathways May Underlie Early Taste Loss and Taste Cell Death Caused by Radiation Therapy

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Taste loss is a distressing side effect experienced by nearly all cancer patients who receive head and neck radiation therapy. There is no treatment. Single dose and fractionated radiation rat and mouse models suggest that inflammation may play a role in the development of early taste loss and ultimately mediate taste cell death. We propose that the radiation-induced inflammation is mediated primarily via NFkB pathways, leading to cell death via oncosis/autophagy programmed cell death. Preliminary histological findings at early post-radiation time points only show the presence of a marked inflammatory infiltrate. Using RT-PCR, following a single 18 Gy dose at 4 days survival, there is an increase in IL-6 in rat fungiform and Bax and ICAM-1 in posterior taste tissue. Following two fractions of 5 Gy in mice, taste tissues show changes in expression levels of certain chemokines, cytokines and other factors involved in an inflammatory response including, for example, IL12b, IL1r1, Fcgr1g, Fcgr1, Scye1, Ccl8, Cxc10, IL1r2, IL5, integrin, and TLR9. Most of these factors are players in inflammatory pathways mediated by NFkB. Immunohistochemistry results demonstrate that the apoptotic indicators TUNNEL and caspase 3 are not elevated at 2 or 25 days following radiation. Electron micrographs of rat taste cells 7 days after a single 18 Gy dose show even margination of chromatin, degenerating membranes, and cytosolic

double membrane vesicles, features of oncosis/autophagy programmed cell death. These data suggest that early activation of inflammatory pathways, perhaps mediated by NFkB, may represent the mechanism underlying early radiation taste loss and subsequent taste cell death.

Poster: Chemosensory Coding and Clinical

Phenylthiocarbamide (PTC) Perception in Patients with Schizophrenia and First-Degree Family Members

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The inability to taste phenylthiocarbamide (PTC) has been associated with a number of medical and neurological illnesses not typically related to taste. We examined PTC sensitivity in schizophrenia patients and non-ill first degree relatives to determine whether taster status could represent a simple vulnerability marker. Method: PTC sensitivity was assessed in 67 schizophrenia patients, 30 healthy-comparison subjects and 31 first-degree family members. Results: A higher incidence of nontasters was seen in patients and family members relative to healthy comparison subjects. Among patients, analysis of clinical symptom ratings revealed that non-tasting patients exhibited increased levels of negative and first-rank symptoms, characteristic of those seen in the deficit syndrome. In addition, non-tasting patients also showed poorer odor identification skills relative to those patients who could taste PTC. These differences were not explained by sex, age, or cognitive impairment. Conclusions: These data demonstrate a higher incidence of nontasters to PTC in patients with schizophrenia and non-ill first degree family members. Phenotypic variation in PTC sensitivity is thought to be genetic in origin and may suggest a somewhat higher risk for illness in those subjects with the recessive alleles.

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Poster: Chemosensory Coding and Clinical

Retronasal Olfaction and Otitis Media

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Retronasal olfaction is perceptually localized in the mouth even though odors are actually stimulating receptors at the olfactory mucosa. Taste plays a role in that localization. Taste also contributes to the perceived intensity of retronasal olfaction for congruent taste/odorant pairs. For example, adding sugar will intensify the sensation of pear from pear juice, adding salt will not. We reasoned that if increasing taste intensity could increase retronasal olfactory intensity, then decreasing taste intensity might decrease retronasal olfactory intensity. At AChemS 2005, we showed data consistent with that idea; nontasters of PROP (who perceive reduced taste intensities) perceived less retronasal olfaction for the same perceived intensity

of orthonasal olfaction. Here we demonstrate another finding consistent with the taste/retronasal connection. Otitis media (OM) can damage taste since the chorda tympani taste nerve passes through the middle ear on its way to the brain. Does this taste damage result in reduced retronasal olfaction? Attendees at lectures sniffed a strawberry candy (orthonasal olfaction) and rated the strawberry sensation on the gLMS. They then chewed and swallowed the candy and rated the strawberry sensation (retronasal olfaction) again. Comparing regression plots of retronasal strawberry intensity as a function of orthonasal strawberry intensity for those with no histories of OM and those with histories of moderate to severe OM showed significantly lower slopes for the OM subjects. That is, for the same orthonasal intensity, the OM subjects perceived less retronasal intensity. This has implications for the way in which sensory alterations alter food preferences and thus lead to weight increases.

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Poster: Chemosensory Coding and Clinical

Retronasal but Not Oral-Cavity Identification of Nontrigeminal Odorants

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Vapor-phase odorants may be stimuli for the olfactory system, the trigeminal system, or both systems. Odorants known to have little or no vapor-phase trigeminal component should be ineffective when present in the oral cavity if retronasal olfactory stimulation is prevented. To study this, retronasal (retro) and oral-cavity-only (trigem) identifications of 67% octane (O) and 10% coumarin (C), octanoic acid (OA), phenylethyl alcohol (PEA) and vanillin (V), delivered in vapor-phase and presented 3 times each in random order, were made by 20 non-smoking unscreened subjects (14 females, median age = 20). Results: Correct identifications (ID) for retro were significantly greater than trigem, $P < 0.0005$. Percent correct retro ID were C, 70%, O, 85%; OA, 73%, PEA, 87%; V, 88%. Percent correct ID for trigem were C, 35%; O, 33%; OA, 12%; PEA, 18%; V, 12%. For these non-trigeminal odorants, retro ID were accurate, but trigem ID, using a nose clip and exhalation through the mouth to prevent retro olfactory access, approximated chance. Conclusions: Retro smelling of some odorants is solely olfactory; a nose clip and oral exhalation prevent retro olfaction.

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Poster: Chemosensory Coding and Clinical

Retronasal and Orthonasal Adaptation: Similar Over 90 Seconds

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Orthonasal adaptation for odorants is well studied but not retronasal adaptation. Retronasal and orthonasal judged intensity were tracked by 22 non-smoking unscreened subjects (14 females, median age = 21).

Five vapor-phase odorants (anise, coffee, orange, peppermint, and strawberry) were 1st matched for intensity orthonasal-only (ortho) and retronasal-only (retro) in a non-tracking task, then tracked over 90 s five times each in random order. Results: Times to initial, maximum, and final intensity did not differ significantly ($P > 0.12$, two-tailed t -test) between ortho and retro, and mean final intensities were less than initial intensities. However, only ortho intensity increased before decreasing. Mean ortho intensity increased 10% between initial and maximum ($P < 0.0001$), with final intensity 19% below initial ($P < 0.0001$). In contrast, mean maximum retro intensity was not significantly different from initial intensity ($P = 0.1324$), but final retro intensity was 17% less than initial ($P < 0.0001$). Another difference was that mean absolute retro intensity was 20–22% less than ortho ($P < 0.0001$). Conclusions: Retro and ortho smelling have similar decreases in judged intensity over 90 sec, although ortho but not retro intensity increases before decreasing.

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Poster: Chemosensory Coding and Clinical

Route of Administration Alters Olfactory Perception

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Olfactory percepts appear to depend on the route by which odorants reach olfactory receptors. Several studies have documented the inability of subjects to identify odorants retronasally (RN) that had previously been identified when delivered orthonasally (ON). However, such paradigms depend not only on subject's inherent sensory acuity, but also their odorant and semantic memory. To obviate the confounding influence of memory associated with identification tasks, we presently used a matching methodology where subjects evaluated a flavor in one condition (ON or RN delivery) and identified the same flavor from a group of 5 unknowns evaluated in either the same or different delivery condition. The delivery conditions included (a) ON delivery of references and unknowns (b) RN delivery of references and unknowns and (c) RN delivery of references and ON delivery of unknowns. In exp 1 subjects matched familiar flavors (orange, lemon, strawberry, pear and grape), in exp 2, unfamiliar flavors (lulo, acerola, guanabana, hibiscus, and papaya) and in exp 3 strawberry flavors having different profiles (fruity, green, woody, ripe & candy). In all 3 exps, subjects correctly matched significantly more flavors when the reference and unknown were delivered via the same route (i.e., RN-RN or ON-ON) than when reference and unknown were delivered via separate routes (RN-ON) supporting the hypothesis that the qualitative content of olfactory percepts are route dependent. Moreover, performance decreased as flavor familiarity decreased and flavor similarity increased, suggesting that specific cognitive strategies used in the matching task influence matching ability.

Poster: Chemosensory Coding and Clinical

Comparison of Responses to Electrical and Chemical Stimuli

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Perceived qualities from electrical and chemical stimuli were compared using physically similar stimulus delivery systems. Twenty female and six male young adult volunteers applied 1.6 v and 3 v batteries, and stainless steel washers of equivalent area holding the following tastants in approximately iso-intense quantities to the tips of their tongues for 1 sec: NaCl (applied twice to provide a measure of reliability, which was high), citric acid, FeSO₄, quinine HCl, alum, NaCl + citric acid, NaCl + quinine HCl, citric acid + quinine HCl. The resulting sensations were rated on 14 attributes utilizing line scales. Analysis by rectangular multidimensional scaling (unfolding) produced a satisfactory 3-d solution (S-STRESS = 0.097; RSQ = 0.982). The dimensions reflected tactile, saltiness, and hedonic qualities. Examination of the inter-point distances showed that the sensations produced by batteries were described best by the attributes "metallic," "copper penny," and "sharp," and that the electrical stimuli were positioned apart from the chemical stimuli and from their common descriptors. Thus the perceived qualities of electric-metallic sensations from the batteries differed from those of chemical stimuli, even when the two kinds of stimuli had similar tactile characteristics. The metallic quality perceived from electrical stimulation is consistent with previous literature.

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Poster: Chemosensory Coding and Clinical

An Extended Version of the "Sniffin' Sticks"

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The "Sniffin' Sticks" test-kit is a validated and commonly used tool to measure olfactory function in patients as well as in healthy subjects. To gain more detailed results the subtests on odor discrimination and odor identification were extended, using 32 instead of the usually applied 16 single tests each. The "new" test was applied to 110 subjects (60 patients with olfactory loss/50 healthy controls). In 55 of them testing was performed again after a mean interval of 4 days. Results revealed significant differences between patients and healthy subjects. Test scores for the first 16 tests were not significantly different from those obtained for the second (newly added) 16 tests for both, odor discrimination and odor identification. In addition, results for "old" and newly parts of the tests exhibited good a correlation (discrimination: $r_{110} = 0.78$; identification: $r_{110} = 0.81$). Test-retest-reliability of first and second session was very high for the complete TDI-score ($r_{55} = 0.92$) and for each subtest ($r_{55} = 0.83-0.94$). In conclusion, the extended test kit allows a precise examination of olfactory function, especially when different olfactory tasks are assessed using the individual subtests. Furthermore, the high test-retest-reliability allows to track even relatively small changes of olfactory function over time.

Poster: Chemosensory Coding and Clinical

Evaluation of US Patients Using the Japanese Odor Stick Identification Test (OSIT-J)

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The Odor Stick Identification Test for Japanese (OSIT-J) has proven effective when administered to control subjects in the United States (US). To determine if the OSIT-J is effective in assessing olfactory function in patients, we administered the OSIT-J and a test frequently used in US clinics, the Connecticut Chemosensory Clinical Research Center (CCCRC) Test, to 50 US patients. We also obtained their opinions regarding the two tests. Scores from both tests and patients' self-assessment were analyzed. Significant correlations were found between the OSIT-J score and the composite score on the CCCRC test ($r_s = 0.802$, $P < 0.0001$, $n = 50$) and patients' self-assessment of their olfactory function ($r_s = 0.734$, $P < 0.0001$, $n = 50$). Overall US patients reported that the OSIT-J was easier, more interesting, and the odors used were more pleasant than in the CCCRC test. The average time required to administer the OSIT-J (8 ± 1 min) was significantly shorter than that required for the standard CCCRC test (21 ± 6 min; $P < 0.0001$, $n = 39$). Findings suggest that the OSIT-J is an effective clinical olfactory function test for use with US patients.

Poster: Chemosensory Coding and Clinical

Administration of the "Sniffin' Sticks" Odor Identification Tests in Japanese Subjects

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Clinical olfactometry is not standardized like audiometry; several different tests have been developed in different countries. For example, the UPSIT is frequently used in the USA, "Sniffin' Sticks" are used in many European countries, and T&T olfactometry (T&T) is common in Japan. Aim of the study was to investigate the usefulness of the "Sniffin' Sticks" in Japanese subjects. T&T was also performed in parallel with the "Sniffin' Sticks" on the subjects who consulted with complaints of smell disorder. Normosmic Japanese subjects ($n = 105$) were studied using the original and a modified "Sniffin' Sticks" 12-item odor identification test with soy sauce odor replacing the odor of cloves which is rarely known in Japan. Average scores with the standard and modified "Sniffin' Sticks" versions were 9.14 ± 1.46 and 9.39 ± 1.46 , respectively ($P < 0.01$). The 50th percentile of the distribution of scores of both tests was 9.0. The lowest identification scores for all odors were obtained for liquorice (32%), leather (46%), and lemon (55%). Results from "T&T" correlated to results from the "Sniffin' Sticks" (Spearman test, $n = 66$, "T&T" detection threshold vs. "Sniffin' Sticks": $r_s = -0.51$, $P < 0.001$; "T&T" recognition threshold vs. "Sniffin' Sticks": $r_s = -0.54$, $P < 0.001$). In conclusion, the Japanese 50th percentile score (9.0) was lower than that obtained in Germany

(11.0). When two or three odors unfamiliar to Japanese people are replaced with more familiar ones, "Sniffin' Sticks" will become a significant clinical test of olfactory function in Japanese patients.

Poster: Chemosensory Coding and Clinical

A Comparison of Methods for Sniff Measurement Concurrent with Olfactory Tasks in Humans

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Appreciation is growing for the role of the olfactomotor system, namely sniffing, in formation of the olfactory percept. To test the effectiveness of various sniff measurement techniques, 16 subjects smelled valeric acid (unpleasant), phenethyl alcohol (pleasant), and clean air (neutral) (10 sniffs each, counterbalanced in order, ISI = 30 s), while their sniffs were concurrently recorded. The measurement devices were pneumotachometer (spirometer), pressure sensor, temperature sensor, and respiratory inductive plethysmograph (RIP). The spirometer and pressure sensors respond to the pressure differential created by the flowing air, and the temperature sensor to the heating and cooling effect of sniffed and exhaled air. The RIP measures thoracic and abdominal volume. We examined the ability of each technique to measure odorant-induced changes in sniffing behavior. We found that temperature had the highest statistical power, followed by the spirometer and pressure. Additionally, RIP failed to show the odorant-induced sniffing modulation that was obvious when using the other techniques. The temperature temporal resolution was significantly worse than spirometer and pressure. With the pressure or spirometer, we were able to detect odorant-induced changes nearly 500 ms faster than with the temperature data. Even though pressure showed the earliest odorant-induced sniff modulation, its front end (cannula) occasionally retreated from the nares and failed to measure sniffs. Based on our findings we recommend using the spirometer or the pressure technique with a carefully secured cannula to determine odorant-induced changes in sniffing behavior. Furthermore, we recommend against use of RIP.

Poster: Chemosensory Coding and Clinical

Olfactory Detection Thresholds in Hunger and Satiety

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Objectives: Several investigators reported the existence of food-related changes in olfactory sensitivity but the findings are highly diverse. We investigated whether olfactory detection thresholds are correlated with food intake. Methods: Using the Sniffin' Sticks, sensitivity to the non-food odor n-butanol and the food-odor isoamyl acetate was assessed when hungry (overnight fast) and satiated (after a standardized breakfast, 668 ± 135 calories) in 24 female subjects with normal olfactory function. Results and Conclusions: We found no consistent pattern of changes in olfactory detection

thresholds related to food intake (n-butanol 10.2 ± 1.9 vs. 10.3 ± 1.9 , isoamyl acetate 11.4 ± 2.5 vs. 12.5 ± 2.7). Ratings regarding valence and arousal of the subjects as well as ratings of pleasantness (n-butanol 5.96 ± 2.07 vs. 6.08 ± 2.06 , isoamyl acetate 2.29 ± 1.00 vs. 2.67 ± 1.13) and intensity of the odors (n-butanol 7.63 ± 1.21 vs. 7.38 ± 1.47 , isoamyl acetate 7.13 ± 1.70 vs. 7.13 ± 1.36) did not differ significantly between hunger and satiety. In summary, we could not find any evidence that food intake has effects on olfactory function in healthy subjects.

Poster: Chemosensory Coding and Clinical

Lateralization of Odor Identification

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Aims of the present study were (1) to investigate the frequency of lateralized differences in olfactory function and (2) to correlate the self assessment of olfactory sensitivity with the results from measured olfactory function. To this end all participants rated their olfactory sensitivity as “complete loss,” “bad,” “normal,” “good,” or “excellent.” Odor identification of over 1700 subjects (652 women, 1063 men) was obtained with a 12-item test from the “Sniffin’ Sticks” test battery. All odors were applied to each nostril in a randomized order while the contralateral nostril was closed. Group analyses revealed that self assessment of olfactory sensitivity correlated well with the results of the olfactory screening test ($r_{1715} = 0.51$; $P < 0.001$). On an individual level, however, ratings of olfactory sensitivity exhibited significant differences from the results of olfactory testing. Ninety-three percent of healthy subjects ($n = 479$) demonstrated a side difference of three or less points. In patients with nasal symptoms ($n = 1236$) this figure was 86%. Lateralized differences were largest in subjects with decreased olfactory function. In conclusion, lateralized differences in odor identification are no rare finding.

Poster: Chemosensory Coding and Clinical

The Clinical Characteristics and Pathogenesis of Dysosmia

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The majority of patients having problems with smell sensation complain of hyposmia or anosmia. Although many of these patients also have dysosmia, a distorted olfactory sensation, the clinical characteristics and pathogenesis of dysosmia remains obscure. In this study, 308 patients who came to our clinic were analyzed to determine the status of their dysosmia using clinical records and questionnaires. The most common cause of their olfactory disturbance was nasosinusitis, however the incidence of dysosmia was not very high. More than 50% of the patients having a sensorineural olfactory disturbance such as post upper respiratory infection (URI) or head injury complained of olfactory distortion. The onset and sensation of dysosmia was different for patients with post URI compared to head injury. The 83% of the patients with post URI

reported that they felt a different odor from the original odor and 46% of post URI patients could only experience a limited number of odor sensations. These sensations started several months from the onset of the olfactory disturbance. On the other hand, the rate of patients reporting distorted smell sensation in the absence of an odor stimulus was higher in patients with head injury than for post URI. Based on these results and recent discoveries regarding odor recognition mechanisms, we hypothesize the following underlying causes of dysosmia for post URI and head injury. In post URI dysosmia is likely to occur due to the misdirection of regenerating axons, while for posttraumatic olfactory disturbances the cause is more likely to be due to damage in olfactory regions of the brain.

Poster: Chemosensory Coding and Clinical

The Hedonic Database of Smell-Frankonia (Hedos-F)—An Analysis of Gender Differences

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The Sniffin Stick Test has been employed widely in order to assess olfactory function in health and disease. The hedonic evaluation of the test odors still remains to be investigated. Thus, the aims of our project were (1) to collect hedonic and intensity estimates in a large human population and (2) to install a hedonic database suited to analyse the influence on hedonic and intensity estimates of the following factors: age, gender, site of stimulation, threshold, odor discrimination and identification. Two hundred and one volunteers participated in our study (mean age: 42.0 ± 16.3 , minimum age: 19 years, maximum age: 83 years, males: 103, females: 98). We executed the Sniffin Stick Test and registered intensity and hedonic estimates using an analogue rating scale. The overall hedonic estimate for all odors was 12.5 ± 18.4 (right) and 11.5 ± 17.4 (left) (unpleasantness/pleasantness scale: -100 to $+100$) indicating that the test is relatively balanced. The statistical analysis of gender differences revealed significant differences for the odors ‘orange’, ‘lemon’, ‘clove’, ‘turpentine’, ‘apple’, ‘pineapple’, ‘rose’, ‘fish’ (Mann-Whitney-U-Test) indicating a gender specific emotional evaluation of these odors. Our results also demonstrate that the Sniffin Stick Test can easily be extended by assessing intensity and hedonic estimates creating a useful tool for investigating the emotional aspects of smell.

Poster: Chemosensory Coding and Clinical

The Hedonic Database of Smell-Franconia (HeDoS-F)—The Influence of Age on the Hedonic Estimates of Odors

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Numerous studies have been conducted collecting normative values dependent on age for the three major components of the Sniffin Stick Test. Especially for the elderly a significant loss of olfactory function could be demonstrated. Less is known about the influence of age on the hedonic evaluation of the standard odors of the test. Thus, the aim of our project was to analyse the influence of age on the hedonic and intensity estimates of a large human population. Data were provided by the Hedonic Database of Smell-Franconia (HeDoS-F) consisting of 201 single data sets with the parameters age, gender, odor threshold, odor discrimination, odor identification, intensity estimates, hedonic estimates and site of odor presentation (mean age: 42.0 ± 16.3 , minimum age: 19, maximum age: 83, males: 103, females: 98). The statistical analysis of the hedonic and intensity estimates was calculated for 6 age intervals (ANOVA; Kruskal-Wallis-Test/for single odors). The relative scaling for 'unpleasantness/pleasantness' was -100 to $+100$ visual analogue rating units (VARU). We found a statistically significant influence of age on the relative values of the overall hedonic estimates whereas the absolute values of hedonic estimates and the intensity estimates were not age dependent. Our results demonstrate that hedonic estimates depend on age while the intensity evaluation of odors is relatively stable over the entire life span.

Poster: Chemosensory Coding and Clinical

Identification of Neurotrophic Factors BDNF, NT-3, and NT-4 in Human Saliva

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Brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), neurotrophin-4 (NT-4), and nerve growth factor (NGF) are structurally related polypeptides necessary for the survival and maintenance of many types of central and peripheral innervating neurons. In rodent lingual epithelium, BDNF and NT-4 are crucial for the innervation of gustatory tissue, while NT-3 supports the innervation of somatosensory tissue. Though initially thought to function only as target-derived factors, these proteins and their receptors are expressed to a certain extent in rodent and human lingual tissue, suggesting additional autocrine or paracrine trophic effects. Of these proteins, NGF is found in rodent salivary glands and was recently discovered in human saliva. This study was undertaken to determine if BDNF, NT-3, and NT-4 are also present in human saliva. SDS-PAGE/Western blot analysis of salivary samples from 14 male subjects was conducted using polyclonal antibodies against each protein. This method revealed the presence of all three proteins in saliva, in both pro- and mature forms. This suggests that the salivary glands may be releasing the proteins to promote survival of the lingual epithelial cells. This is supported by previous findings in rodents that removal of the salivary glands can lead to a decrease in fungiform papillae and taste bud number. Alternatively, the gustatory and somatosensory cells themselves might be the source, in which case the proteins may be acting in an autocrine or paracrine function.

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Poster: Chemosensory Coding and Clinical

Metabolomic Analyses of Human Skin: Age and Disease Biomarkers

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Skin odorants should change with age, differ with gender and may provide biomarkers of abnormal changes. We performed comprehensive metabolomic analyses of skin constituents from 23 healthy human subjects to identify volatile metabolites and assess how they vary between subjects. Understanding the natural variation of metabolites from healthy subjects provides a baseline from which to assess abnormalities and diagnose disease. Skin metabolites were collected by solid-phase microextraction (SPME) and solvent extraction and analyzed via gas chromatography/mass spectrometry (GC/MS). Skin secretions were also probed for the presence of human odor-binding proteins. Qualitatively, most individuals yielded similar constituents: both exogenous and endogenous components were seen. SPME-GC/MS analyses revealed the presence of C₈-C₁₂ aldehydes and C₈-C₁₂ ketones in almost every subject's volatiles. Analysis of the GC/MS data collected by solvent extraction primarily showed the presence of large amounts of C₁₂ to C₁₈ acids, and squalene. Extract data also suggest some quantitative and perhaps qualitative differences between young and older subjects. In addition, we analyzed the co-expression patterns of metabolites to identify sets of metabolites that are potentially co-regulated. Western blot analysis demonstrated the presence of apocrine secretion odor-binding proteins in all age groups and genders. By characterizing the nature and abundance of skin-derived components in healthy individuals, this study provides a database of normal constituents against which comparisons can be made with similar samples obtained from individuals with basal cell and other skin carcinomas.

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Slide: Chemosensory Coding and Clinical

Analyses of Human Axillary Odors and Their Precursors in Normal and Stressful Situations

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Our laboratory previously reported the 3-hydroxy-3-methylhexanoic acid (3H3MH) and 3-methyl-3-sulfanylhexan-1-ol (3M3SH) as key odorants in human axillary sweat. We had also elucidated the occurrence of cysteine-linked precursor of 3M3SH on the axillary skin and applied for a patent for the use of body odor indicator. One objective of this study is to analyze amino acid linked axillary odor precursors quantitatively. A second objective is to evaluate the effect of mentally stressful situation on axillary odor. In this study, we evaluated axillary odor of healthy American women ($n = 26$, aged 18–63) and collected their sweat samples

in both normal (day 1) and mentally stressful situation (day 2). During 10-min stress session trained moderator asked math, trivia and word questions to subjects continuously. LC-MS/MS analyses showed there was correlation between the precursor levels on skin surface and odor intensity. Whereas the ratios of glutamine-linked two odor precursors, Gln-3H3MH to Gln-3M2H (3-methyl-2-hexenoic acid) were approximately 7:1 with no inter-subject variation, the ratios of Gln-3H3MH to Cys-3M3SH were unique to subjects. This finding indicates a presence of common synthetic pathway for the glutamine-linked 3H3MH and 3M2H. The temperature and other circumstances remained unchanged for testing 2 days. However, 70% of subjects of the day 2 had higher odor and precursor levels compared to day 1. Some subjects had more than three times higher amounts of precursors. This result suggests mental stress induced perspiration acts as an accelerator for strong odor formation.

Poster: Chemosensory Coding and Clinical

Volatile Constituents of Human Skin: Genetic Factors and Biochemical Individuality

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Human skin surface contains different types of glands that excrete numerous compounds, including polar and nonpolar lipids and peptides, but also small volatile organic compounds (VOCs) which can be olfactorily active. Human body odors can reflect physiological state and mood of individuals. Body odors appear to have their genetic attributes (e.g., MHC-related odors), while resident microflora can contribute to their occurrence. The studies of VOCs in human emanations have been limited by the lack of quantitative techniques for comparing a large number of individuals. We have recently developed a high-throughput and highly quantitative technique for VOC profiling, which allowed to monitor precisely about 400 compounds by gas chromatography/mass spectrometry. Repeatedly collected VOC samples of 195 subjects were analyzed. Advanced chemometric methods were employed for evaluation of the VOC profiles. Numerous marker compounds distinguishing gender, families and individuals were located. Various oxygenated compounds were identified as prominent marker metabolites. Their biochemical and genetic significance will be discussed.

Poster: Chemosensory Coding and Clinical

Probing the Cerebellar Role in Sniffing with Transcranial Magnetic Stimulation (TMS)

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Sniffs are modulated in response to odor concentration; higher concentrations of odor induce lesser-volume sniffs. Studies using func-

tional magnetic resonance imaging (fMRI) and lesion patients both suggest a cerebellar role in this olfactomotor response. However, fMRI is a correlational technique and lesion patients may have developed compensatory strategies that differ from healthy subjects. To probe whether cerebellar function is essential to the olfactomotor response in healthy subjects, we will use transcranial magnetic stimulation. We first set out to determine the relevant time-window for TMS application. We found that sniff volume was concentration-independent for the first 150 ms, but inversely proportional to odorant concentration after 160 ms [$t(8) = 3.12$, $P < 0.014$]. TMS has been shown to have a temporal resolution of 50 ms in visual tasks, suggesting that single-pulse TMS can probe the time course of cerebellar involvement in the olfactomotor response. To probe this time-course, single-pulse TMS will be applied to the cerebellum at 100 ms preceding the start of the sniff, the start of the sniff, 100 ms after the start of the sniff and 200 ms after the start of the sniff. Influence of the TMS pulse on both sniffing and olfactory performance will be assessed.

Slide: Taste Chemoreception

"Fatty"—A Primary Taste

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Preliminary psychophysical data indicate that long-chain fatty acids of varying saturation are effective taste stimuli. This is consistent with electrophysiological and animal data. The present study sought to isolate the taste property of three 18-C fatty acids by masking other sensory attributes. Linoleic, oleic, and stearic fatty acids were sonicated in deionized water in concentrations ranging from 0.00028% to 5% (w/v). To minimize oxidation, samples were stored under nitrogen. Stimuli were prepared fresh daily and 0.01% EDTA (w/v) was added to each sample. The contribution of viscosity was minimized by addition of 5% Acacia (w/v) to the vehicle. Lubricity effects were reduced by addition of 5% mineral oil (w/v). To determine if the effective stimulus was an oxidation product, oxidized linoleic acid was included among the test stimuli. Testing was conducted with participants wearing nose clips and under red light to eliminate olfactory and visual cues. Detection thresholds were obtained using a three-alternative, forced-choice ascending concentration presentation procedure. The criterion stopping rule was three consecutive correct identifications of a target sample. Incorrect identification resulted with presentation of the next higher fatty acid concentration. The mean detection threshold for linoleic was 0.11% (SD = 0.24), for oleic 0.02% (SD = 0.04), for stearic 0.09% (SD = 0.21) and oxidized linoleic 0.05% (SD = 0.11). The results are indicative of a gustatory component to fat perception in humans.

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Slide: Taste Chemoreception

Taste Cells in the Gastro-Intestinal Tract

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Cells that resemble taste bud cells have been described in the gastrointestinal tract and are thought to play a role in gut chemoreception.

To understand the role of these cells, we set out to determine which genes, and in particular which taste signal transduction elements, they express. For bitter, sweet and umami, the taste signaling cascade is initiated in the taste receptor cells by activation of G-protein coupled receptors (T2rs for bitter, T1rs for sweet and umami). The signaling pathways downstream the receptors include gustducin, a G-protein expressed selectively in taste cells, phospholipase C β 2 (PLC β 2) and Trpm5, a calcium activated cation channel. Using immunohistochemistry and RT-PCR we found that T1r1, T1r3, α -gustducin, PLC β 2 and Trpm5 are expressed in solitary cells disseminated throughout the villi and the glands of the mouse gastro-intestinal tract. Real time PCR showed that T1r1, T1r3 and Trpm5 are also expressed in the human intestine. Co-localization studies showed a large degree of co-localization of T1r3, T1r1, α -gustducin and Trpm5 in the villi of the duodenum, whereas PLC β 2 is expressed in a different subset of cells. In the glands of the duodenum, Trpm5 rarely co-localizes with T1r3 or α -gustducin, but about 30% of Trpm5 expressing cells also express PLC β 2. In the colon Trpm5 co-localizes with α -gustducin but very few cells express T1r3, T1r1 or PLC β 2, and these cells do not express Trpm5 or α -gustducin. Our data show that the "taste cells" in the gut are heterogeneous and suggest that the duodenal "taste cells" may respond to L-amino acids.

Slide: Taste Chemoreception

The Regulation of Neural Targeting in the Developing Gustatory System

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During development, axons of the chorda tympani nerve must navigate to specific locations, the fungiform papillae, in the lingual epithelium. We quantified the accuracy with which gustatory fibers innervate fungiform papillae from embryonic day 14.5–18.5. By E14.5 chorda tympani fibers penetrate the epithelium of most fungiform papillae forming a "neural bud". Initial targeting was incredibly accurate: specifically, 94% of the fungiform papillae on the tongue are innervated at E14.5. Targeting accuracy increased from E14.5 to E18.5 of development as more papillae became innervated and inappropriate innervation was withdrawn. We have determined that one factor regulating the accuracy of initial neural targeting in the taste system is the neurotrophin, brain-derived neurotrophic factor (BDNF). BDNF is produced by developing gustatory epithelia. In mice lacking BDNF, the chorda tympani branches extensively below the epithelium at E14.5 and E16.5, but does not penetrate the epithelium forming a neural bud. This increased branching occurs even though BDNF knockout mice are losing geniculate neurons between E14.5 and E16.5. A few neural buds finally form at E17.5, 3 days after they are present in wild type mice. In mice overexpressing BDNF in non-gustatory epithelium, chorda tympani fibers are misdirected to inappropriate locations. These targeting effects are specific to BDNF and do not occur with other neurotrophins (NT3 and NT4). Taken together these findings demonstrate that initial targeting of chorda tympani fibers is extremely accurate and that this accuracy is regulated by BDNF.

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Slide: Taste Chemoreception

Searching for Genes Affecting Preferences for Sweet Foods; a Finnish Family Study

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One hundred and thirty-five members (31% male, 69% female, 19–78 years old) of 24 Finnish families, genome-scanned with 360 microsatellite markers, were phenotyped for chemosensory traits (intensity of PROP by filter paper method, intensity and pleasantness of 3, 7.5, and 18.75% sucrose solutions) and for use and pleasantness of sweet foods (questionnaire). Intensities were rated using the labeled magnitude scale (LMS) and pleasantness using the labeled affective magnitude scale (LAM). Phenotypes for use frequency and pleasantness (7-point scales) of sweet foods were constructed as means of ratings given to 5 items (chocolate, candies, ice cream, sweet pastry, sweet desserts). Program MERLIN was used for variance component linkage analysis of these quantitative traits. Heritabilities for PROP intensity, sweetness intensity and pleasantness of 18.75% sucrose solution, and use frequency and pleasantness of sweet foods were 65, 3, 48, 54, and 46%, respectively. The highest LOD score of 4.28, located on Chr16p11.2 was obtained for use frequency of sweet foods, suggesting that this locus harbors variation(s) influencing this trait.

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Slide: Taste Chemoreception

Molecular Mechanisms of Human Sweet Water Taste

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Sweet water taste is elicited by chemicals such as the sweet allosteric inhibitor lactisole that only tastes sweet when rinsed from the mouth with water. Similarly, saccharin, which has only a negligible sweetness at high concentrations, tastes intensely sweet during water rinses. Intriguingly, these effects were mimicked in calcium imaging experiments of HEK293T G16Gust44 cells expressing the human sweet receptor. Saccharin activated these cells at low sweet-tasting concentrations (EC₅₀ of ~0.1 mM), but at higher concentrations attenuated the response (IC₅₀ ~50 mM) suggesting the presence of a low affinity inhibitory allosteric site. This effect was reversible since saccharin washout elicited robust signals. This suggests that the sweet water taste of saccharin is caused by the release from inhibition of the allosteric site, which activates the receptor. While lactisole application on sweet receptor expressing cells does not cause any receptor activation, rinsing away lactisole does. Further analysis revealed that cells expressing the sweet receptor have elevated basal calcium levels relative to mock transfected cells that are reduced by the application of lactisole. Therefore, lactisole may act as an inverse agonist by shifting the constitutively active sweet receptor into the inactive form. The

signals upon lactisole removal may be explained by a rebound of the receptor to its constitutively active form. Thus, sweet water-taste in humans can be elicited by the release of the constitutively active sweet receptor during the wash out of an allosteric inhibitor.

Slide: Taste Chemoreception

HTAS2R38 Haplotypes Determine Bitterness Ratings of Glucosinolate Containing Vegetables

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Our previous psychogenomic studies show that variations in human TAS2R38 receptor haplotypes determine individual differences in bitterness perception of compounds that contain a thiourea (N-C = S) moiety, such as PTC and PROP. Structurally related chemicals are contained within all glucosinolate generating vegetables, such as mustard greens, kale, and brussel sprouts. The aim of this sensory study was to investigate the effect of subjects' TAS2R38 haplotype on the bitterness ratings of 28 commercially available vegetables and plant products, which were presented randomly and in triplicate over three sessions. Trained subjects ($n = 36$) were screened for having TAS2R38 haplotypes of: PAV/PAV (sensitive), AVI/AVI (insensitive), or PAV/AVI (heterozygotes); subjects with other TAS2R38 haplotypes were not recruited. Overall, PAV/PAV subjects rated the glucosinolate generating vegetables as much more bitter than did the AVI/AVI subjects. Bitterness ratings of non-glucosinolate generating foods, such as bitter melon, endive, and radicchio, did not differ as a function of TAS2R38 haplotype with the notable exception of one food. Heterozygous subjects' vegetable ratings were closer to those of PAV/PAV than AVI/AVI subjects. These results demonstrate the importance of individual human taste gene alleles when specific food preferences are studied. In this case, a broad family of plants that produce compounds containing the N-C = S moiety are perceived as especially bitter by people who possess even a single 'sensitive' allele of only one bitter taste receptor gene.

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Slide: Taste Chemoreception

Influence of Response Variability on the Coding Performance of Central Gustatory Neurons

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We explored how variability in responding to taste stimuli could impact the ability of central gustatory neurons to signal taste quality. Taste responses to (in M) 0.5 sucrose, 0.1 NaCl, 0.01 HCl and 0.01 quinine-HCl were recorded from cells in the nucleus of the solitary tract of anesthetized rats. We attempted to test each neuron 6 times with each stimulus. On each trial, the instantaneous firing rate (spikes/s) was repeatedly sampled during the first 2 s of taste responding to build histograms of spike rates for each stimulus. For each neuron, pairs of histograms were compared using an analysis based on statistical decision theory to estimate the probability (P) that an observer with knowledge of the means of these distribu-

tions could discriminate between firing rates to different stimuli. This analysis bears on whether the mean firing rates to different stimuli are reliably different. This technique was also applied to pairs of distributions between neurons to explore relative response relationships to tastants. Data from 172 trials recorded from 8 neurons of different categories (sucrose-, NaCl- or HCl-oriented) were analyzed. For each cell, a failure to discriminate between firing rates to the most effective stimulus and at least one other tastant was found ($P < \text{detection threshold}$). Yet analyses of relative firing between heterogeneous neurons revealed that different stimuli produced detectably different response relationships that could be used to identify stimulus quality. Results suggest that taste quality could be signaled by the relative firing of different kinds of neurons in parallel.

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Symposium: Trp Channels

TRP Channels: Mediators of Sensory Signaling and Roles in Health and Disease

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The TRP superfamily is distinct from other ion channel families in displaying an unusually diverse set of activation mechanisms and cation selectivities. However, one unifying theme is that so members of this superfamily have critical roles in sensory physiology. The founding member of this superfamily, *Drosophila* TRP, is critical for phototransduction and null mutations in this channel result in light-dependent retinal degeneration. Conversely, constitutive activation of TRP results in profound cell death. The molecular bases for the degenerations resulting from either decreased or increased TRP channel activity will be presented. Influx of Ca^{2+} via the TRP channels is countered by rapid Ca^{2+} extrusion and we have found that the primary extrusion mechanism is via the $\text{Na}^+/\text{Ca}^{2+}$ exchanger, CalX. Other *Drosophila* members of the TRP family are essential for a variety of sensory functions and we will describe recent work indicating that the TRPA2 channel functions in the gustatory response. Finally, mutations in TRP channels underlie a variety of human diseases, such as polycystic kidney disease and mucopolipidosis. We will present our work establishing *Drosophila* as an animal model to characterize these diseases.

Symposium: Trp Channels

ThermoTRP Channels and Chemesthesis

A. Patapoutian

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Mechanical forces, chemical stimuli, and temperature are perceived by the sense of touch, but the molecules that mediate this ability have been a long-standing mystery. Temperatures above 43°C and capsaicin were shown to activate the ion channel TRPV1 (VR1), a member of transient receptor potential (TRP) family of cation channels. Taking advantage of the human genome project, we mined for additional TRP channels. Our work has led to the characterization of a warm-activated TRP channel, TRPV3 (33°C threshold) and two cold-activated TRP channels, TRPM8 (25°C threshold) and TRPA1 (17°C threshold). These ion channels

are also the receptors for natural sensory compounds such as camphor, menthol, allicin, and cinnamaldehyde. We have also shown that the *Drosophila* sequence orthologue of TRPA1 is activated by temperature, suggesting an evolutionary conserved role of TRP channels. We are using a combination of genetic and pharmacological studies to elucidate the role of these ion channels *in vivo*.

Symposium: Trp Channels

TRPM5 and Taste Transduction

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The transduction of taste is a fundamental process that allows animals to discriminate nutritious from noxious substances. Three taste modalities, bitter, sweet, and amino acid, are mediated by G-protein-coupled receptors that signal through a common transduction cascade: receptors activate phospholipase C $\beta 2$ which hydrolyzes PIP₂ into DAG and inositol IP₃, leading to release of calcium from intracellular stores. The ion channel, TRPM5, is highly expressed in taste cells and is an essential component of this cascade, however its precise role in taste transduction is not well understood. Our previous studies showed that in heterologous cell types TRPM5 forms a nonselective channel, that is opened by intracellular calcium. We have also shown that TRPM5 currents are regulated by PIP₂ and sensitive to block by protons. The relationship between the properties of heterologously expressed TRPM5 and native channels and possible models for the role of TRPM5 in taste transduction will be discussed.

Supported by DC04564 and DC05000.

Symposium: Trp Channels

Functional Properties of a Native TRP-Related Ion Channel in Lobster Olfactory Receptor Neurons

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Lobster olfactory receptor neurons express a novel, Ca²⁺/Mg²⁺-permeable non-selective cation channel with physiological properties consistent with its being a member of the TRP family. The channel is not extracellular ligand- or cyclic nucleotide-activated, but can be activated by intracellular sodium in a concentration dependent manner. Phosphoinositides, and especially D-3 phosphorylated phosphoinositides, also activate the channel as well as modulate the sensitivity of the channel to sodium. The channel occurs naturally in calcium-sensitive and calcium-insensitive forms. Calcium does not activate the channel directly, but modulates the sensitivity of the channel to sodium. The channel can be blocked by 2APB, SKF96365 and trivalent cations, all known non-specific blockers of TRP channels, as well as by H⁺ and pyrazine derivatives of amiloride. Blocking the channel pharmacologically and/or removing extracellular sodium *in situ* reduces the receptor current, suggesting the native channel is a downstream target for phosphoinositide signaling and serves an important, signal amplifying role in these cells.

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Poster: Multimodal, Chemosensory Measurement, Psychophysical, Clinical Olfactory, and Trigeminal

Mechanism of New Ultrasonic Real-Time Gas Molecule Sensor

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The observation of odor and air exchange with high temporal accuracy is indispensable to obtain strict chemosensory event-related potentials (CSERPs) or magnetic fields as proposed by Evans (1993). There have been no suitable methods, however, for real time observation of gas stimuli by using previously proposed gas detecting technique. We have, therefore, developed brand new technique to realize of accurate measurement of gas molecule concentrations with millisecond temporal resolution by utilizing ultrasound. But principles of this ultrasonic gas sensor had not still been unclear. We tried to clarify the principle of measurement by observation of changing from CO₂ to nitrogen and vice versa slowly, and changing the distance between sounder and receiver. And we found the key of this measurement is change of the multiplex interference pattern between the ultrasonic sounder and the receiver, relevant to the molecular weight. We succeeded in detecting including 1% hydrogen from pure nitrogen with high signal to noise ratio (42 dB), and nitrogen including 0.1% hydrogen from pure nitrogen with 33 dB S/N. This means that our gas sensor has the sensing capability to detect very low concentration gas change.

Poster: Multimodal, Chemosensory Measurement, Psychophysical, Clinical Olfactory, and Trigeminal

Determination of the Smell Threshold Using a Piezoelectric Microdispenser for Neurodegenerative Disease Diagnostics

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In its early stages Alzheimer's disease attacks medial temporal lobe structures critical to smell identification. Further research has shown that smell identification and detection abilities declines with the progression of the disease. Scratch-and-sniff tests were created to take advantage of the early smell deficit exhibited by Alzheimer's disease as a diagnostic tool. Progression as a clinical tool however, has been hindered by the complexity of delivering a controlled dose of different odorants to a patient in a controlled manner. MicroFab's clinical olfactometer prototype is designed to overcome these challenges through ink-jet dispensing technology. This technology allows for precise, data-driven delivery of multiple odorants into an airstream presented to a patient for testing. The research presented here describes early human subject olfactory threshold testing using MicroFab's prototype clinical olfactometer. MicroFab's prototype olfactometer consists of the headpiece, the odorant assembly, and control microprocessor. The headpiece holds the patient's head level

to the odorant assembly positioning the nose within an airstream flowing from the odorant assembly. Within the odorant assembly resides multiple piezo-electric microdispensing devices and their reservoirs, a fan to generate airflow, and a heated wick that vaporizes the droplets ejected from the micro-dispensers. The control micro-processor resides in a handset that allows the tester to change the number of drops dispensed per trigger and trigger dispensing. Once triggered, the dispensers eject a number of drops that are vaporized on the heated wick and enter the airstream presented to the patient.

Poster: Multimodal, Chemosensory Measurement, Psychophysical, Clinical Olfactory, and Trigeminal

DNA Structural Changes Associated with DNA Hydration and Odor Polarity Are Mechanistic Factors in Odor Response by Novel DNA-Based Fluorescent Sensors

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Our laboratory has developed an artificial nose that exploits 22 principles of biological olfaction. Importantly, broadly tuned sensor arrays are used to achieve odor detection. In previous presentations at AChemS, we have shown that 20–30 base, solid-state, single-stranded DNA-Cy3 (ssDNA) conjugates can respond to odors. DNA-Cy3 conjugates have the combinatorial potential to provide large arrays of different sensors, but their mechanism of odor response is unknown. Our experiments demonstrate that odor response in DNA-Cy3 sensors correlates with DNA conformational changes in a sequence and odor specific manner. Other hypotheses (Stokes shift, pH, etc) have shown null results. The data are consistent with DNA structural changes as the primary modulators of Cy3 responses in DNA-Cy3 odor detection. DNA conformation appears to be altered by most odors through a mechanism governed by odor polarity, although the positively charged amines seem to have other specific interactions with DNA. In general, non-polar odors lead to ssDNA shortening and polar odors lead to an increase in ssDNA length as measured by resonance energy transfer (FRET). FRET data also show that drying leads to changes in DNA length similar to the effect of non-polar odors. We hypothesize that this occurs through odor effects on the hydration layer associated with solid-state DNA-Cy3 sensors. Changes in DNA conformation upon odor exposure may then lead to changes in Cy3 stacking with adjacent DNA bases or changes in Cy3 interactions with downstream bases, thus altering the local environment of Cy3 and leading to a change in Cy3 fluorescence.

Supported by grants from NIDCD, ONR, and NSF.

Poster: Multimodal, Chemosensory Measurement, Psychophysical, Clinical Olfactory, and Trigeminal

Single Protein Nanobiosensor Grid Array, IST-2001-38899-SPOT-NOSED European Project

E. Pajot

European SPOT-NOSED Consortium, Institut National de la Recherche Agronomique, Jouy-en-Josas Cedex, France

European Consortium: INRA Jouy-en-Josas and Ecole Centrale de Lyon, France—U. Barcelona and Campus UAB Bellaterra, Spain—U. Lecce and Politecnico di Milano, Italy. The objective of SPOT-NOSED is to develop a nanobioelectronic sensor based on the electrical properties of single olfactory receptors anchored between nanoelectrodes. ORs within yeast-membrane nanosomes are specifically immobilized onto mixed-SAM functionalized nanoelectrodes, using an anti-OR antibody and avidin-biotin interactions. Nanotransducers with nanoelectrodes were fabricated using electron beam lithography. An OR is modelled by an equivalent impedance network, predicting a detectable impedance change upon odorant binding-induced conformational change. A transimpedance preamplifier suited for low-noise wide-bandwidth measurements of small electrical signals was designed. The new instrumentation for multi-modal DC/AC measurements is validated in an AFM. These nanobioelectronic sensors should benefit from ORs individual properties: high specificity and reproducibility, low detection thresholds, large odor spectrum. Integration of individual nanosensors into multisensors arrays could further increase sensitivity and widen detection spectrum, providing a new concept for powerful electronic noses/tongues mimicking *in vivo* odorant detection/discrimination. Numerous applications are anticipated: rapid detection/characterization of toxic/dangerous compounds and pathological agents, food safety, medical diagnosis, follow-up of processes, de-orphanization of receptors.

Poster: Multimodal, Chemosensory Measurement, Psychophysical, Clinical Olfactory, and Trigeminal

Use of Ink-Jet Microdispensing to Create Concentration-Critical Chemical Laden Vapors for Sensor Calibration

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Ink-jet micro-dispensing technology has been used in applications where discrete picoliter volumes of fluids have been required. The inherent precision of ink-jet based dispensing made it an ideal candidate as a calibration tool for sensor technologies. In this application, a prototype calibrator was built for the National Institute for Standards and Technology to test if MicroFab's microdispensing prototype can deliver explosives laden fluids precisely enough to perform as a calibration tool. The same system can be used to train dogs and test olfaction thresholds. MicroFab's calibrator is made of three subsystems: the PC controller, the drive electronics, and the vapor generator. The PC controller is simply a personal computer that runs the drive electronics and controls the overall operation of the calibrator. MicroFab's proprietary drive electronics contain circuitry that sends a specific waveform to each microdispenser that subsequently ejects the correct drop(s). For this system, the vapor generator is made up of six ink-jet microdispensers and their reservoirs, a drop heater, and other temperature controlling hardware. In operation, the devices inside the vapor generator delivers and vaporizes the fluid droplet. This vapor enters the ambient airflow within the vapor generator and flows to the vapor generator outlet. Maintaining the interior surfaces at an elevated temperature prevents vapor condensation inside the vapor generator. Testing at NIST has shown that vapor concentration of explosives can be varied almost continuously from 0 to hundredths of parts per trillion.

This range covers current standards in detection limits and will enable real time sensor calibration.

Poster: Multimodal, Chemosensory Measurement, Psychophysical, Clinical Olfactory, and Trigeminal

Development of the OLFACT-RL

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Assessment of olfactory detection threshold is a frequently used clinical test for evaluating olfactory function. While administration of the test is time consuming and cumbersome and reliability is purported to be low, the measure nevertheless provides important information concerning olfactory function. We report here the development of the OLFACT-RL™ test, the first economical, computer-administered clinical olfactory detection threshold test. Stimuli are produced by an olfactometer originally developed for administering the OLFACT™ odor identification test. The protocol employed is similar to other threshold tests, e.g., CCCRC or Sniffin' Sticks, except this test is computer-administered. A dilution ratio of 1:2 butanol (highest concentration, 4%) is used to generate 16 different stimuli. Stimuli are produced by blowing air through glass reservoirs containing the appropriate concentrations and the subject records their response via a computer after sampling the stimulus. A single staircase with reversal paradigm is used. Three stimuli (2 blank, 1 butanol) are presented sequentially and the subject is asked to identify which stimulus is different from the other two. If a concentration is correctly identified twice in a row, the next lower concentration is presented. A miss triggers presentation of a higher concentration on the next trial. After 7 reversals, threshold is determined as the mean of the last 4 reversals. Alternatively, if a miss occurs at step 1, or two consecutive hits occur at step 16, the test is terminated. Testing of ~100 subjects resulted in a fairly normal distribution of scores.

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Multimodal, Chemosensory Measurement, Psychophysical, Clinical Olfactory, and Trigeminal

The Credibility of Measured Odor Thresholds

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Assume that investigators in two labs measure odor detection for the same chemical. Each lab uses accepted methodology that differs between the two. One measures a threshold of 100 ppb and the other a threshold of 10 ppb. Which, if either, would you accept? All things being equal, logic says to accept 10 ppb on grounds that weaknesses in methodology, even if unrecognized, would generally increase the measured threshold. What if a coherent data set for more than 200 materials, studied with accepted methodology and analytically verified, implied that thresholds in most compilations (US EPA, AIHA) overestimated the true values, i.e., underestimated sensitivity, by an order of magnitude? Would you accept the new results? Nagata (2002) measured such thresholds in connection with rule making

in Japan. These data could make thresholds used for rule making by the US EPA and most states obsolete, as systematically too high. In experiments on such diverse VOCs as ethyl butyrate, hexanoic acid, limonene, and glutaraldehyde delivered via a system designed to yield stable detection, we obtained thresholds consistently below those in most compilations. Our analytically verified values lie closer to those of Nagata. The results imply that lower measured thresholds should generally take precedence over higher. They also suggest a need to rebuild the data sets that currently guide decisions regarding odor detection of hazardous chemicals.

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Poster: Multimodal, Chemosensory Measurement, Psychophysical, Clinical Olfactory, and Trigeminal

Selection of Subjects for Chemosensory Studies: Criteria

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It has become common to collect chemosensory data in studies of environmental chemicals. It is then relevant to ask whether demographic variables influence outcome. Results from Woskie (1998) of chemosensory effects in workers exemplify such: "Ironically, those who may appear most "susceptible" to borate exposures, because of their greater reactivity, were the healthy non-smoking workers not using nasal spray/drops, not reporting allergies or colds on the test day or any history of bronchitis. This finding seems to contradict the common view of susceptibility as representing compromised health status." Whether we seek normals or others, we inevitably need to screen for health. We describe outcomes for screening for normal health, chronic rhinosinusitis, and allergic rhinitis. In screening for normals, a phone interview regarding medical history disqualified 1/6th of 299 applicants. Screening in the lab disqualified another 1/6th via interviews to confirm medical history and to learn of current symptoms, as well as to perform rhinomanometry, spirometry, examination of the eyes, nose, and oropharynx, and to collect of samples for cytological analysis. The principal reason for disqualification was evidence, by one means or another, of inflammation. In screening for the inflammatory conditions chronic rhinosinusitis and allergic rhinitis, we have added measurement of NO from the lungs and nose. From the comparison of normals with patients with diagnosed inflammatory disease, we have come to see that chronic inflammation can endow subjects with chemical intolerance.

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Poster: Multimodal, Chemosensory Measurement, Psychophysical, Clinical Olfactory, and Trigeminal

NaCl Sensation and Hedonics: Relationships with Sex, Taste Genetics, and Sodium Intake

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NaCl imparts saltiness and blocks unpleasant tastes to enhance flavor, yet sodium intakes exceed health recommendations. In 87 subjects (45 men), we tested, via regression and structural equation

modeling (SEM), if markers of taste variation [bitterness 6-n-propylthiouracil (PROP), fungiform papillae (FP)] explained variability in: saltiness and liking for NaCl concentration series in solution and broth; preference for sampled and surveyed salty foods; and salty food intake. Females preferred lower NaCl levels in solution or broth, regardless of PROP status. Men who tasted PROP as least bitter preferred the highest levels. Saltiness positively predicted liking for cheese and snack foods, but negatively for soy sauce. Compared to supertasters, PROP nontasters liked these foods more; nontasters also tasted low sodium cheese as less bitter and liked it more. More frequent intake of high-salt foods was seen in those who liked high levels of NaCl in broth. SEM showed liking of sampled broth and surveyed salty food liking both predicted intake frequency of 38 salty foods, and PROP bitterness predicted intake via saltiness and liking of broth at sodium levels commonly found in commercial soups. Via χ^2 , PROP nontasters were more likely to report 'usually adding salt to food' than were supertasters. PROP effects on NaCl sensation may be mediated through FP number as the intensity of concentrated NaCl was associated with greater FP, not T2R38 genotype. In summary, liking for predominantly salty foods influences sodium intake, with men and nontasters showing greater liking for these foods. In foods where NaCl is added to enhance flavor, nontasters may require less to mask unpleasant tastes.

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Poster: Multimodal, Chemosensory Measurement, Psychophysical, Clinical Olfactory, and Trigeminal

Detection of Weak Gustatory-Olfactory Flavor Mixtures

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Previous research has shown that the detectability of flavor mixtures containing olfactory and gustatory components can exceed the detectability of either individual unmixed component. A variety of models, however, can predict increased detectability, including models of cross-modal interaction (enhancement), cross-modal summation (summation across independent channels), and probability summation of independent channels. Using a two-alternative forced-choice method and a sip procedure, subjects first evaluated near threshold solutions of sucrose alone and vanillin alone at several concentrations, to establish baseline psychometric functions for each, after which the same subjects also detected sucrose-vanillin mixtures. The results showed clear evidence of increased detectability of mixtures, but also suggested the possibility of interindividual differences that may make it difficult to establish a single general model of mixture detection.

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Poster: Multimodal, Chemosensory Measurement, Psychophysical, Clinical Olfactory, and Trigeminal

Individual Taste and Smell Sensitivity, and Their Effects on Salivary Flow Rates and Food Perception

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The objective was to investigate the relationship between taste and smell psychophysics and salivary flow rates of individual consumers to their perception of the quality of semi-solid foods. Tests on 120 subjects of pure solutions showed thresholds of 14.3, 38.6, 0.086 and 1.14 mM for the 4 tastants NaCl, sucrose, quinine sulfate, and citric acid, respectively, and 0.47 mM for the odorant, phenylethyl alcohol. Salivary flow rates, measured as spit, increased by as much as 10 ml/min depending on tastant and concentration. Relationships were determined by linear regression. Among subjects, sensitivities to one tastant were related to those of the other tastants ($r > 0.24$, $n = 120$), but not to the odorant ($r < 0.09$). Except for NaCl ($r = 0.21$), taste sensitivities were poorly related ($r < 0.1$) to salivary flow rates. Those subjects who had relatively high salivary flow rates in response to one tastant, also showed relatively high flow rates in response to the other tastants ($r > 0.71$). Across subjects, salivary flow rates varied more with perceived intensity than with tastant, i.e., at iso perceived intensities, all tastants elicited fairly similar flow rates. Within certain foods, some sensory profile attributes (e.g., dairy taste) varied with the consumers' sensitivities, whereas others (e.g., stickiness) varied with salivary flow rate whilst 'creaminess' varied with both. In conclusion, variations in both taste psychophysics and salivary flow among consumers accounted at least some of the variation in food sensations.

Poster: Multimodal, Chemosensory Measurement, Psychophysical, Clinical Olfactory, and Trigeminal

Molecular Structure Predicts Human Judgments of Pleasantness and Similarity

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The organization of olfactory perceptual space and its relation to properties of odorant molecules is a long-standing problem. We used olfactory descriptive attributes and asked: (1) how is the space of attributes organized, (2) can an attribute perceptual space predict similarity judgments and behavior, and (3) does the perceptual space predict physical molecular properties. A principal component analysis (PCA) of set of 160 odorants described by experts using 146 attributes revealed that the first principal component (PC) of the attribute space corresponds to pleasantness (valence). Empirically, the first PC values correlated with judgments of pleasantness and to judgments of pleasantness of each attribute. An olfactory space (Euclidean metric over the first 4 PCs) predicted subjects' judgments in a pairwise similarity task and reaction times on a speeded same-different task. To compare the results to molecular properties, we used >1500 molecular descriptors (Dragon, <http://www.taletе.mi.it>) for each of 144 monomolecular odorants, divided randomly into a test set and a cross validation set. The molecular descriptors were grouped into 18 blocks, and within each we conducted a PCA to reduce the dimensionality in the test set and built a regression model to predict the first 4 PCs of the perceptual data. We then tested the model we derived in the cross-validation set. We found that a linear combination of fewer than 20 molecular features predicted the first PC of the perceptual space ($r = 0.41$, $P < 0.001$). These results underscore the primacy of valence as an organization scheme for

olfactory perception, and its importance in understanding the representation of molecular structures in olfactory coding.

Poster: Multimodal, Chemosensory Measurement, Psychophysical, Clinical Olfactory, and Trigeminal

Hedonic gLMS: Valid Comparisons for Food Liking/Disliking Across Obesity, Age, Sex and PROP Status

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Labeled food preference scales like the Natick 9-point category scale or the visual analogue scale (VAS) provide valid within subject comparisons. However, to provide valid across-group comparisons, the labels on these scales must denote the same affective intensities to each group. For example, a VAS might be labeled “zero” to “maximum liking of foods”; this assumes that “maximum liking of foods” denotes the same intensity of liking to all. We used the hedonic general Labeled Magnitude Scale (hedonic gLMS) to demonstrate that this is often not true. Using this scale, subjects ($N = 4299$) rated liking/disliking for 26 foods in the context of all affective experience rather than just affect for foods. The maximum liking and maximum disliking were determined for each subject. These values were significantly correlated with body mass index (BMI). As BMI rose, both the maximum liking and maximum disliking rose; that is, the obese in this group of subjects live in an affectively more pleasant food world than do the non-obese. For age, maximum liking did not rise, but maximum disliking did. For sex, women showed greater extremes than did men; that is, the maximum liking for women was higher and the maximum disliking was lower. For PROP (propylthiouracil) status, supertasters showed greater extremes than did nontasters. Since the boundaries of liking and disliking were not constant over BMI, age, sex and PROP status, conventional labeled scales are invalid for comparisons of food liking/disliking across these groups.

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Poster: Multimodal, Chemosensory Measurement, Psychophysical, Clinical Olfactory, and Trigeminal

Psychosocial Predictors of 6-*N*-Propylthiouracil Ratings in a General Population Sample

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There is variability in rated sensitivity to the bitter compound, 6-*n*-Propylthiouracil (PROP). While polymorphisms of the *TAS2R38* gene account for between 55 and 85% of the variance observed, it is likely that other factors also influence PROP ratings. In order to examine some of these factors, the participants in the Dunedin Multidisciplinary Health and Development Study rated PROP intensity. This study has followed a birth cohort of 1037 individuals since 1972. Ninety-six percent ($n = 972$) of living participants were re-assessed in 2004–2005 (aged 32) and their responses to 0.0032M of PROP were measured using the general Labeled Magnitude

Scale (gLMS). Participants were also asked to imagine the brightest light ever seen and to rate this sensation on the gLMS. The data were analysed separately by sex. Factors predicting PROP ratings were: childhood IQ scores (higher IQ predicting lower PROP rating), childhood SES scores (higher SES predicting lower PROP rating for women) and scores on the gLMS for the imagined brightest light (higher light rating predicting higher PROP rating) (final models: $r^2 = 0.168$ for women, $r^2 = 0.087$ for men). Neither childhood IQ nor childhood SES predicted ratings for the imagined brightest light. These findings indicate that psychosocial factors may account of some of the variance in PROP ratings not explained by genetics.

Funding was provided by the Health Research Council of New Zealand.

Poster: Multimodal, Chemosensory Measurement, Psychophysical, Clinical Olfactory, and Trigeminal

Experience with Na-Cyclamate, but Not Acesulfame-K, Induces Increased Taste Discrimination Ability for Glucose

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Experience with fructose induces increased taste discrimination ability for glucose in humans. To investigate potential peripheral loci for the induction, we tested the effects of experience with acesulfame-K or Na-cyclamate on discrimination for glucose. Subjects briefly treated their tongues at home each day for 10 days with either blue-colored isosweet concentrations of Na-cyclamate (40 mM) or acesulfame-K (0.4 mM), or blue distilled water. On day 11 or 12, they tasted paired samples of green glucose (17.5, 27, 43, 65, and 100 mM) and water in the laboratory and identified the “sweetener” in each pair. There was a significant difference in glucose discrimination among the groups ($P < 0.004$). Subjects experienced with Na-cyclamate discriminated glucose as sweetener at lower concentrations than those experienced with either water ($P < 0.013$) or acesulfame-K ($P < 0.002$). There was no difference in discrimination ability between water and acesulfame-K experienced subjects ($P > 0.51$) (Wilcoxon/Kruskal-Wallis Tests). The differential effects support a peripheral locus for the induction. Viewed in light of *in vitro* responses of human sweet receptor subunits to fructose, glucose acesulfame-K and cyclamate, it would appear that experience via hT1R2/hT1R3 can but does not necessarily lead to, while experience via hT1R3 alone can be sufficient for, the induction. Yet changes in transduction pathways and other mechanisms for the different stimuli are possible. Experiments are in progress to clarify the mechanisms.

Poster: Multimodal, Chemosensory Measurement, Psychophysical, Clinical Olfactory, and Trigeminal

Women and Smoking: Effects on Sucrose Taste Preference and Thresholds

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Previous research revealed that the sensory properties of cigarettes play a greater role in women’s smoking behaviors than in men.

Moreover, the reduced body weight of female smokers has been partially explained by changes in the consumption of sweet tasting foods. The present study aimed to determine the effects of cigarette smoking on sucrose taste preferences and thresholds in female smokers. The women were on average 28.5 years of age and all had normal BMIs. Each subject participated in a 2-day study separated by 1 week. In counterbalanced order, women smoked a cigarette with nicotine on one testing day and a nicotine-free cigarette on the other. On both days, subjects refrained from smoking for 12 h prior to testing as verified by CO₂ levels. Sucrose taste thresholds and preferences were assessed within 50–80 min after smoking. To allow for comparisons, we also determined sucrose preference and thresholds in a group of women who are nonsmokers. Preliminary analyses revealed that women who smoke preferred significantly higher concentrations of sucrose after smoking the nicotine cigarette when compared to the nicotine free cigarette ($P < 0.025$). The sucrose preferences of non-smoker women did not significantly differ on the 2 days of testing ($P > 0.35$) and were similar to levels preferred by women on the day they smoked the nicotine-free cigarette. Although sucrose thresholds were not significantly different between the two conditions in women who smoke, their thresholds were significantly higher when compared to women who do not smoke ($P < 0.005$).

This project was funded by a grant from the Pennsylvania Department of Health.

Poster: Multimodal, Chemosensory Measurement, Psychophysical, Clinical Olfactory, and Trigeminal

Exposure to a Variety of Fruits Increases Fruit but Not Vegetable Acceptance in Infants

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Research in humans and animal models suggested that infants' acceptance of novel vegetables such as carrots can be enhanced by repeated exposures to either the target vegetable or a variety of vegetables. The present study followed from these findings to determine whether fruit acceptance can be enhanced by similar exposure strategies. To this end, we randomized 38 infants into one of two groups and evaluated their acceptance of puréed pears and green beans before and after an 8-day home exposure. During the home exposure period, one group ($N = 20$) was fed only pears, the target fruit, whereas the other group ($N = 18$) was fed a variety of puréed fruits that did not include pears. Preliminary findings revealed that both groups significantly increased their acceptance of the target fruit after the home exposure period ($P = 0.03$). This increased acceptance of fruits did not generalize to the green vegetable. Practical implications will be discussed.

This research was supported in part by NIH Grant HD37119.

Poster: Multimodal, Chemosensory Measurement, Psychophysical, Clinical Olfactory, and Trigeminal

Feeding Vegetables and Fruits to Infants: Does the Type of Exposure Matter?

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Concerns about the timing and order of solid food introduction represent one of the primary matters discussed by mothers with their child's pediatrician. Although some health professionals recommend that foods can be introduced in no particular order, others contend that vegetables should be introduced before fruits because the infant's inherent preference for sweet tastes may hinder vegetable acceptance. Ultimately, the goal is to gradually accustom children to a varied diet that meets nutritional needs for growth and development. To this end, we evaluated the acceptance of a novel vegetable (puréed green beans) and fruit (puréed peaches) before and after an 8-day home exposure period in three groups of infants. The length of the exposure period was based on the finding that infants of this age require 8–10 exposures to a new food to increase acceptance. The three groups differed in the type of foods they were fed during the exposure period at home. The first group was fed only puréed green beans at the same time of day on each home exposure day; the second group was fed only puréed peaches; and the third group was fed puréed green beans followed by puréed peaches. Preliminary findings revealed that regardless of whether the green beans were presented alone or with peaches, infants increased their acceptance of the green beans after the home exposure. These findings suggest that contrary to popular belief, exposure to a sweet-tasting fruit does not hinder acceptance of a green vegetable.

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Specific Anosmia for Selected Nor-Isoprenoid Flavor Compounds in Orange Juice

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Thresholds for flavor volatiles have been traditionally calculated in water or air, but they may vary widely in more complex food matrices. Thresholds of orange flavor compounds were measured in a deodorized orange juice matrix (pumpout) using the three-alternative-forced-choice (3-AFC) method (ASTM: E-679). A bimodal distribution was found among panelists for sensitivity to β -ionone and β -damascenone whereas thresholds for other tested compounds followed a normal distribution. Orthonasal thresholds for β -ionone and β -damascenone were, respectively, 985 and 690 times higher for non-perceivers than perceivers. Panelists who could not perceive β -ionone were otherwise good perceivers of most compounds tested, including α -ionone, a constitutional isomer of β -ionone. All three compounds were re-tested in water using the same panelists, and with another set of panelists. Differences between non-perceivers and perceivers of β -ionone were 4900 and 4600 times higher for ortho- and retronasal thresholds, respectively. No such differences were found for β -damascenone when measured in water. Results for β -damascenone indicate that half of the panelists could not differentiate the compound from the background when tested in pumpout. Differences between low and high thresholds for β -damascenone in pumpout indicate that the response to that stimulus could be processed at the cognitive level in the complex matrix. In conclusion, specific anosmia was only observed for β -ionone, but not for α -ionone or β -damascenone.

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Human Odor Detection of Homologous Carboxylic Acids and Their Binary Mixtures

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Does structural similarity of odorants influence detectability of their mixtures? To address this question, psychometric (probability of correct detection vs. concentration) functions were measured for aliphatic carboxylic acids and selected binary mixtures thereof. Unmixed stimuli included acetic (C₂), butyric (C₄), hexanoic (C₆), and octanoic (C₈) acids. Mixtures included C₂ + C₄, C₂ + C₆, and C₂ + C₈. Vapor-phase concentrations of individual compounds, as measured by a combination of SPME and GC/MS, were always the same, whether presented singly or in a binary mixture. A response-additivity model (independent processing of mixture-components) was applied to the data for unmixed compounds to generate theoretical predictions for the psychometric function for each binary mixture. For C₂ + C₆ and C₂ + C₈, psychometric functions agreed well with theoretical predictions from near-chance detection to near-perfect detection. These results suggest independent processing of mixture-components. For C₂ + C₄, however, detection deviated from predictions in a concentration-dependent fashion. At low concentrations, proportion correct exceeded additivity (synergy). At higher concentrations, proportion correct fell below additivity (suppression). Thus, results with C₂ + C₄ partially agree with past findings that detection tends to fall below additivity for more easily detected mixtures. Unlike past research, the current results suggest that a high degree of structural similarity is needed for mixture-interactions, i.e., deviations from independent processing, to occur. Future studies can determine if this result is particular to carboxylic acids.

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Sweet Odours Increase Pain Tolerance

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Several studies in humans have documented an impact of odours in reducing measures of pain. One hypothesis is that odour pleasantness influences pain, possibly via its impact on mood. Sweet tastes reduce pain, and we tested whether odours that are sweet-smelling through prior association with such tastes might similarly reduce pain. In the study reported here, adult subjects (Ss) underwent a pain-inducing cold-pressor test (CPT) during which they inhaled air containing a sweet-smelling odour. To test for potential odor-induced mood effects on pain, and to distinguish between effects due to pleasantness versus those due to a specific sweet smell (necessary, since many sweet-smelling odours are also pleasant), Ss in two control groups also underwent the CPT with other non-sweet odors of similar intensity, but varying in pleasantness. In the CPT, Ss immersed their dominant forearm in water at ~5°C for up to 4 min

on two occasions, 15 min apart: once with the odour present (CPT+), and once without (CPT-), order balanced across Ss. For each S, we then determined the impact of the different odours by comparing latencies (secs) to remove their arm in the two CPTs. Ss also rated the pain intensity immediately after immersion, again after another 30 s, and then immediately on withdrawing their arm from the water. The group receiving the sweet odour had a significantly longer mean latency during the CPT+ than the CPT- condition, and a longer latency than both control groups for either CPT+ or CPT-. There were no group differences in pain ratings at any of the rating periods. Hence, these results most likely reflect differences in pain tolerance rather than pain reduction per se. These results are discussed in terms of associative conditioning models.

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The Influence of Smelling Coffee on Olfactory Habituation

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Perfume stores often encourage customers to clear their olfactory pallet by smelling coffee in between samples of perfume. We set out to test this in the laboratory. Sixteen subjects participated in an experiment where a computer controlled olfactometer was used to deliver one of three perfumes, clean air, or coffee, in a design that maximized habituation. Each trial consisted of 7 consecutive sniffs separated by judgments on a VAS scale. An example of a "perfume A" trial is: 1. sniff perfume A > estimate intensity > 2. sniff perfume A > estimate pleasantness > 3. sniff coffee or air > estimate intensity > 4. sniff perfume A or B or C > estimate same/different (as previous) > 5. sniff perfume as previous > estimate intensity > 6. sniff perfume as previous > estimate pleasantness > 7. sniff coffee or air > estimate pleasantness. An experiment consisted of 24 trials (ITI = 20 s) containing all possible orders of perfumes A, B, and C. 12 consecutive trials were "coffee trials" (coffee in sniffs 3 and 7), and 12 were "air trials" (air in sniffs 3 and 7). The order of air and coffee trials was counterbalanced across subjects. Accuracy at match-to-sample was the same following sniffs of air or coffee (mean coffee = 74 ± 13%, mean air = 70 ± 15%, binomial $P < 0.4$). Whereas estimates of odorant pleasantness were similar after sniffing coffee or air (10 of 16 subjects, binomial $P < 0.4$), estimates of odorant intensity were preserved following sniffs of coffee yet reduced (habituated) following sniffs of air (13 of 16 subjects, binomial $P < 0.02$). In further testing we will use other odorants to ask whether this influence on intensity perception is specific to coffee, and if yes, what component of coffee exerts this effect.

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Predicting Nostril-Specific Detection Thresholds

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Evidence from frog electrophysiology and human psychophysics suggest that both the chemical properties of an odorant, and the velocity with which it passes over the olfactory epithelium affect the magnitude of the olfactory response. Based on these past results we constructed a simple model of olfactory response as a function of airflow velocity and chemical sorption rate. We hypothesized that the percent coverage of the olfactory epithelium would be directly related to the olfactory response. We then propose a simple equation that might model the epithelial coverage as percent coverage = $100(c/c_{maxcov}) - k(FR - SR)^2$, where c is the odorant concentration, c_{maxcov} is the concentration that would be needed for maximal coverage of the epithelium, FR is the airflow velocity of odorant over the epithelium, and SR is the sorption rate of the odorant. This simple model predicts that for a given odorant, the detection threshold should vary with airflow velocity. Considering that airflow velocity is different across nostrils, this model further predicts nostril-specific detection thresholds. In order to test this hypothesis we have measured monorhinal detection thresholds using the MLPEST method (maximum likelihood parameter estimation by sequential testing). To date we have collected monorhinal detection thresholds from 7 subjects using the odorant octane, a low sorption rate odorant. As predicted by our model, 5 of 7 subjects had a lower detection threshold in their low airflow velocity nostril compared to their high airflow velocity nostril. Data from additional subjects and additional odorants will be presented as a test of the model.

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Odor Memory and Labeling in Adults and Children

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Children are well documented to perform poorly on odor identification tasks, but the reasons are not well understood. One reason for poor memory performance in children may be inaccurate odor labeling. Lumeng, *et al.* (2005) investigated the role of verbal labeling in flavor memory in preschool-aged children using flavors of jelly beans in an “old”-“new” flavor recall task. It was found that recall for flavors improved with age and was associated with the ability to correctly label the flavor. However, the children generally performed poorly on the flavor recall task, with mean performance being only slightly better than chance, and no comparative data on adult performance was collected. The purpose of the present study was to determine how healthy adults perform on the flavor recall task used by Lumeng *et al.* (2005) so as to further explore memory for flavors and the role of verbal labeling on flavor memory. Healthy young adults received 10 randomly selected flavors of jelly beans during the initial phase of the experiment and were asked to identify the flavor. After a 15-min retention interval, the participants were asked to taste 20 more jelly beans; 10 previously tasted and 10 new distractors. Participants were first asked if they had previously tasted the jelly bean flavor and again asked to identify the flavor. Responses were scored

for hits, false alarms, accuracy and consistency of labeling. Striking similarities were observed when the performance of the children and adults was compared. In general, the study confirms that odor memory tasks are difficult for both adults and children.

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Verbal Associations and Odor Memory

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Are verbal associations to uncommon odors helpful in remembering these odors? Two groups of young subjects (each 12 female and 12 male) learned to associate three one-syllable nonsense words to three uncommon odors and were exposed equally often to three other odors in a same-different test. The odors in the learning condition of the first group were the odors in the same-different test in the second group and vice versa. The same words were used for learning in both groups. The order of learning and exposure was systematically varied over subgroups. Association learning performance, odor memory and odor-word association memory were measured. No significant difference in odor memory between the odors in the “verbally associated” and the “same-different” conditions was found. Odor memory and verbal association learning performance were unrelated. Odor memory was almost perfect; verbal-odor association memory was not. When both the targets and the distractors of the first memory test were used again the next day in a memory test with new distractors under the instruction to find only the originally learned stimuli, the odor memory for the original targets was still high for stimuli from both learning conditions. The much less frequently encountered first-day distractors were also well remembered as was shown in the large number of false alarms they produced. The implications of these findings for understanding odor memory are discussed.

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It Smells So Good I Can Almost Taste It: Evidence that Food and Nonfood Odors Are Localized Differently Within the Nasal Cavity

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We investigated whether perceiving an odor as a food or nonfood affects where in the nose it is localized. We hypothesized that experience with an odor as a flavor that emanates from the mouth causes the odor to be localized toward the posterior region of the nasal cavity. We defined odor localization as where in the nasal cavity a subject perceives a sniffed odor to be distributed. Subjects were shown three MRI cross-sections of the head depicting hypothetical distributions of odor within the anterior, mid or posterior portions of the nasal cavity. They were told that that these images represent possible

distributions of an odor in the nose. Subjects sniffed six food and six nonfood odors and selected the distribution that best matched the perception of each. In two separate subjects ($n = 14$ and $n = 20$), Chi square analyses showed that subjects selected the posterior distribution more frequently for food compared to nonfood odors ($P < 0.02$ and $P < 0.05$). These results are consistent with the general proposal that experience influences the neural representation of odors, and specifically that experience with food odors affects their spatial distribution. Interestingly, the effect only occurs if the odors are not identified as foods before perception begins, indicating that verbal information changes the way olfactory signals are processed.

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Asparagus Malodor in Urine—A True Polymorphism?

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It has long been recognized that urine from some individuals who have eaten asparagus smells like vegetable soup or rotten cabbage. Previous authors have suggested that there are individual differences in production of the malodor or that everybody produces the odor, but some are insensitive to it. We asked: Are there individual differences in perception of asparagus malodor in urine? Are there individual differences in the production of asparagus malodor in urine? and Are there both non-producers and non-perceivers? 37 adults served as urine donors on two separate days. On one day, they ate roasted asparagus and on the other day they ate bread. They gave a urine sample right before and 2 h after each snack (4 samples, total). 31 adults were sensory evaluators. Urines from a single donor were evaluated in each session. Evaluators performed a two-alternative forced choice in which their task was to identify (by smell) the after-asparagus urine (AAU). The AAU was compared to the before-asparagus urine and the after-bread urine from the same donor. We found evidence for individual differences in both perception and production of asparagus malodor in urine, but at lower levels than previously reported: 2/31 evaluators were not able to distinguish the AAU from other samples at a better than chance level and an additional 2/31 were accurate significantly less of the time than were other evaluators. All evaluators with poor discrimination had otherwise normal olfactory function. For 2/37 urines, the evaluators were not able to distinguish the AAU from other samples at a better than chance level.

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Working Memory for Odors

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Little is known about working memory for odors. Dade *et al.* (2001) compared working memory for odors and faces in a two-back same-different task and found similar levels of performance. Because the

odorants in their study were highly identifiable odorants, verbal coding is likely to have supported the memory performance. In our study, using a similar two-back same-different task, we compared odors that could be verbally described to a variable degree. Twenty-two paid participants (16 women), with a mean age of 28.09 (SD = 9.17) judged a series of 36 odors. The probability of an odor on trial n to be same as the one on trial $n-2$ was 0.33. Complementary judgments of the odorants' perceived intensity, familiarity and verbal category were also required. Preliminary results indicate that memory performance varied with both the level of familiarity and the level to which the odor could be verbalized, with higher levels yielding higher memory performance. Familiarity and verbalisation on trial n and $n-2$ were equally important for memory performance. VR-HS:2005-1779.

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The Inhibition of Stress—Odor Conditioning

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Odors, especially novel ones, are often readily associated with people, places and even emotional states. In a series of studies, we have been investigating the ability of odors to acquire such learned associations to either positive or negative emotions. In the present study, we evaluated the degree to which a negative association between an odor and a stressor could be prevented by simply pre-exposing the individuals to the odor while they were in a non-stressful or relaxing state. Using a latent inhibition paradigm, individuals were exposed to galbanum on three occasions. On the first two occasions, the odor was paired with either a stressful public speaking and mathematical task or a non-stressful slideshow. On the final session, individuals were merely re-exposed to the odor while measures of autonomic arousal and subjective health and well-being reports were obtained. Individuals who experienced the odor in the non-stressful condition first exhibited lower levels of autonomic arousal and fewer adverse symptoms in the test session than did individuals whose first odor experience occurred during the stressful condition. If novel or salient odors that are likely to be experienced under stressful or dangerous conditions can be identified prior to that event, the findings suggest that the ability of these odors to acquire and potentiate conditioned responses can be reduced by pre-exposure under non-stressful conditions. This technique could be useful for minimizing the persistence of odor-elicited memories and adverse responses that are often experienced by disaster relief workers, military personnel and other individuals.

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Adverse Effect of Air Pollution on Olfactory Detection of a Contaminated Food by Residents of Mexico City

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Recently we reported that residents of Mexico City (MC; high air pollution) are poorer in detecting, describing and discriminating odors of beverages than residents of the state of Tlaxcala (Tx; low air pollution). Here we ask if air pollution also affects performance on a real-life task - detecting contamination of a common food. Healthy non-smokers 18–30 years old (MC $n = 30$, Tx $n = 30$, equal numbers of men and women) were presented with odorants in squeeze bottles. When tested with ascending concentrations of stimuli in a 3-way oddball paradigm, Tx subjects detected the odor of orange drink (Tang, Kraft), milk (Nido, Nestlé), and dimethylsulfide (D) at significantly lower concentrations than MC subjects. When presented with milk in its commercially recommended concentration but mixed with increasing concentrations of D (a component of “off” milk), Tx subjects detected the presence of D and provided descriptions and negative hedonic judgements at significantly lower concentrations than MC subjects. Also, while Tx subjects began to respond negatively to D at the same low concentration whether presented alone or in milk, MC subjects first responded negatively to it in milk at significantly higher concentrations, that is, detection of D was masked by milk odor to some extent in MC but not in Tx subjects. Thus, air pollution may affect an olfactory function as basic as judging the edibility of foods.

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Pathophysiology of the Olfactory Neuroepithelium in a Murine Model Of Allergic Rhinitis

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Background: Olfactory dysfunction is present in 15–20% of patients suffering from allergic rhinitis, but the specific pathophysiology is poorly understood. Traditionally, smell deficits in patients with sinonasal disease have been attributed to diminished airflow to the olfactory cleft. Recent studies however, have demonstrated inflammatory changes in the olfactory epithelium (OE) and increased apoptosis of olfactory sensory neurons (OSNs) in patients with chronic rhinosinusitis and anosmia. The effect of inflammation in allergic rhinitis alone has not been examined. Objective: This study examines the effect of allergy on the OE and OSN apoptosis employing a murine model of allergic rhinitis. Methods: 7- to 8-week old-week old C57BL/6 mice were sensitized by means of intranasal application of a protein extract of *Aspergillus fumigatus* three times a week for 3 weeks, rested for 1 week, and then challenged either acutely or chronically with the same allergen. The olfactory neuroepithelium of these mice and wild-type controls was assessed for immunohistochemical evidence of apoptosis and inflammation. Results: Sensitized mice in both the acute and chronic groups had significantly greater eosinophil infiltration of the OE and activation of the apoptotic effector enzyme Caspase-3 as compared to the wild-type control. The chronically exposed group demonstrated the greatest apoptotic activity,

while the acute group had a more intense inflammatory influx. Conclusion: Taken together this data supports the hypothesis that the olfactory deficits in patients with allergic rhinitis may be the result of inflammatory changes resulting in OSN apoptosis within the OE.

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Neuronal and Inflammatory Changes in Nasal Tissues of Chronic Rhinosinusitis Patients

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Our on-going project examines the impact of inflammation on the olfactory mucosa (OM) due to chronic rhinosinusitis (CRS) pre- and post-treatment. Nasal lavages were collected and evaluated for the presence of inflammatory cells and level changes in specific interleukins and chemokines via cytokine array. Morphological characteristics of the OM from biopsies were analyzed by quantitative measurements and alterations of neuronal cells were assessed by immunocytochemistry. We observed both similar and unique inflammatory, morphological and neuronal changes across CRS patients and within individuals before and after treatment. Cytokine array results yield specific chemokine patterns. OM histopathology reveals cellular proliferation based on Ki67 immunoreactivity (Ki67-ir) and OMP-ir neurons in most samples; however, there are alterations in cellular structure and anatomical distribution. We also observed a deficit of ck18-ir supporting cells (SCs) in biopsies with the most severe inflammatory pathology and keratinization of the superficial epithelium. The absence of a normal SC population in CRS OM is a previously unreported phenomenon, and may indicate that these cells are particularly susceptible to inflammation-related damage. These initial findings provide an insight into the complex changes induced by chronic inflammation on the OM. Psychophysical and clinical data collected from these patients will enable correlation of molecular and patient outcome measures.

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Laser Scanning Microscopy of the Nasal Mucosa: A Preliminary, Ex Vivo Study

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A confocal microscopic approach was performed to establish and to differentiate between chronic inflammation and hyperplasia of

the nasal mucosa without inflammation. Aim of this study was to assess the potential use of laser scanning microscopy (LSM) for *in vivo* diagnosis of nasal diseases. Nineteen specimens (2 different regions of the middle turbinate) of 10 patients with chronic rhinosinusitis (CRS) were investigated using LSM. The findings were compared with both, the corresponding histopathological sections and hyperplastic nasal mucosa obtained from patients who underwent turbinate surgery (12 specimens of 7 patients). The following criteria were used for characterization of inflammation: thickness of the epithelium, motility of the kinocilia of the respiratory epithelium within 20 min after biopsy and identification of inflammatory cells. LSM enables differentiation between respiratory and squamous and olfactory epithelium, respectively. The cilia of the respiratory epithelium and the directed mucous transport can be observed *ex vivo*. LSM exhibited differences for the parameter "inflammation cells" and "thickness of the epithelium" being highest in CRS compared to hyperplasia. Among lymphocytes, koilocytes were identified in CRS. They are characterized by their swollen ballooned appearance combined with a vacuolated cytoplasm. Further investigations are needed to assess the potential role of this technology to evaluate nasal mucosa *in vivo*.

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The Relationship between Human Nasal Anatomy and Olfactory Ability

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The nasal airspace of 6 subjects was delineated from T1-weighted coronal MRIs under 4 conditions (pre-dilator, post-nasal dilator, 4 h after dilator application and following dilator removal). As expected, the nasal dilator increased the overall size of the nasal cavity compared to the undilated condition, with increases seen in both the nasal valve region and in the more posterior bony regions. The changes seen in the nasal valve area suggest wearing a dilator directs more incoming air toward the upper part of the nose. The increase in volume seen in the bony region generally results in a widening of the area around the middle turbinate with some increase in the early anterior section of the area around the superior turbinate. These changes in the bony region are likely reflexive (i.e., initiated as a result of the dilation of the nasal valve region) and are more pronounced 4 h after dilation as compared to what is observed immediately after dilator application. The relationship between these changes and laminar and turbulent flow in the various parts of the nose is still not clear, but, at the least, nasal dilators would seem to proportionally increase airflow to the airspace around the olfactory receptors. This change in airflow patterns is certainly part of the explanation of the decrease in olfactory threshold, increase in magnitude estimation and better identification seen when wearing nasal dilators. The results of the present study give added support to the use of nasal dilators for better describing the relationship between nasal anatomy and olfactory ability.

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Odor and Lateralization Thresholds for Ammonia: A Comparison Across Static and Dynamic Olfactometry

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Lateralization thresholds (LT), in which irritancy is assessed by the ability to localize to the stimulated nostril, have been used in the indoor air field in the context of setting occupational exposure limits. LT's are typically obtained using bottles (static olfactometry: SO) with single chemical compound stimuli. However, if we wish to assess irritancy for complex mixtures, dynamic olfactometry (DO), in which odors are diluted in a clean air stream would be more appropriate. To this end, we compared the performance of both methods using a single chemical, ammonia (NH₃). Methods: Odor detection thresholds (ODT) and LT's for NH₃ were collected using SO and DO (see above). A two-alternative forced choice procedure was employed. 22 Females were tested on each method twice in a within-subjects design. Results: For the SO method, the (geometric) mean ODT = 2 (SEMs = 1.7), and LT = 31 (SEMs = 1.3) ppm. For the DO method, the mean ODT = 2 (SEMs = 1.2), and LT = 50 (SEMs = 1.3) ppm. There was no significant difference between methods ($F < 1.0$). Test-retest reliability was reasonable to high within methods ($0.18 < r < 0.59$) but low between methods ($-0.09 < r < 0.23$). LT's were significantly higher than ODT's ($P < 0.0001$), thresholds measured at T = 1 higher than at T = 2 ($P < 0.05$). Conclusion: Both methods yielded very comparable mean results, and thus can be used interchangeably for estimates at the population level. However, on the individual level, results should not be compared across methods.

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Irritation, Ammonia and Asthma

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Sensitive subpopulations, such as those with asthma and other respiratory diseases, commonly attribute the exacerbation of asthmatic symptoms to exposure to chemical odors and irritants. However, many of the odors reported to cause asthma symptoms do not necessarily reach concentrations capable of stimulating irritant receptors. The goal of the present study was to evaluate the irritation potential of ammonia (NH₃), and to determine whether there are any differences in nasal or ocular irritant sensitivity between healthy individuals and those with mild-moderate asthma. 25 healthy and 15 mild-moderate asthmatic volunteers (age 29.7 ± 10.8) were evaluated for their ability to detect odor and irritancy of NH₃. Ammonia vapor was delivered to either side of a set of specially-configured goggles (for the ocular exposure) or to either nostril or both simultaneously for durations of 10 s for the thresholds and up to 30 s for the supra-threshold exposures. Additionally, 13 healthy and all asthmatic

volunteers were evaluated throughout the testing sessions for pulmonary function using spirometry. There were no significant differences in the sensory irritation thresholds or rated intensity of irritation between asthmatic subjects and healthy controls. Exposure to NH₃ didn't alter pulmonary function in either group. However, asthmatic individuals exhibited significantly lower odor detection thresholds (93.24 ± 13.99 ppm, asthmatic, 153.35 ± 25.64 ppm healthy, $P < 0.09$). This implies that, in some instances, an adverse response to volatile chemicals among asthmatics may be triggered by the perception of low-level odor, not irritation, and may reflect a psychogenically-mediated symptom response to a perceived health risk.

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Poster: Multimodal, Chemosensory Measurement, Psychophysical, Clinical Olfactory, and Trigeminal

Breathing Responses of Normosmic and Anosmic Individuals to Stimuli Presented in an Environmental Chamber

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To investigate the effects of airborne contaminant exposures on breathing in humans, we exposed 20 normosmics and 4 anosmics to 8 conditions, each presented in 100-min sessions conducted in a 10 m³ environmental chamber: environmental tobacco smoke (ETS)—0, 15, 100 and 800 µg/m³ RSP; propionic acid (PA)—0, 1, 10 and 15 ppm. Except with 15 ppm PA, for which the exposure plateau ended at min 30, concentrations rose mins 11–20, were maintained through min 70 and then declined. With normosmics, all PA concentrations caused a modest increase in inhalation duration (InDur). With the two lowest PA concentrations, inhalation volume (InVol) was maintained whereas this parameter declined slightly with 15 ppm PA and even more with clean air. Thus, minute ventilation (MnVnt) was maintained over the course of the session with 1 and 10 ppm PA while this parameter declined with clean air and, to a slightly less extent, with 15 ppm PA. With anosmics small increases and decreases, respectively, in InVol were seen with 1 and 15 ppm PA. In normosmics, ETS increased InDur and decreased InVol, resulting in a drop in MnVnt. Effects of ETS on anosmics were quite different. InDur was unchanged but InVol increased, with the magnitude of change following the order: $15 > 800 > 100$ µg/m³. In the absence of an InDur effect, the InVol pattern was repeated with the MnVnt parameter. This work will contribute to an improved understanding of the principles underlying the integration of chemosensory inputs, including ocular trigeminal, that yield changes in specific breathing parameters under environmentally realistic conditions.

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Temporal Integration in Nasal Lateralization of Ethanol

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Two experiments examined how one can trade stimulus-duration with concentration of n-ethyl alcohol to maintain a fixed level of

performance in detection of nasal irritation. Irritation threshold was measured via nasal lateralization, a technique in which subjects receive chemical vapor in one nostril and clean air in the other. Subjects try to determine which nostril received the chemical. Concentration was fixed within experimental runs, and stimulus-duration varied to find the briefest stimulus subjects could reliably lateralize. Concentration varied between runs (1650–5000 ppm). Experiment 1 involved a small, intensively-tested group of subjects to obtain stable individual data. Experiment 2 involved a larger group and employed more rapid methods. In both cases, a fixed-ratio increase in stimulus-duration could compensate for a fixed-ratio decrease in concentration. However, an increase in duration of more the two-fold was required to compensate for a two-fold decrease in concentration. These results suggest that a simple, but imperfect, mass-integrator model (i.e., an exponentiated form of Haber's rule) can describe short-term integration of nasal lateralization of ethanol.

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Pain Intensity Related Cortical Activation Following Trigeminal Stimulation of the Nasal Mucosa: fMRI Study

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Objectives: The application of carbon dioxide (CO₂) stimuli to the nasal mucosa is a well-established model of acute experimental trigeminal pain. We studied the brain activation correlated with the intensity of painful trigeminal stimulation of the bilateral nasal mucosa without concomitant tactile or thermal stimulation. **Methods:** Functional images following CO₂-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:** Following painful trigeminal stimulation, 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 (thalamus, S1, S2, amygdala). Cortical activations correlated with the intensity of the induced pain included anterior and middle cingulate gyrus, S2, thalamus, caudate nucleus, insula, and trigeminal nuclei. Our data indicate that the experimental pain model of CO₂-stimulation of the nasal mucosa specifically activates the nociceptive cortex. The cortical network we found coding pain intensity is consistent with results from studies using peripheral pain models.

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Poster: Multimodal, Chemosensory Measurement, Psychophysical, Clinical Olfactory, and Trigeminal

Effects of Irritant Chemicals on Oral Heat and Cold Pain Perception

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Some thermosensory transient receptor potential (TRP) channels also respond to common irritant chemicals. Capsaicin acts at the noxious heat-sensitive TRPV1 channel, and menthol activates and enhances cold-evoked currents through TRPM8. We tested if irritants enhance perceived hot and/or cold pain. One of the following was applied to one side of the tongue by filter paper: menthol (0.3%), capsaicin (0.001%), mustard oil (1%), or cinnamaldehyde (0.2%). The subject then pressed the tongue against a preheated Peltier thermode maintained at 49°C or 9.5°C. In a 2-alternative forced choice (2-AFC) paradigm, subjects stated which side of the tongue had greater heat or cold pain, and then rated the intensity on each side, at 0, 1.5, 5, and 10 min after chemical application. The method was validated by showing that ~90% of subjects correctly identified a 0.5°C temperature difference. Capsaicin and mustard oil enhanced heat pain at 0, 1.5 and 5 min, as a significant majority ($P < 0.05$, binomial test) chose the irritant-treated side as more painful in the 2-AFC and assigned significantly higher intensity ratings to that side ($P < 0.05$, paired t -test). Cinnamaldehyde initially enhanced heat pain. Neither capsaicin nor cinnamaldehyde affected cold pain, while mustard oil significantly enhanced cold pain initially. Menthol significantly enhanced cold pain at 1.5 and 5 min but did not affect heat pain. The results are consistent with the hypothesis that capsaicin and menthol enhance thermal gating of TRPV1 and TRPM8, respectively, and additional experiments are underway to further test this.

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Is Overall Trigeminal Sensitivity Impaired in Patients with Olfactory Dysfunction?

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To investigate the relationship between cutaneous somatosensory and intranasal chemosensory trigeminal sensitivity, patients with olfactory dysfunction ($n = 17$ following URTI; $n = 31$ following head trauma) were tested and compared to 48 healthy controls. Trigeminal chemosensory function was tested using electrophysiological methods (negative mucosal potential—NMP; trigeminal event-related potentials—tERP) and psychophysical techniques (lateralization task, CO₂-thresholds). To test cutaneous somatosensory sensitivity, detection thresholds and pain tolerance thresholds for electrical DC stimuli were assessed unilaterally on the subjects' cheeks. Patients had smaller tERP amplitudes and increased CO₂-thresholds indicating decreased chemosensory sensitivity (no difference for ratings and NMP amplitudes). In contrast, pain tolerance thresholds were lower in patients indicating increased somatosensory sensitivity (no difference for detection thresholds). As a conclusion, with regard to intranasal chemosensory measures, patients showed either decreased or similar sensitivity when compared to healthy controls. With regard to somatosensory measures, however, they showed either similar sen-

sitivity or higher responsiveness than healthy controls. This indicates that changes of trigeminal sensitivity in patients with olfactory dysfunction are specific to chemosensory sensations.

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Feel from Soluble Dusts

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Subjects judged the feel of the soluble mineral dusts boric acid (2.5, 5, 10 mg/m³), calcium oxide (2.5 mg/m³), and sodium borate [pentahydrate] (10 mg/m³) during 47 min episodes of light exercise. The Ss indicated perceived sensory magnitude in the eyes, nose, and throat by the concentration of carbon dioxide that matched the sensations. Consistent with previous work, the nose led with the highest magnitude, followed by the throat, then the eyes. Perceived magnitude increased for periods up to 1/2 h, then either held at a plateau or declined. This was true for all 3 agents, though with some differences in temporal signature. Accompanying measures implied that the decline of perceived magnitude in the nose occurred neither because of an increase in dilution of the dissolved dusts in newly secreted mucosal fluid nor from any increase of consequence in nasal resistance. Most likely, sensory adaptation principally determined the non-monotonic change of increase then decrease over time. The outcome across agents showed, as expected, that calcium oxide exceeded sodium borate in potency. On the basis of mass exposure, boric acid behaved similarly to sodium borate, viz., the perceived magnitude of 10 mg/m³ boric acid fell just slightly and insignificantly below 10 mg/m³ sodium borate. Boric acid also showed a relatively flat dose-response relationship, i.e., a change in level caused only a small change in perceived magnitude.

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Determination of Oral Trigeminal Sensitivity in Humans

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The aim of this study was to establish a clinical test for determination of oral trigeminal sensitivity. Capsaicin impregnated filter paper strips (5 concentrations: 0.0001–1%) were used for threshold tests of the dorsal anterior tongue. The strips were placed on the tongue for 10 s and the subjects were asked for onset of any sensation, quality (9 trigeminal and 4 taste descriptors), and duration of sensation. Intensity ratings were assessed after 10s stimulation. Thresholds were estimated in two ways: (1) the lowest concentration where subjects consistently indicated that they perceived a “burning,”

“stinging,” or “hot” stimulus (THRESH1), and (2) the lowest concentration the pain intensity of which was rated 2 and higher on a 10-item scale (THRESH2). The test was applied to 63 nondesensitized healthy subjects (mean age 40 years; 34f, 29m). These data were correlated to measures of gustatory sensitivity obtained with “taste strips” (filter papers impregnated with tastants). With regard to whole-mouth testing THRESH1 and THRESH2 exhibited a significant correlation ($r_{63} = 0.41$, $P < 0.001$). Coefficients of correlations between test and retest were $r_{25} = 0.67$ ($P < 0.001$) for THRESH1 and $r_{25} = 0.73$ ($P < 0.001$) for THRESH2. Younger subjects (<40 years) had significantly lower THRESH2 scores than older subjects ($t[61] = 2.25$, $P = 0.028$) while no such differences were found for THRESH1. No sex-related differences were found ($P > 0.22$). Neither THRESH1 nor THRESH2 measures revealed differences between the left and right side of the tongue ($P > 0.25$). In conclusion, capsaicin threshold test appears to be a useful diagnostic tool for assessment of the intraoral trigeminal sensitivity.

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Concentration-Detection Functions for Eye Irritation from Homologous N-Alcohols Approaching a Cut-Off Point

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The study aims to measure and to model concentration-detection functions for eye irritation. Based on previous studies, we selected homologous n-alcohols with carbon chain length reaching values where ocular detection begins to fail (cut-off effect). Failure of a vapor to elicit eye irritation could rest on a chemical-structural or a concentration limitation. The stimuli comprised 1-nonanol, 1-decanol, and 1-undecanol delivered to the eye for 6 s at 2.5 L/min by a computer-controlled vapor delivery device. Delivered vapor concentrations (ppm by volume) were measured by gas chromatography. Twenty-two subjects (16 females) were tested using a 3-alternative forced-choice procedure against humidified air blanks. As expected, detection probability (P), i.e., detectability, increased with vapor concentration. Close to vapor saturation, nonanol approached perfect detection, whereas decanol and undecanol barely reached halfway ($P = 0.5$) between chance ($P = 0.0$) and perfect ($P = 1.0$) detection. In fact, undecanol reached a ceiling in detectability ($P = 0.5$) even below vapor saturation, and further increases in concentration failed to increase detectability. The outcome provides additional support to the notion that the cut-off in eye irritation at the level of 1-undecanol rests on a chemical-structural limitation rather than on a concentration limitation.

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TrpV1 Receptors and Nasal Trigeminal Chemesthesis

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The trigeminal nerve responds to a variety of irritants in the environment and serves a protective role. Trigeminal nerve fibers express several receptors including TrpV1 (vanilloid receptor 1), ASICs (acid sensing ion channels), and P2X (purinergic receptors). TrpV1 is activated by capsaicin (CA) and acids, although its role in the transduction of other irritants has not been determined. The irritants: amyl acetate, AA; cyclohexanone, CY; acetic acid, AC; toluene, TO; benzaldehyde, BE; (-)-nicotine, NI; (R)-(+)-limonene, LI; (R)-(-)-carvone, RCR; (S)-(+)-carvone, SCR, and CA all stimulate the trigeminal nerve when delivered in solution to the nasal cavity of rats, but their mechanism of action is unclear. We have used standard calcium imaging techniques to examine responses of TrpV1 receptors to these chemical irritants. For these experiments, TrpV1 and GFP constructs were co-transfected into HEK293t cells. Three irritants (AC, SCR, and RCR) stimulated non-transfected controls and were not tested further. Two irritants (CA and CY) stimulated only transfected cells, and the response could be eliminated with capsazepine, a TrpV1 blocker. The five remaining irritants (NI, BE, AA, LI, and TO) were nonstimulatory in both non-transfected and transfected cells, suggesting they utilize a different receptor mechanism. These results suggest that TrpV1 serves as a receptor for both CY and CA in trigeminal nerve endings.

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Food Flavors and the Sweetener Saccharin Activate the Transient Receptor Potential Vanilloid Subtype 1 (TRPV1) Channel

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Chemosensory perception of food relies on olfaction, taste and trigeminal sensation and several volatile organic compounds naturally present in food stimulate these senses. Many artificial sweeteners activate two taste modalities (sweet and bitter at higher concentrations), but it is not known whether these molecules can also induce chemesthesis. The sensation of irritation is initiated by pungent molecules activating Trp channels expressed in sensory nerve endings of the trigeminal nerve. Capsaicin, the pungent molecule in hot chilli peppers, and other irritant molecules are known to activate the heat gated vanilloid receptor TRPV1. We investigated whether several aromas and artificial sweeteners activate TRPV1, using Fura-2-based calcium imaging of a HEK293 cell line heterologously expressing TRPV1. Aromas at 1 mM (thujone, geraniol, linalool, coumarin, citral, p-anisaldehyde, menthone) elevate intracellular $[Ca^{2+}]_i$; and this response is decreased in the presence of the TRPV1 inhibitor capsazepine. At 1 mM concentration limonene, β -pinene, safrole, (+) and (-) carvones, cyclohexanol, and thymol do not activate the TRPV1 channel. Saccharin, a common artificial sweetener, strongly activates TRPV1 at 1 mM and at 10 mM. This response is partially inhibited by capsazepine. Aspartame at 1 mM does not activate the channel. All agonists listed here are lacking the vanilloid group characteristic of capsaicin but their

hydrophobicity suggests they might bind TRPV1 by diffusion through the cell membrane as does capsaicin. Taken together the data show that several food flavors and saccharin can stimulate the trigeminal system by activating the vanilloid receptor.

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TRPM5-Expressing Solitary Chemoreceptor Cells in the Mouse Nasal Cavity Respond to Odors at High Concentrations

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The trigeminal system in the respiratory epithelium of the nasal cavity detects airborne irritants. Previously, we reported that the transient receptor potential channel TRPM5 is expressed in a large population of solitary chemoreceptor cells (SCCs) in the mouse nasal cavity; a subset of which also express α -gustducin, indicating that diverse populations of SCCs could be trigeminal sensors. In this study, we further characterized SCCs using immunohistochemistry and Ca^{2+} -imaging. Many TRPM5-expressing cells also reacted with antibodies against elements of the phospholipase C (PLC) pathway including PLC β 2 and G γ 13. We also found that synaptobrevin-2, a key component in synaptic vesicle release, was present in these cells. In Ca^{2+} -imaging studies, we observed that some isolated GFP-marked TRPM5-expressing SCCs responded to high concentrations of various odorants, and the PLC inhibitor U73122 suppressed the odor-evoked Ca^{2+} responses, suggesting the TRM5-expressing SCCs respond to these odors via the PLC transduction pathway. These results show that diverse populations of SCCs are present in the respiratory epithelium of the mouse nasal cavity, and that these cells are able to detect odorants at high concentrations that act presumably as trigeminal irritants.

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Oleocanthal, an Anti-inflammatory and Anti-oxidant Compound of Olive Oils, Elicits Activity in Isolated Trigeminal Neurons

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Premium extra virgin olive oils are characterized by a distinctive pungency that is unusual because it is sensed primarily in the pharynx or throat and much less in the mouth. The compound responsible for this irritation is (-)-deacetoxy-dialdehydic ligstroside aglycone, which we termed oleocanthal (oleo = oil; canth = sting; al = aldehyde). This restricted throat irritation is remarkably similar to that elicited by the non-steroidal, anti-inflammatory drug ibuprofen. Cyclooxygenase and lipoxygenase assays conducted with synthetic (-)-oleocanthal demonstrated that it is a natural NSAID.

This compound may thus play a significant role in the well-known health benefits associated with a diet high in extra virgin olive oil. In order to investigate the physiology underlying the unusual pharyngeal sensation of oleocanthal, we measured intracellular calcium in rat trigeminal and nodose ganglion neurons. We found that oleocanthal induces increases in intracellular calcium in a calcium- and sodium-dependent manner in selected cells. Because the compound activates neither all of the capsaicin- nor all of the cool-sensitive neurons, it is unlikely that either TRPV1 or TRPM8 mediate the trigeminal or nodose response to oleocanthal. Future studies will further define the pharmacology of oleocanthal receptors.

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Topographical Differences in the Trigeminal Sensitivity of the Human Nasal Mucosa

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Background: Previous work suggests differences in the distribution of human intranasal trigeminal receptors. The aim of this study was to investigate these topographical differences using an electrophysiological measure of trigeminal induced activation, the negative mucosa potential (NMP). Material and Methods: A total of 29 young, healthy volunteers participated (16 men, 13 women; age 19–42 years). CO_2 (60% v/v; stimulus duration 500 ms; interstimulus interval 30 s) was used for trigeminal stimulation. For stimulus presentation we used a computer controlled olfactometer (OM6b, Burghart Instruments, Wedel). Recording of the NMP was performed with a tubular electrodes (AgAgCl, outside diameter 0.8 mm, 1% Ringer-agar). Recording sites were the anterior septum, the lower turbinate, and the rima olfactoria. Results: Maximum amplitudes of the NMP were found at the anterior septum, lowest amplitudes were recorded at the rima olfactoria. Conclusions: The present data suggest that there are topographical differences in the arrangement of trigeminal neurons with the highest sensitivity in the anterior part of the nasal cavity. This finding is compatible with the idea that the trigeminal system acts as a sentinel of the human airways.

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Pet-Based Investigation of Cerebral Activation Following Intranasal Trigeminal Stimulation

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The present study aimed to investigate cerebral activation following intranasal trigeminal chemosensory stimulation using O15-H₂O-PET. A total of 15 healthy male volunteers participated (age range 30–58 years). Using a PET scanner (ECAT EXACT HR+; Siemens, Erlangen, Germany) subjects underwent 4 sessions of 5 min each with an interval of at least 15 min. During 2 of the sessions subjects received left-sided CO₂-stimuli (duration 1 s, interstimulus interval 3 s) embedded in a constant stream of air (36°C, 80% rH). Stimulation started 20 s before intravenous injection of 1.7 GBq O15-H₂O for the entire duration of the sampling period of 2 min. During the other two sessions subjects received odorless air only. SPM99 was used for analysis of the data. In 12 subjects measurements were analysed for all 4 sessions, in 3 subjects only one stimulation session and one resting session could be analysed. There was a pronounced activation of the trigeminal projection area at the base of the post-central gyrus which was more intense for the right hemisphere, contralateral to the side of stimulation. In addition, activation was also found in the orbitofrontal and the piriform cortex, respectively, which are typically found to be active following presentation of odors. In conclusion, the present data suggest that intranasal trigeminal stimulation not only activates somatosensory projection areas, but that it also leads to activation in cerebral areas associated with the processing of olfactory information. This may be interpreted in terms of the intimate relation between intranasal trigeminal and olfactory sensations.

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Mouse Strain Differences in Fat Appetite: Initial Orosensory Response and Long-Term Intake

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The interaction of orosensory, experiential and genetic factors in fat appetite was examined in mice. The orosensory appeal of fat was compared in 7 inbred strains of mice by measuring initial licking responses to a range of concentrations of intralipid (IL), a stable emulsion of soybean oil in water, and long-term intake of a range of concentrations of IL in 24-h oil vs. water tests. Initial licking responses reliably predicted strain differences in 24-h intake of 1% IL, but not of 5, 10 or 20% IL. Additional tests with 2 strains examined the response to nonnutritive (olestra) and nutritive (IL) oils in 24-h oil vs. water tests. Compared to 129P3 mice, C57BL/6 (B6) mice showed greater acceptance of and preference for all concentrations of olestra (0.3125–2.5%), and the low (0.3125–5%) but not the high concentrations of IL (10 and 20%). When retested with IL, both strains showed increased intakes of and ~90% preferences for all concentrations, although the B6 mice still consumed more 0.625–2.5% IL than the 129P3 mice. These latter findings, together with results obtained in a previous intragastric IL infusion study, indicate that positive post-oral feedback increases fat preference and acceptance in mice. Overall, our findings indicate that mouse strains differ significantly in their intake of fat, and that these strain differences are due to a complex interaction between orosensory responsiveness and post-oral nutritive feedback (positive and negative).

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Weight Gain, Olfactory Sensitivity and Kv1.3 Expression: Is There a Link?

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Mice deficient in the voltage-gated potassium channel, Kv1.3, have recently been shown to have increased odor sensitivity (both threshold and discrimination) and are resistant to high fat diet-induced weight gain. These observations lead us to question whether the control of weight and olfactory acuity are interrelated through Kv1.3 signaling. To address this issue, melanocortin 4 receptor (MC4R) deficient mice (an animal model of obesity due to hyperphasia and reduced metabolic rate) were bred with Kv1.3-null mice to produce various allelic combinations of both genes. Use of metabolic chambers revealed that Kv1.3-null mice were more active, have an elevated dark phase metabolism, and exhibited altered ingestive behavior. Body weight monitored over a 10 month interval demonstrates that the significant weight gain of MC4R-null animals beyond 2 months of age is suppressed in the Kv1.3-null background (10 month weights: 52.8 ± 3 g MC4R^{-/-}; 29.2 ± 1 g WT; 32 ± 1 g double mutant; ANOVA, snk). General anosmia tests were conducted on WT, MC4R-null, double mutant, and diet-induced obese mice. Whereas the ratio of time to find a cracker/marble was significantly reduced for Kv1.3-null compared to WT mice, that for MC4R-null mice was not altered compared to WT controls (Arc-Sin percentage Student's *t*-test). Object recognition tests performed on the same four types of mice indicated that MC4R-null mice displayed impairment of object memory after 1 or 24 h and diet-induced obese mice displayed impairment at 24 h. These data demonstrate that an ion channel prevalent in the olfactory system intercepts hormonal pathways essential to the regulation of energy homeostasis.

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Mitral Cells in Postnatally Undernourished Rats

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Mitral cells (MC) are one of the main elements conforming the olfactory glomeruli (OG), the functional unit of the olfactory bulb. Perinatal undernourishment modifies the cytoarchitectonic organization of the nervous system, whereby the aim of this study was to characterize some morphometric parameters of MC cells in lactating rats chronically undernourished. Male rats were used, 24 control (C) and 24 undernourished (U) distributed in three ages: 7, 14, 21 D. Undernourishment was done by the nipple ligation of one of a pair of dams. Brains were Golgi-Cox impregnated and cut into coronal sections (140 μm). The soma and dendritic parameters

of MC were obtained by using an image analyzer. Ear and eye opening delaying was observed in U group (U-Mann-Whitney, $P < 0.05$). Significant alterations in soma and decreased dendritic processes were observed (ANOVA, $P < 0.01$). The soma area of MC was lower on day 7, and larger on 14 in the U rats. Distal dendritic orders (5th, 6th and 7th) were absent in the U group at the three ages as well as lower dendritic length was also observed. Postnatal organization of OG in the olfactory bulb depends mainly on MC characteristics; thus, dendritic alterations during this critical period of development should probably modify the neuronal communication conforming the OG with effects that could remain at later ages.

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Poster: Multimodal, Chemosensory Measurement, Psychophysical, Clinical Olfactory, and Trigeminal

Crossmodal Associations between Olfaction, Vision, and Touch

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We investigated crossmodal associations between odours, colours, and fabrics using a variant of the Implicit Association Test (IAT). In Experiment 1, participants made speeded discrimination responses to a series of unimodal target stimuli (strawberry odour, spearmint odour, a pink colour patch, or a turquoise colour patch) by pressing one of two response keys. The stimulus-response assignments of targets onto the two response keys were varied in order to generate both compatible (e.g., strawberry and pink) and incompatible (e.g., spearmint and pink) response mapping blocks of trials. The results showed that the odour-colour pairings sharing the stronger association (compatible response mappings) resulted in faster ($F(1,15) = 22.14$, $P < 0.001$) and more accurate responses ($F(1,15) = 12.59$, $P < 0.01$) than those sharing a weaker association (incompatible response mappings). In Experiment 2, we used the IAT to demonstrate the existence of crossmodal associations between odour and touch (fabric swatches ranging in softness) as well. The pairings of lemon odour-‘feels soft’ and animal odour-‘feels rough’ onto the same response keys resulted in more rapid responses than the opposite pairing (e.g., lemon with rough; $F(1,23) = 5.70$, $P < 0.05$). These results suggest the existence of systematic odour-colour and odour-touch associations that are robust enough to be highlighted indirectly. Our results also provide a novel extension of the IAT paradigm to the crossmodal study of olfactory-visual and olfactory-tactile associations.

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Poster: Multimodal, Chemosensory Measurement, Psychophysical, Clinical Olfactory, and Trigeminal

fMRI of Subthreshold Integration of Odors and Tastes: A Study of Learned Congruency

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Sub-threshold integration of a congruous smell and taste pair, but not an incongruous smell and taste pair, showed that flavor perception might arise from the central neural integration of this multi-modal input. One question that remained, however, was why an incongruous pairing of stimuli would fail to integrate. To determine whether prior exposure played a role in summation of tastes and odors, we tested the sub-threshold integration of otherwise incongruous pairs of stimuli prior to, during, and following their 3-week administration in gum form. Following exposure, the integration threshold for the exposure pair fell, demonstrating newly acquired summation, while the integration thresholds of combinations not experienced during exposure remained constant. The failure of the incongruous taste and smell stimuli to integrate may be attributed to a lack of prior experience with their pairings. fMRI of brain BOLD signal in subjects throughout the study reveal that subthreshold stimuli activate traditional brain regions for taste and smell and show enhanced activation when integrated.

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Poster: Multimodal, Chemosensory Measurement, Psychophysical, Clinical Olfactory, and Trigeminal

Human Cortical Activity of Touch Sensation and Laterality

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In daily life, touch or texture sensation of food will always present simultaneously with taste or flavor. The interaction between touch sensation and gustation, however, is still little known. In order to investigate interaction, we tried to establish basic method for presentation touch sensation that is suitable for evoked potential or magnetic fields. Air puff or electric stimulation is generally used for measuring evoked potential of touch sensation in hands or legs. In case of measurement for tactile sensation of tongue, however, electrode will give artifact to MEG sensors, because tongue is near to sensors. Additionally, air puff method will generate explosion noise at stimulation and will evoke auditory sensation simultaneously. In order to solve these problems, we have developed new method, not puffing air but pulling tongue mucosa by utilizing vacuum chamber. We succeeded to obtain evoked magnetic fields without any artifact. We presented touch stimulation to right and left edge of participants' tongue, which was located about 2 cm from the center. We presented tactile stimulus during 2 ms and average inter stimulus interval was 2 s, which was randomized in range 1000 ms. Two hundred trials were presented to each side, and sampling rate was 625 Hz. Clear magnetic distribution pattern was observed about 90 ms after stimulus, and estimated equivalent current dipoles (activated area) were located in bottom of central sulcus. More precise analyses are in progress.

Poster: Multimodal, Chemosensory Measurement, Psychophysical, Clinical Olfactory, and Trigeminal

'Active' Tasting Selectively Enhances Perception of MSG on the Front of the Tongue

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Tasting is an active process that occurs in the context of mechanical stimulation. The present study followed up our recent finding that active tasting enhanced perception of MSG more than other tastes, particularly in the front of the mouth. In the prior study sucrose, NaCl and MSG were swabbed onto the tongue tip or both the tongue tip and hard palate (which contains no taste buds), and taste intensity was rated after passively receiving the stimulus or touching the tongue to the roof of the mouth and swallowing once. Active tasting increased perception of sucrose and MSG when stimulus was applied to both surfaces, but enhanced only perception of MSG when it was applied just to the tongue. Because MSG is perceived more strongly in the back of the mouth, it was possible that swallowing caused MSG to spread to posterior taste areas. We tested this hypothesis by swabbing sucrose, NaCl, citric acid, QSO₄ and MSG onto the tongue tip and asking Ss ($n = 22$) to rate taste intensity on the gLMS under two conditions: with the tongue immobile and after Ss said the word "taste" three times. Articulating "taste-taste-taste" produced mechanical stimulation at the tongue tip while limiting stimulus spread to the anterior hard palate. The articulation condition led to higher intensity ratings relative to the passive condition only for the savory taste of MSG [$F(4,168) = 2.78$; $P < 0.05$]. This result rules out stimulus spread as the primary cause of savory taste enhancement during active tasting and implies that enhancement is caused by a mechanical or tactile effect that is specific to perception of MSG.

Supported in part by NIH grant DC005002.

Poster: Multimodal, Chemosensory Measurement, Psychophysical, Clinical Olfactory, and Trigeminal

Overexpression of K⁺ Channel Subtypes Alters Responsiveness to Fatty Acids in a Chemosensory Cell Line

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Our studies in obesity-prone and -resistant rats suggest that the ratio of fatty acid-sensitive (fa-s) to fatty acid-insensitive (fa-i) delayed rectifying K⁺ (DRK) channels contributes to differences in dietary fat preference. Using heterologous expression, we have determined that the KCNA and KCNB DRK families are fa-s channels, while the KCNC family is fa-i. To test the hypothesis that the ratio of fa-s:fa-i DRK channels alters fatty acid responsiveness, we have attempted to overexpress a fa-i channel (KCNC1) or a fa-s channel (KCNA5) in an enteroendocrine cell line (STC-1) using lipofectamine-mediated transfection. STC cells respond to polyunsaturated fatty acids (PUFAs) in a similar fashion to taste receptor cells (TRCs). However, unlike TRCs, PUFAs (10 μ M) inhibit only ~40–50% of the total DRK current in STC cells. Using patch

clamp, we have examined the effect of linoleic acid (10 μ M) on DRK currents in STC cells cotransfected with GFP and either KCNA5 or KCNC1. Overexpression of the fa-i KCNC1 channel leads to an increase in total DRK current and a marked reduction in fatty acid responsiveness consistent with our model. Currently, we are exploring whether overexpression of the fa-s KCNA5 channel enhances the fatty acid induced inhibition of DRK currents. Our data support the idea that fatty acid responsiveness in chemosensory cells is determined by the relative expression of fa-s and fa-i DRK channels and that this ratio may help determine the magnitude of sensory signals conveyed by dietary fat.

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Poster: Multimodal, Chemosensory Measurement, Psychophysical, Clinical Olfactory, and Trigeminal

Measures of Confusion and Similarity between Bitter Taste and Burning Sensation

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Although it has long been studied as a pure irritant, capsaicin can also evoke and desensitize bitter taste. This suggests that bitter taste and burning sensation might be closely related perceptually. The current study investigated the psychophysical relationship between bitterness and burning using two different approaches. In Exp. 1, spatial discrimination of four basic tastes was measured in the presence or absence of capsaicin. Subjects reported which of three swabs spaced 1 cm apart and presented to the tongue tip contained a taste stimulus when (1) water was presented on the other two swabs, or (2) when 10 μ M capsaicin was presented on all three swabs. The presence of capsaicin did not change performance on the 3-AFC task for sweet, sour and salty stimuli, while the localization error for 1.8-mM QSO₄ significantly increased ($P = 0.03$). In Exp. 2, the overall similarity/dissimilarity of taste stimuli and capsaicin was measured directly. All combinations of four taste stimuli and capsaicin were applied in pairs to opposite sides of the tongue tip on swabs separated by 2 cm. Multidimensional scaling analyses applied to the similarity ratings showed that capsaicin fell nearer to QSO₄ than to any other taste stimuli. Cluster analysis corroborated this finding: capsaicin was closely linked with QSO₄, and the capsaicin-QSO₄ group was separated from the other taste stimuli. The results also indicated that bitterness was more similar to burning than to the other basic taste qualities. These findings imply that bitterness and burn may be functionally related as sensory signals of potentially dangerous stimuli.

Supported in part by NIH grant DC005002.

Poster: Multimodal, Chemosensory Measurement, Psychophysical, Clinical Olfactory, and Trigeminal

Gustatory–Olfactory Mixtures: A Confusion Matrix Study

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Gustation may dominate olfaction in taste-odor mixtures. We measured identification of odors and tastes in binary mixtures after water or chlorhexidine rinses with a 10-stimulus chemosensory confusion matrix (CCM): 100 mM NaCl, 300 mM sucrose, 100 μ M phenethyl alcohol, 30 μ M vanillin, 4 taste-odor mixtures, 1 odor-odor mixture and water. The odorant concentrations used did not linger and were reliably identified retronasally. Stimuli were presented to 10 subjects twice in separate sessions with different treatment rinses: 1.34 mM chlorhexidine, an oral antiseptic that reduces salty taste intensity, or deionized water, in a cross over design. Percent correct identification and two measures derived from information theory: T10 and T2, were calculated and analyzed with ANOVA and post hoc *t*-tests. In taste-odor mixtures, tastants were identified more frequently (92% correct) than odorants (60% correct) [$t(7) = 5.2, P = 0.001$]. Response consistency (T10) was lower after chlorhexidine (1.96 ± 0.12 bits) than after water (2.36 ± 0.16 bits) [$t(9) = 4.92, P = 0.0008$] and chlorhexidine rinse decreased discriminability (T2) of NaCl solutions [$F(23,207) = 5.1, P = <.000001$]. For this CCM, results were consistent with gustatory dominance in taste-odor mixtures and a weakened taste of NaCl after chlorhexidine rinse. Thus, a CCM is useful for study of olfactory-gustatory interactions.

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Symposium: Olfaction in Neurodegenerative Disease

The Anatomical Dissection of Hyposmia in Parkinson Patients

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Hyposmia is one of the earliest symptoms in Parkinson's disease (PD). The olfactory bulb and the vagus motor nucleus are the first brain structures where alpha-synuclein positive neurites and Lewy bodies can be observed in the earliest stages of PD. The alpha-synuclein aggregates are mainly located in the anterior olfactory nucleus (AON). This nucleus also seems to atrophy in PD patients. In addition we found in each PD-case a few ectopic olfactory glomeruli in the olfactory bulb. Usually only one or two such glomeruli were found in the external plexiform layer. Unlike normal glomeruli, no dopaminergic periglomerular neurons are present around these structures. Since only a very limited number of ectopic glomeruli is present in Parkinson patients it is unlikely that these ectopic glomeruli are responsible for the general hyposmia in these patients. Although it was generally believed that dopaminergic neurons disappear in PD, we found that the number of dopaminergic cells in the olfactory bulb of Parkinson patients increases. This increase is more outspoken in females than in males. In female controls the number of dopaminergic cells in the olfactory bulb is significantly lower than in male controls. In Parkinson patients the number of dopaminergic cells in females equals that of male patients. Since dopamine is known to inhibit the transmission of olfactory input on the mitral cells, the increase of dopamine around and in the glomeruli may at least be partly responsible for the hyposmia in PD patients.

Symposium: Olfaction in Neurodegenerative Disease

Olfactory Dysfunction as an Early Index of 'Pre-motor' Parkinson's Disease

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Olfactory dysfunction is among the first clinical signs of idiopathic Parkinson's disease (PD). In this presentation, I will review research done at the Smell and Taste Center over the past few decades that has assessed the influences of PD on olfactory function. Among such research is a recent study of 24 early-stage PD patients in which relationships between (a) scores on the UPSIT, (b) scores on the Unified Parkinson Disease Rating Scale (UPDRS), and (c) indices of SPECT imaging of the dopamine transporter [99mTc]TRODAT-1 (TRODAT) were evaluated. In this study, a correlation of 0.66 ($P = 0.001$) was found between the UPSIT scores and TRODAT uptake in the striatum as a whole. A 0.74 ($P = 0.001$) correlation was noted between UPSIT scores and TRODAT uptake within the putamen. These findings suggest that olfactory testing may be sensitive, in early PD, to the same neurodegenerative changes as measured by dopamine transporter imaging.

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Symposium: Olfaction in Neurodegenerative Disease

Detection of Preclinical Parkinson's Disease along the Olfactory Trac(t)

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In order to determine whether olfactory impairments are a sign of incipient or preclinical Parkinson's disease (PD), we set up a prospective study involving a cohort of 361 non-parkinsonian first-degree relatives of PD patients, in whom likely alternative causes of olfactory dysfunction were excluded. Baseline performance on a combination of olfactory tasks (odor detection, odor identification and odor discrimination) was used to select groups of hyposmic ($n = 40$) and normosmic ($n = 38$) individuals for clinical follow-up and sequential [123 I]-CIT SPECT (single-photon emission computed tomography) scanning to assess nigrostriatal dopaminergic function. A validated mail questionnaire was used in the follow-up of the remaining 283 relatives. Baseline SPECT scans demonstrated a subclinical loss of striatal [123 I] β -CIT binding in some of the hyposmic but in none of the normosmic relatives. Two years from baseline, 10% of the individuals with unexplained hyposmia, who also had strongly reduced baseline striatal [123 I] β -CIT binding, had developed clinical PD as opposed to none of the other relatives in the cohort. In the remaining non-parkinsonian hyposmic relatives, the average rate of decline in striatal [123 I] β -CIT binding was significantly higher than in the normosmic relatives. Presently, we are analyzing the clinical and imaging results of the 4-year follow-up visits. The data obtained so far indicate that otherwise unexplained hyposmia in first degree relatives of patients with sporadic PD is associated with an increased risk of developing clinical PD.

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Symposium: Olfaction in Neurodegenerative Disease

Long-Term Changes of the Olfactory System in Idiopathic Parkinson's Disease

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Olfactory dysfunction is an early sign of idiopathic Parkinson's disease (IPD). While this has been established some 30 years ago relatively few studies investigated long-term changes of the sense of smell/structures related to olfactory function. A study on 50 PD patients followed up for 6–12 months revealed that there was no major difference between olfactory function in IPD subtypes, while all olfactory tests differentiated IPD from non-IPD. Follow-up of cases with *de novo* IPD did not yield a significant decrease of olfactory function. This was further corroborated by a study in a larger group of IPD patients being followed up over a period of more than 3 years. Again, these patients did not exhibit a significant change of their olfactory function but remained, on average, at the level of hyposmia. Further, an investigation in 11 IPD patients and 9 healthy, age-matched controls suggested that there is little or no difference between IPD patients and healthy controls in terms of olfactory bulb volume. Based upon the relation between loss of olfactory input to the olfactory bulb and consecutive decrease in volume, these data support the idea that olfactory loss in IPD is not a primary consequence of damage to the olfactory epithelium but rather results from central-nervous changes beyond the olfactory bulb—which is emphasized by recent studies in patients with Wilson's disease.

Slide: Molecular Genetic Approaches to Chemoreception

Molecular Identification of PACAP-Sensitive K Channel Expression in OE

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A-type K⁺ currents (I_A) in olfactory sensory neurons have been characterized electrophysiologically but the molecular identities of the underlying channel subunits have not been determined. Using RT-PCR, immunoblot and immunohistochemistry, we found that the channel families underlying I_A, shaker and shal, are expressed in mouse olfactory epithelia (OE). Specifically, Kv1.4, from the shaker family, and Kv4.2 and Kv4.3 from the shal family were expressed, but Kv4.1 mRNA was not amplified from the OE. Immunoblot and immunohistochemical studies confirmed the existence of Kv1.4 and Kv4.2/3 subunits. Furthermore, quantitative RT-PCR showed that PACAP reduced the expression of Kv1.4 and Kv4.2 but not Kv4.3. The PACAP-induced reduction of Kv4.1 and Kv4.2 expression was completely blocked by inhibiting the PLC pathway. Inhibition of the cAMP pathway had no effect. In addition, calcium mediated the reduction of both Kv1.4 and Kv4.2 expression and I_A current density. PKC activation did not affect

Kv1.4 and Kv4.2 mRNA expression, even though PKC reduced I_A current density. Together with our previous studies, our data suggest that A-type K⁺ currents in OSNs are comprised of multiple K⁺ channel subunits, among which Kv1.4 and Kv4.2 are subject to transcriptional modulation by PACAP. We also found that PACAP predominately uses a PLC-calcium pathway to modulate Kv4.1 and Kv4.2 expression. Modulation of A-type K⁺ current expression may contribute to the previously observed neuroprotective effects of PACAP on olfactory sensory neurons.

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Slide: Molecular Genetic Approaches to Chemoreception

The Functional Properties of Mammalian Odorant Receptors

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In order to elucidate the functional properties of mammalian odorant receptors (ORs), we are performing high-throughput screening of active ligands for human and mouse ORs using HEK293-T cells expressing RTP 1 and 2, and REEP1. We first generated both mouse and human OR libraries. Based on Olfactory Receptor DataBase (ORDB; <http://senselab.med.yale.edu/senselab/ORDB/default.asp>) and the published data from Firestein's lab, 300 human and 250 mouse odorant receptors were chosen and subcloned into the expression vectors. The screening was done by a cAMP-dependent luciferase assay. First the activity of these receptors was tested with mixture of odorants and then examined with individual odorants at several different concentrations. From these subsequent experiments, 20 human and 80 mouse ORs were found to be activated by some of the compounds from a group of 78 ligands tested. These data are now being further examined to complete the profile of ORs-ligand interaction sets and quantify the potency of the receptors by EC₅₀. The results show that each individual receptor was activated by several different odorant molecules, which is in agreement with several previous reports. Furthermore, the data indicate that there are certain roles in the combination of receptor-ligand sets which are apparently related to the sequence similarity of receptors and the structure of odorant molecules.

Slide: Molecular Genetic Approaches to Chemoreception

Neuron-Specific Odor Receptor Gene Choice in *Drosophila*

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We have uncovered a combinatorial code of *cis*-regulatory elements critical to the process of odor receptor (*Or*) gene choice in *Drosophila*. The *Or* gene family in *Drosophila* consists of 60 members. Individual members are expressed in specific functional classes of

olfactory receptor neurons (ORNs) of either the antenna or the maxillary palp. 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. In *Drosophila* a special subset of ORNs expresses not one but two *Or* genes. We have identified two distinct mechanisms by which such co-expression can be achieved. First, we demonstrate that some co-expressed *Or* genes share specific sequence motifs in their flanking DNA that can direct expression in a certain neuronal type. Second, for some of the tightly linked *Or* genes, we have found evidence that the downstream gene can share the same promoter as the upstream one by virtue of alternative splicing of the mRNA. The majority of ORNs, however, express a single *Or* gene. We have used comparative genomics to identify evolutionarily conserved *cis*-acting neuron-specific elements that act in specifying the expression of these classes of palp ORNs. *In vivo* analysis of these elements has led to a model for *Or* gene choice. We find that within an olfactory organ a given *Or* gene has the ability to express in a few different ORN classes; however, transcriptional repressors restrict expression to the appropriate neuronal class. Finally, we have identified transcription factors that are required for this highly regulated process of *Or* gene choice.

Slide: Molecular Genetic Approaches to Chemoreception

Altering Olfactory Neuron Identity with Ectopic Expression of G-Protein Coupled Receptors

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In the mammalian olfactory epithelium (OE), each olfactory sensory neuron (OSN) is thought to express only one allele of a single olfactory receptor (OR) gene. It has been proposed that the expressed OR protein represses activation of additional OR genes through feedback inhibition. The resulting singular OR expression effectively determines the identity of the OSN by defining its odorant response properties and influencing its axonal projection. The mechanisms by which ORs influence OSN identity are unknown. We have taken a gain-of-function approach using ultrasound-guided injection of the embryonic OE with retroviral vectors to study the role of ORs in the development of OSN identity. We find that the ectopic expression of functional GPCRs, either OR17 or beta-2-Adrenergic Receptor, perturb odorant response profiles and axonal sorting without a noticeable effect on endogenous OR expression. Additionally, expression of an amino-terminally mutated OR17 that encodes a full-length and properly trafficked OR does not confer octanal responsiveness or perturb axonal sorting. Our work supports a model wherein modulation of G-protein signaling, at least in part, encodes OSN identity. In conclusion, embryonic expression of GPCRs using retroviral vectors offers a new gain-of-function approach for studying the role of ORs in olfactory development.

Support contributed by: NIDCD.

Slide: Molecular Genetic Approaches to Chemoreception

Ubiquitous Expression of an Odorant Receptor Impairs Proper Axon Targeting to the Olfactory Bulb

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In order to study the effects of widespread expression of an odorant receptor (OR) in the olfactory epithelium, we have generated a transgenic mouse line in which we placed the coding region of the *mI7* OR gene into the transcription factor *O/E3* locus. Expression of this transcription factor occurs early in olfactory neuron development and persists in mature olfactory neurons in the adult mouse. In the *O/E3-mI7* line, we detect widespread expression of *mI7* OR, encompassing all four zones of the olfactory epithelium, from birth (postnatal day 0) up to adulthood (8 week-old mice), both at the mRNA, by *in situ* hybridization, and protein level, by immunofluorescence. Electroolfactogram recordings show a 100-fold increase in the dose response to the *mI7* ligand, heptaldehyde (-4.59 ± 1.17 mV at 1×10^{-6} M 7-al in *O/E3-mI7* and -5.35 mV \pm 0.25 mV at 1×10^{-4} M 7-al in wt mice, $P < 0.05$, Student's *t*-test, $n = 4$). The olfactory neurons from these animals express high levels of *mI7* protein and provide a useful model for examining adaptation and OR protein biochemistry. Although ubiquitous expression of *mI7* does not abolish the expression of endogenous ORs (*M72*, *mOR28*), it dramatically affects the targeting of *M72*-expressing axons. Moreover, stereotyped alterations in the position of convergent axons to generate glomeruli suggest a model for sorting axons and glomerular formation. Supported by Howard Hughes Medical Institute and NIH.

Symposium: Changing the Development of Taste and Olfaction

Flavor Programming During Infancy

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A major factor that has inhibited progress in understanding the origin of human flavor preferences is the absence of a robust, experimental paradigm. In addition to demonstrating that experience with flavors in amniotic fluid, mothers' milk and formulas contribute to individual differences in flavor preferences, we have identified a naturally occurring flavor variation that can be exploited to study this important, yet under-investigated, research area. Using as a model system a class of infant formulas that are hydrolyzed-protein based and have distinctive flavors which are unpalatable to older-aged infants and adults, we found that early exposure to this formula (Nutramigen) resulted in a complete shift in hedonic tone to the flavor of this formula from one of absolute distaste to eager acceptance. The effects of early exposure were particularly persistent, leading to heightened preferences for sour tastes, as well as for the taste and aroma of the formula and similarly flavored foods, several years after the child's last exposure. These findings provided evidence of the clearest example of a sensitive period in the development of responses to flavors in humans thus far identified.

This research is supported by NIH Grant HD37119.

Symposium: Changing the Development of Taste and Olfaction

Early Environmental Events Shape the Neurobiological Development of the Gustatory System

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Impressive morphological, physiological, and behavioral changes characterize the postnatal development of the rat gustatory system. While much has been learned by studying developmental processes in control rats, complementary experiments using early dietary sodium restriction have been of great value in learning how the taste system is organized. For example, recent findings from our laboratory demonstrate that the dramatic diet-related differences in the organization and size of gustatory afferent terminal fields in the rat brainstem are due to a reorganization of terminal fields with age in control rats and a lack of an age-related reorganization in experimental rats. Due to large differences in primary afferent taste responses between groups, this finding suggests that activity-dependent "pruning" of axonal arbors may occur in controls but not in early sodium-restricted rats. This symposium presentation will provide a summary of early developmental diet-related changes in peripheral taste function and changes in central structure and function. An emphasis will be placed on more recent experiments in which we have examined the interrelationships among the terminal fields of three gustatory nerves in the rat NTS during normal development and how various dietary manipulations instituted during embryonic development differentially alters this development.

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Symposium: Changing the Development of Taste and Olfaction

Ontogenetic Emergence of Learned/Natural Fear and Development of the Odor Pathway to the Amygdala

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Infant rats do not express fear to natural predator odors, nor do they learn to avoid odors from olfactory fear conditioning until postnatal day 10. This is coincident with the emergence of walking suggesting fear emerges when the risk of predation increases. The ability to express fear appears to be due to the functional emergence of the amygdala at PN10 in both natural fear and conditioned fear. Fear to predator odor and learned fear can be either retarded or advanced by manipulating corticosteroid (CORT) levels. Moreover, CORT is capable of changing the neural pathway of the odor and presumably underlies the behavioral change. Specifically, before the emergence of fearful behaviors (learned and unlearned), odors are relayed from the bulb to the anterior piriform cortex. For the expression of fear, either in older pups or younger pups given CORT, the olfactory circuit is bulb, posterior piriform and amygdala, although a direct bulb-amygdala pathway also exists. We will present data suggesting odor-shock fear conditioning in 14-day-old pups produces an odor aversion with amygdala

participation when the mother is absent but an odor preference with amygdala nonparticipation when the mother is present. The ability of maternal presence to alter olfactory fear learning appears due to the mother's regulation of pups' CORT with maternal odor and tactile stimulation maintaining pups low CORT levels, even during stressful presentations of 0.5-mA shock.

This work is supported by NIH-NICHHD, NSF-IBN and OCAST.

Symposium: Changing the Development of Taste and Olfaction

Neural Networks Involved in Olfactory Fear Conditioning in Rats from Infancy to Adulthood

A. Mouly

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In a recent study, we investigated whether synaptic changes could be detected at different levels of the olfactory pathways following an odor fear conditioning learning. For this, evoked potentials induced in four recording sites (anterior piriform cortex, posterior piriform cortex, cortical and basolateral nuclei of the amygdala) in response to electrical stimulation of the olfactory bulb were collected before odor-shock training (baseline) and during the retention test. The data showed that learning was accompanied by a lasting increase in signal amplitude in cortical amygdala, and a transient increase in posterior piriform cortex and basolateral amygdala, suggesting a differential involvement of these structures in recognition of the learned odor. Paradoxically early olfactory fear conditioning causes odor preferences in rat pups during a temporally defined sensitive period. We assessed the effects of this early experience on adult olfactory fear conditioning. Specifically, infant rats were trained daily from 8-12-days old in an odor fear conditioning paradigm. In adulthood, these animals were trained again in the same paradigm and tested for freezing to the presentation of odor alone. In parallel, amygdala activation following acquisition was assessed using 2-DG mapping. The data showed that, compared to control animals, early trained animals presented lower levels of freezing and no detectable amygdala participation in the adult fear conditioning paradigm. This suggests that early neonatal experience has lasting consequences on both the behavior and the neural network observed in adulthood in odor fear learning.

Symposium: Changing the Development of Taste and Olfaction

Neural Analysis of Predator Odor-Induced Fear and Emotional Memory

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Infant and adult rodents exposed to predator odor exhibit diverse fear-related responses including freezing, avoidance, and stress hormone secretion. Brain structures, with prominent binding of stress hormones, such as the hippocampus and the amygdala likely play key roles in the development and modulation of fear

behavior. However, very little information is known on the neural basis of emotional learning and memory associated with predator odor. We are especially interested in the amygdala, a brain region implicated in fear conditioning using aversive electric shock, in mediating predator odor-induced emotional behavior. Our predator odor studies in adult rats have shown that basolateral amygdala (BLA) lesions impair the elicitation of cat odor-induced unconditioned and conditioned fear responses. In addition, the medial amygdala (MeA), which is associated with aggressive and sexual behavior, plays an important role in modulating cat odor-induced fear. MeA lesions were as effective as BLA lesions in attenuating predator odor-induced unconditioned and conditioned behavior. The specific roles of the BLA and MeA in predator odor fear consolidation and memory retrieval were further evaluated using temporary brain inactivation methods. Muscimol injections made immediately after exposure to cat odor produced a subsequent impairment in conditioned fear behavior but only when injected in the BLA. In contrast, the retrieval of conditioned fear behavior was attenuated but only when lidocaine was injected into the MeA just prior to the conditioned fear test. Our novel results underscore the unique participation of the BLA and MeA in consolidation and retrieval processes underlying predator odor emotional memory.

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Poster: Central Olfaction and Chemical Ecology

Two Distinct Classes of Excitatory Glutamatergic Inputs onto Olfactory Bulb Granule Cells

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Granule cells mediate lateral and self-inhibition of mitral cells and are critical for sculpting mitral cell output patterns. Despite their importance in controlling olfactory bulb output, little is known about the fundamental properties of excitatory synaptic transmission onto granule cells. In addition, the functional properties of excitatory inputs onto proximal granule cell spines remain a mystery. We used minimal stimulation techniques and quantal analysis combined with whole cell patch-clamp recording in olfactory bulb slices to study excitatory transmission at single granule cell spines. We found two distinct classes of excitatory inputs onto granule cells. Inputs onto distal spines in the external plexiform layer (presumably from mitral cell secondary dendrites) showed strong paired-pulse depression due to an increase in transmission failures on the second stimulus. In contrast, EPSCs from proximal inputs in the granule cell layer (possibly from mitral cell axon collaterals or centrifugal feedback inputs) showed paired-pulse facilitation accompanied by a decrease in failure rate on the second stimulus. These two types of synapses also showed markedly different responses to trains of EPSCs that mimic bursts of mitral cell action potentials during sniffing. 50 Hz stimulus trains rapidly silenced transmission at distal synaptic contacts after 2–3 shocks, while the same stimuli produced initial facilitation followed by steady state depression at proximal synapses. These two classes of synapses are thus expected to have distinct effects on granule cell output and the time course of feedback inhibition onto mitral cells.

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Poster: Central Olfaction and Chemical Ecology

Activation of Metabotropic Glutamate Receptors (MGLUR1) in the Glomerular Layer (GL) and Granule Cell Layer (GCL) of the Olfactory Bulb Enhances Synaptic Inhibition of Mitral Cells (MCs)

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mGluRs are densely expressed on granule cells (GCs) and juxtaglomerular neurons and may modulate inhibitory dendrodendritic synapses onto MCs. We investigated the actions of the group I mGluR agonist DHPG on spontaneous IPSCs (sIPSCs) and TTX-insensitive miniature IPSCs (mIPSCs) recorded in MCs in rodent olfactory bulb slices. Bath-applied DHPG in intact slices increased sIPSC frequency at 1 μ M, and increased mIPSC frequency at 3 μ M. IPSCs were blocked by gabazine (10 μ M). The mGluR1 antagonist LY367835 (100 μ M) blocked DHPG's enhancement of mIPSC frequency in most MCs. Focal pressure application of DHPG (1 mM) in the GL or GCL increased sIPSC frequency; however, an increase in mIPSC frequency was only observed when DHPG was puffed in the GL. In slices in which the GL was excised, bath-applied DHPG at 100 μ M did not alter mIPSC frequency, but it increased sIPSC frequency at 10 μ M. Taken together, these results suggest that DHPG-evoked excitation of GCs or periglomerular neurons increases spike-dependent GABAergic inhibition of MCs. Further, DHPG appears to presynaptically facilitate spike-independent release of GABA from periglomerular cells.

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Poster: Central Olfaction and Chemical Ecology

Group I Metabotropic Glutamate Receptors Are Differentially Expressed by Two Populations of Olfactory Bulb Granule Cells

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At least two classes of main olfactory bulb granule cells (GCs) can be distinguished based on soma location, either deep in the GC layer (dGCs) or superficially in the mitral cell layer (MCL) interspersed with mitral somata. Little is known about the physiological properties of the dGCs vs. superficial GCs (sGCs). We explored the role of mGluRs in regulating activity GC in slices from wildtype (WT) and mGluR knockout (KO) mice using patch-clamp electrophysiology. In WT mice, bath application of the group I/II mGluR agonist ACPD or the selective group I agonist DHPG, but not Group II or III agonists, depolarized and increased the firing rate of both populations of GCs. The two GC populations responded differentially to DHPG in mGluR1 and mGluR5 KO mice. DHPG activated sGCs in slices from mGluR5, but not from mGluR1, KO mice. By contrast, dGCs responded to DHPG in slices from

mGluR1, but not from mGluR5, KO mice. Both GC populations lacked an axon and had apical dendrites that extended into the external plexiform layer (EPL). dGCs had a long apical dendrite that crossed the MCL and then ramified in the superficial EPL. sGCs branched almost immediately, i.e., close to the cell body and sent dendrites into the deep EPL. Dendritic spines were observed on both dGCs and sGCs. These anatomical results agree with previous studies that the morphological properties of GCs vary with laminar depth. The present pharmacological results suggest that sGCs are more similar to mitral cells than dGCs in terms of mGluR expression, i.e., both mitral and sGCs express mGluR1 but not mGluR5.

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Poster: Central Olfaction and Chemical Ecology

Glutamate Autoreceptors on Dendrites of External Tufted (ET) Cells

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In principal neurons of the main olfactory bulb (MOB), glutamate autoreceptors provide a presynaptic mechanism for modulating neuronal activity during dendrodendritic neurotransmission. Action potential synchronization of mitral cells projecting to one glomerulus relies on AMPA autoreceptors on dendritic tufts. Here we show that AMPA autoreceptors are also expressed on tufts of another type of MOB principal neuron, the ET cell. In whole-cell voltage clamp recordings of ET cells in rat MOB slices, with 1 μ M TTX, 50 μ M bicuculline, 100 μ M APV, depolarizing voltage pulses (100 ms, -60 mV to 0 mV) activated Ca^{2+} currents plus a slow tail current that was potentiated by 100 μ M cyclothiazide (charge transfer 35 ± 5 pC, decay $\tau = 78 \pm 37$ ms, $n = 13$) and abolished by NBQX. In 300 μ M NAS, this current was strongly attenuated (charge transfer $43 \pm 10\%$ of control), accelerated ($\tau = 39 \pm 15$ ms) and restored after drug wash out ($77 \pm 14\%$, $n = 8$). This indicates a major contribution from Ca^{2+} -permeant AMPA receptors. In 500 μ M Cd^{2+} , uncaging Ca^{2+} in ET cell tufts loaded with 6 mM DM-nitrophen evoked a biphasic current (durations 3.8 ± 1.2 ms, 16 ± 7 ms, $n = 4$) which we attribute to ET autoreceptors. The ET cells projecting to one glomerulus fire periodic spike bursts synchronized in part by gap junction couplings. We suggest that slow AMPA autoreceptor EPSPs may help maintain burst synchrony, analogous to their role in mitral cell spike synchrony. During bursts, Ca^{2+} auto-permeation may regulate or sustain glutamate exocytosis initially triggered by voltage-gated Ca^{2+} channels as backpropagating action potentials invade the ET cell tuft.

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Poster: Central Olfaction and Chemical Ecology

Measuring Olfactory Sensory Neuron Synaptic Vesicle Release in Zebrafish Using the Genetically Encoded Exocytosis Marker Synaptophluorin

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Use of the genetically-encoded exocytosis indicator synaptophluorin (spH) permits direct examination of presynaptic vesicle release dynamics in targeted neurons. In acidic synaptic vesicles spH fluorescence is quenched; upon neutralization following exocytosis fluorescence increases approximately 20-fold. We have developed a zebrafish line stably expressing spH under control of the zebrafish olfactory marker protein (OMP) promoter to examine olfactory input to the adult and developing olfactory bulb (OB). spH expression in the developing OB is detectable 28–48 hpf in F1 or F2 embryos. Labeling is highest in the presynaptic terminals, evident in the distal axonal processes but nearly undetectable in the OSN soma. An increase in fluorescence following neutralization of the synaptic vesicles with NH_4Cl confirmed function. An odor mixture, forskolin (an adenylate cyclase activator) and electrical stimulation of the olfactory nerve elicit OSN synaptic vesicle exocytosis at developmental stages as early as 48 hpf. Suppression of the second response was observed during paired pulse stimulation (ISI 400 ms) of olfactory nerve bundles entering the glomerular layer of F1 adult OBs indicating that the intrinsic inhibitory mechanisms previously noted in the mouse pOMP-spH transgenic line are likely functional in the zebrafish OB. Ionotropic glutamate receptor antagonists partially reduced the suppression. The pOMP-spH transgenic zebrafish line provides an important tool for investigations of bulbar circuitry development.

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Poster: Central Olfaction and Chemical Ecology

Tyrosine Hydroxylase and cFOS Expression in Mouse Olfactory Bulb Slice Cultures Requires an L-type Calcium Channel

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Expression of the olfactory bulb (OB) dopamine (DA) phenotype, as reflected by the level of the first enzyme in DA biosynthesis, tyrosine hydroxylase (TH), requires either receptor afferent stimulation or equivalent depolarizing conditions. Previous studies suggested a role for L-type calcium channels in development of the DA phenotype as well as a causal relationship between cFOS and TH expression. To show that cFOS is involved in the signal transduction mechanisms underlying the activity-dependent expression of TH in OB, forebrain slices were prepared from postnatal day 2–3 transgenic mice expressing enhanced green fluorescent protein (GFP) driven by 9 kb of TH promoter (TH/GFP). Slices were treated with: (1) a depolarizing concentration of potassium chloride (KCl, 50 mM) to simulate receptor afferent activity; (2) KCl plus an L-type calcium channel blocker, Nifedipine (10 μ M); or (3) as a control, sodium chloride (NaCl, 50 mM). cFOS and TH/GFP expression, detected immunohistochemically, were quantitated using MetaMorph Imaging software. cFOS expression was widespread in OB, peaking at 3 h after stimulation, whereas, TH/GFP levels were highest at 48 h. The increase in the number of TH/GFP expressing cells was greater in the superficial granule than in periglomerular regions. Nifedipine prevented the increase in both cFOS and TH/GFP expression. These findings suggest that the same signal

transduction pathway regulates cFOS and TH expression and, despite the temporal disparity, supports the hypothesis that cFOS plays a role in OB TH expression.

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Poster: Central Olfaction and Chemical Ecology

Olfactory Bulb Specific Regulation of Tyrosine Hydroxylase Gene Expression by ER81 in Mice

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Tyrosine hydroxylase (TH) is both the rate limiting enzyme in the biosynthesis of the neurotransmitter dopamine (DA) and a well established marker for DA neurons. The DA phenotype shows region specific brain development and regulation. In the mouse olfactory bulb (OB), peak generation of DA neurons occurs primarily in early postnatal development, and new DA neurons are produced throughout the adult lifespan from stem cells maintained in the sub-ventricular zone. Recent evidence suggests that the transcription factors, Pax6 and ER81, may be necessary for the OB-specific expression of TH in DA neurons. To determine whether either Pax6 or ER81 act directly to regulate TH expression, we have examined the upstream mouse, rat and human TH promoters for potential Pax6 and ER81 binding sites. The analysis identified consensus ER81, but not Pax6, binding sites in the TH promoter. Chromatin immunoprecipitation pull down assays with mouse OBs suggested that these sites in the TH promoter are bound by ER81 *in vivo*. Immunohistochemical staining revealed that ER81 is broadly expressed in most periglomerular cells and overlaps with the subpopulation that also contains TH. Perturbations that profoundly reduce TH expression in the OB, such as odor deprivation, decrease, but do not eliminate, ER81 expression. Together these results suggest that ER81, but not Pax6, is a direct regulator of TH. ER81 expression is not sufficient to activate TH expression, however, and OB-specific TH expression may require a combinatorial set of transcription factors that include ER81.

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Poster: Central Olfaction and Chemical Ecology

Serotonin Increases GABA Release from Periglomerular Cells in Mouse Olfactory Bulb

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Periglomerular (PG) cells, the most populous neuron type in the glomerular layer, have physiological and morphological properties that distinguish them from external tufted (ET) and short axon (SA) cells. PG cells are small interneurons whose dendrites are generally restricted to a single glomerulus. Subpopulations of PG cells express GABA and/or dopamine. Proposed functions of PG cells are (1) presynaptic inhibition of ON terminals and (2) postsynaptic inhibition of mitral/tufted cells, including ET cells. PG cells receive monosynaptic glutamatergic input from and monosynaptically feed

back onto ET cells. This glomerular circuit suggests that modulation of PG cell activity affects ET cell activity. Glomeruli are heavily targeted by 5-HT fibers arising from the raphe nuclei. We have shown that 5-HT, via 5-HT_{2C} receptors, causes a depolarizing current in ET cells when pharmacologically isolated from excitatory and inhibitory inputs. Here we show that when PG cells are isolated from ET and other glutamatergic inputs, 5-HT, via 5-HT_{2A} receptors, induces GABA release from PG cells observed as IPSCs in postsynaptic ET cells. This increased inhibitory input is action potential independent as it is unaffected by TTX. 5-HT modulation of PG cells may function to inhibit glomerular excitation through suppression of bursting activity in ET cells. Alternatively, 5-HT's combined actions on PG and ET cells may enhance the signal to noise ratio of glomerular throughput.

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Poster: Central Olfaction and Chemical Ecology

The Phylogeny of a Putative Circadian Modulator of Olfactory Sensitivity

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Insects are among the most widely used olfactory models and their odor-driven behaviors and olfactory systems are quite variable. This diversity highlights the importance of comparative studies to determine the ubiquity of anatomical and functional traits across taxa. In the sphinx moth *Manduca sexta*, a single serotonergic neuron innervates each antennal lobe and serves as a circadian modulator of olfactory sensitivity. The cell body resides in one antennal lobe, while the axon crosses the posterior midline to innervate the contralateral antennal lobe. To determine the phylogenetic breadth of this neuron's characteristic bilateral morphology, brains of 40 insect species (38 families, 9 orders) were labeled using serotonin immunocytochemistry. Structurally homologous neurons were found in the Lepidoptera, Trichoptera, Diptera, Coleoptera and Neuroptera, but not in the Hymenoptera nor in the hemimetabolous orders examined. Within the hemimetabola, serotonergic antennal lobe neurons are strictly unilateral, projecting only to the ipsilateral antennal lobe and protocerebrum. Our data suggest that the phenotype common to the Lepidoptera most likely originated near the divergence of the holometabola from the hemimetabola some 300 million years ago. This study provides a list of candidates within the insects for which serotonin may act as a circadian modulator of olfactory sensitivity.

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Poster: Central Olfaction and Chemical Ecology

Application of Magnetic Resonance Spectrometry in the Olfactory System

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Magnetic resonance spectrometry (MRS) is a non-invasive technology that measures *in vivo* concentrations of individual metabolites. We have developed MRS methods for determining cerebral metabolic fluxes associated with glutamate and GABA neurotransmitters in olfactory bulb (OB). In present study we tested whether, in terms of neurotransmitter releasing rates, the OB is “quiet” or “noisy”. Overnight fasted urethane anesthetized animals were infused with [1,6-¹³C₂]glucose for different time. Amino acids ¹³C labeling was measured using ¹H-¹³C NMR *in vivo* as well as *ex vivo*. GABA and taurine level in the OB were the highest in the measured brain region. Under resting conditions, the neurotransmitter cycling rates in OB were slightly lower for glutamate and higher for GABA compared with the cerebral cortex, indicating that overall the OB is ‘quieter’. The cycling rates for glutamate and GABA increased significantly with odor stimulation and the increase correlated with stimulation strength. Since the cycling rates for neurotransmitters reflect synaptic activity, the data can be used to correlate/calibrate the results from other studies, such as the BOLD signal in fMRI, the optical signal in intrinsic imaging, and the radioactive signal in 2-deoxyglucose mapping. The methods can be readily used to study synaptic properties at different developmental stages, and the effects of transgenic manipulations in various types of diseases and physiological conditions.

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Poster: Central Olfaction and Chemical Ecology

Oscillations, GABA and Spike Timing in the Moth *Manduca sexta*

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Odor stimulation drives spatial responses, slow patterns of spike bursting, and local field potential oscillations (LFPOs). These phenomena are observed in vertebrate and invertebrate models and have been correlated to odor discrimination. However, oscillatory-based temporal models remain highly controversial. To further explore this model, whole *M. sexta* moths were restrained and a window was opened in the head capsule. Physiological saline was bath applied and 16 channel electrodes implanted into one AL. Multiunit and multi-LFPs recordings were made in response to 9 closely related odorants, each presented at high concentrations in twenty 100-ms stimulations. This panel of stimuli was repeated before, during, and after bicuculline (BMI; 200 μM in saline) application. Spikes were sorted and LFP data filtered using standard techniques. We observed odor-driven high frequency LFPOs with a spectral peak at ~80 Hz. These LFPOs were: multi-phasic, BMI sensitive, coherent across stimulations, initiated at ~50 ms and were highly coherent across recording sites, suggesting a distributed phenomenon. Furthermore, we observed unit-specific crosscorrelations between LFPOs and spiking with spikes from units typically preceding the peak of the LFPO. Interestingly, application of BMI abolished early phase LFPOs. In contrast to the temporal model, BMI also appeared to amplify and enhance the coherence of later phase LFPOs and enhance the coherence between LFPOs and spikes. These results indicate a fundamentally different relationship between GABA LFPO control and spike timing.

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Poster: Central Olfaction and Chemical Ecology

Cell Type Specific Activity-Dependent Regulation of GAD Isoforms in the Glomerular Layer of the Mouse Main Olfactory Bulb

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The inhibitory neurotransmitter aminobutyric acid (GABA) is expressed by subpopulations of interneurons in the mouse main olfactory bulb (MOB). The majority of these GABAergic interneurons are periglomerular (PG) cells located in the glomerular layer and granule cells located in the granule cell layer. GABA is synthesized from L-glutamic acid by the enzyme glutamic acid decarboxylase (GAD). In adult, there are two major isoforms of GAD protein, 65 kDa (GAD65) and 67 kDa (GAD67). There is heterogeneity in the expression of the 65 kDa or 67 kDa isoforms among PG cells. Of the approximately 1.2 million cells in the glomerular layer, 24% express only GAD65, 19% express only GAD67 and 5% express both isoforms. Preferential use of GAD65 or 67 by PG cells correlates with expression of other neurochemicals. For example, ~60% of dopaminergic PG cells, identified by expression of tyrosine hydroxylase (TH) express only GAD67, ~6% express only GAD65, and ~12% express both GAD65 and GAD67. Lesion of the olfactory receptor neurons (ORNs) decreases expression of TH in PG cells. Expression of GAD65 is generally reported to be activity independent in most brain regions while the 67 kDa isoform can be modulated by activity, e.g., in the hippocampus. We find that ORN lesion has no effect on the number of cells expressing GAD65, but reduces the number of cells expressing GAD67 by 70%. These data show different populations of PG cells preferential use the 65 or 67 kDa isoform of GAD and that the presence of ORN axons modulates only expression of the 67 kDa isoform.

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Poster: Central Olfaction and Chemical Ecology

Age-Dependent Modulation of mEPSCs by Carbachol in Rat MOB Granule Cells

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The main olfactory bulb (MOB) of rodents undergoes synaptic development postnatally. Specifically, granule cells (GCs), the major GABAergic neurons of the MOB, form dendrodendritic synapses with glutamatergic mitral cells (MCs) postnatally. During this period of active synaptogenesis, the MOB is thought to play a role in odor-guided behavior critical for survival. Does this behavior correlate with dendrodendritic synapse development? We used carbachol (CCh) to probe the development of dendrodendritic synaptic signaling between MCs and GCs during the first few postnatal weeks. CCh has extensive effects on signaling in the MOB. We report an increased frequency of postsynaptic currents during bath application of CCh in 1 μM TTX and 100 μM picrotoxin, recorded in whole-cell voltage clamp from GCs in MOB slices from 7- to 9-day old rats (*n* = 3, fold-change range: 3.2–20). These currents were sensitive to 10 μM DNQX and 50 μM APV indicating they were

mEPSCs. By contrast, we found no change in mEPSC frequency recorded in GCs from slices of 11- to 17-day old rats ($n = 3$ out of 4 cells, range: 0.9–1.3, one cell showed ~10-fold increase). Conversely, earlier experiments have shown an increased frequency of mIPSCs, sensitive to GluR blockers, in recordings from MCs of OB slices of p7-9 rat pups and GluR blocker insensitive mIPSCs from the older age group. These results suggest plasticity in presynaptic mechanisms at dendrodendritic synapses during the first 10 days of life. Experiments exploring the mechanism/s underlying these age-dependent synaptic changes will be presented.

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Poster: Central Olfaction and Chemical Ecology

Nitric Oxide Is Necessary for Maintaining *Manduca sexta* Antennal Lobe Neuron Activity and Odor Responsiveness

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Despite many studies in several species showing the presence of NO and its signaling components in the olfactory system, the function of NO in the processing of olfactory information remains elusive. In order to better understand the function of NO in the olfactory system, we are using the moth *Manduca sexta* as a model. We have previously shown that enzymes involved in NO signaling, including nitric oxide synthase (NOS) and soluble guanylyl cyclase (sGC), are expressed in subsets of neurons within the *M. sexta* olfactory system and, moreover, that NO is produced in olfactory glomeruli in response to odor stimulation. The function of NO in the olfactory system was examined in individual olfactory neurons with intracellular recording techniques while manipulating levels of NO signaling with pharmacological agents. Blocking NOS with either L-NAME or 7-NI resulted in changes in the behavior of both local interneurons (LNs) and projection neurons (PNs). Both PNs and LNs showed changes in baseline activity, including both increases and decreases in spike firing rate in LNs and the presence of bursts in many PNs. The odor-evoked activity in both neuron types was either missing or altered. The effects were mimicked in several neurons when sGC signaling was blocked using ODQ. However, some of the neurons that were affected by NO blockade did not contain detectable levels of sGC as measured by immunohistochemistry of the recorded and dye-filled neurons. These results indicate that NO has a variety of effects on olfactory neurons and that these effects are mediated by both sGC-dependent and sGC-independent mechanisms.

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Poster: Central Olfaction and Chemical Ecology

Nitric Oxide Signaling in the Rodent Olfactory Bulb

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In the brain, the gaseous messenger nitric oxide (NO) is synthesized by the neuronal isoform of nitric oxide synthase (nNOS). In the

main olfactory bulb (MOB), both nNOS and soluble guanylyl cyclase, an NO target, are highly enriched. However, production of NO in the MOB has not been directly measured, and its actions on olfactory bulb neurons are unknown. Here we report direct electrochemical and optical detection of NO production in the mouse MOB. Using an NO-selective microsensor, we recorded transient extracellular signals from the granule cell layer *in vivo*, in response to odor stimuli. In MOB slices, the microsensor detected steep endogenous NO gradients, and slow transient signals evoked by electrical stimulation of glomeruli. Loading slices with the fluorescent NO indicator DAF-FM revealed a staining pattern consistent with known patterns of NOS immunoreactivity, i.e., strong labeling of periglomerular (PG) cells and granule cells. Step increments in PG cell fluorescence were evoked by stimulating the olfactory nerve layer, and spontaneous spiking of mitral cells was transiently potentiated by L-nitroarginine, an inhibitor of nNOS. Our data show that NO signaling occurs both endogenously and in response to exogenous stimuli, and NO can affect the activity of MOB neurons. We suggest that NO signaling plays a dynamic role in olfactory information processing, perhaps by modulating synchronous oscillations of the mitral-granule network during odor recognition and odor memory formation.

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Poster: Central Olfaction and Chemical Ecology

Inhibitory Interactions among Olfactory Glomeruli in the Moth *Manduca sexta*

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Inhibitory synaptic interactions are important in shaping the activity of output (projection) neurons (PNs) in primary olfactory centers. Such interactions promote coincidence among PNs and contrast enhancement of odor representations. We expect inhibitory interactions to occur among glomeruli belonging to sets or clusters that are functionally related. In initial tests of this idea, we recorded intracellularly the responses of PNs arborizing in 3 neighboring glomeruli in the antennal lobe (AL) of male *M. sexta*. Two of these glomeruli (the Toroid and Cumulus) are part of the male-specific macroglomerular complex (MGC), respectively, processing the 2 main components (E10, Z12-16-A1 and E10, E12, Z14-16-A1, or A and B for simplicity) of the conspecific female's sex pheromone. The third glomerulus (G35) is sexually isomorphic, processes information about a plant volatile (Z3-6-Acetate, Z3HA), and in males is adjacent to the MGC. All PNs gave excitatory responses to stimulation with their respective key odor input. As shown previously, Cumulus-PNs and Toroid-PNs, respectively, were inhibited by stimulation with A and B—i.e., each by the input to the other glomerulus. However, these PNs were not significantly inhibited by Z3HA. By contrast, preliminary results showed that stimulation with A and/or B inhibits G35-PNs, suggesting that inhibitory interactions may not always be reciprocal. These results indicate that inhibitory interactions are not necessarily dictated by spatial position but rather by response properties, chemical relatedness, or functional relationships.

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Poster: Central Olfaction and Chemical Ecology

Ionic Mechanisms Regulating Intrinsic Bursting in Mouse Olfactory Bulb External Tufted Cells

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External tufted (ET) cells in olfactory bulb glomeruli receive mono-synaptic olfactory nerve (ON) input and excite periglomerular and short axon cells thus forming the basic glomerular local circuit. ET cells exhibit spontaneous bursting at the range of frequency (1–8 Hz) which spans the range of rodent sniffing frequencies. Thus ET cells spontaneously drive the glomerular circuit in a range of frequencies ideally matched to the periodic sampling of odorant stimuli. ET cell bursting is mediated by intrinsic conductances. A persistent Na^+ current (I_{NaP}) active near resting membrane potential is required for burst generation but the mechanisms that regulate the duration of a burst, its termination and the inter-burst interval are unknown. The threshold for action potentials in mouse ET cells is ~ -44 mV. When the membrane is depolarized to intermediate levels (~ -38 mV) a Ca^{2+} spike is generated. NiCl_2 (1 mM) and NNC55-0396 (50 μM), a selective T-type calcium channel blocker, completely abolished this low-voltage activated calcium spike and Ca^{2+} current. We hypothesized that activation of T-type Ca^{2+} channels might activate Ca^{2+} -activated K current, which terminates bursts by re-polarizing the membrane. Consistent with this, ET cells exhibit large-conductance calcium-dependent potassium (BK) currents which were blocked by iberiotoxin (IBTX, 200 nM), a selective BK channel blocker. Furthermore, IBTX significantly broadens the evoked burst duration. These results support the hypothesis that the T-type calcium channel plays a critical role in the burst firing activity of ET cells by activating BK channels, which contribute to the termination of the burst.

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Poster: Central Olfaction and Chemical Ecology

Binaral Interaction Modulates Olfactory Bulb Responses to Odorant History

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While odorant-evoked oscillations in the vertebrate olfactory bulb have been studied extensively, information about which neural circuits generate and modulate them has been missing. In particular, it is unclear to what extent oscillations are a product of the olfactory bulb alone, or if they reflect coactivation of the olfactory bulb and other central odor-processing regions. Using voltage-sensitive dye imaging, we show that paired-pulse odorant presentations with interstimulus intervals of 2–5 s had dramatic and diverse effects on the DC depolarization and oscillations that occur in the turtle olfactory bulb. If the same odorant is presented on each pulse, the DC depolarization is depressed while the power of the low-latency 14 Hz oscillation is enhanced in response to the second stimulation. If different odorants are presented on the first and second pulse, then all components of the response are depressed. These effects

are present if both pulses are delivered to the same naris, or if the first pulse is delivered contralaterally to the second. The similarity of uninaral and binaral effects suggest that the history-dependent modulation of the olfactory bulb response is mediated by brain structures sending bilateral projections to both olfactory bulbs.

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Poster: Central Olfaction and Chemical Ecology

Multi-Single Unit and Local Field Oscillatory Dynamics from *In Vivo* Brain Stimulation Focused on Parallel Connections between the Anterior and Posterior Piriform Cortices and the Entorhinal Cortex

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A major question in neuroscience concerns the computational advantages of parallel processing in the brain. The olfactory-limbic circuitry contains multiple examples of parallel inputs from multiple areas onto a single upstream center. The current study was undertaken to determine the neural correlates of olfactory information flow between two output sites in the piriform to the medial entorhinal cortex. This poster focuses on stage 1 of these ongoing experiments, conducted in the anesthetized preparation. Our prior results from recording simultaneously from multiple nodes within the olfactory-motor circuit during a GO/NO-GO olfactory task found the importance of transient frequency modulation and spike inhibitory activity in predicting trial outcome. Our hypothesis states that specific frequency bands from theta to gamma facilitate both the transfer of an artificial stimulus pulse or a stimulus odor, in a site-specific manner, from the posterior and anterior piriform to distinct laminar layers of the medial entorhinal cortex. We used grid microelectrode wires for anterior and posterior piriform stimulation and vertical silicon multisite electrodes for recording current sources in the entorhinal cortex layers. Results currently indicate we have detected layer-specific long lasting complex waveforms generated by one or both piriform sites to the entorhinal, with distinct frequency dependent modulation. These results will aid in characterizing how the flow of information from the periphery, influences feedforward dynamics as exemplified by the parallel inputs from the piriform to the entorhinal cortices.

Poster: Central Olfaction and Chemical Ecology

Neuromodulatory Role for Post-synaptic Density 95 (PSD-95) in the Olfactory Bulb

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Previous work by our laboratory has demonstrated a pivotal role for the voltage-gated *Shaker* potassium channel (Kv1.3) highly expressed in the olfactory bulb (OB) in acuity, threshold, and odorant discrimination. The insulin receptor (IR) kinase and the adaptor protein, PSD-95, are expressed at high levels in the OB whereby PSD-95 disrupts insulin-evoked Kv1.3 current suppression in an

activity-dependant manner. We now show that all three proteins are co-localized in the OB, with PSD-95 showing heavy labeling across all neurolamina including the glomeruli. We found that PSD-95 coimmunoprecipitates with Kv1.3 as well as the IR kinase, demonstrating a multiple protein-protein interaction. PSD-95 clusters Kv1.3 in HEK293 cells, as well as clusters the IR kinase, but only in the presence of Kv1.3. A PSD-95 mutant lacking the SH₃ and guanylate kinase (GK) domain (PSD-95 SH₃) was constructed, as well as use of previous mutants created by A. El-Husseini (2002) to dissect the interaction of these three proteins. PSD-95 PM (palmitoylation) was also used to demonstrate that PSD-95 may be involved in the trafficking and distribution of Kv1.3. We propose a model of interaction of Kv1.3, IR kinase, and PSD-95 where Kv1.3 channels are bound by PDZ domains 1 and 2 of PSD-95 and the IR kinase is bound by the SH₃ domain. These data demonstrate that PSD-95 may influence the excitability of synaptic connections in the OB via K channel interaction and subsequent modulation.

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Poster: Central Olfaction and Chemical Ecology

Differences in Odor Responses between Anterior and Posterior Piriform Cortex

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Single pyramidal cells in piriform cortex respond to a large number of structurally dissimilar odors, can distinguish between highly similar odorant compounds, and develop responses to non-olfactory components of an odor-guided behavioral task. Together with observed anatomical features, such complexity of responding suggests an information processing scheme in piriform cortex that combines convergent information from the olfactory bulb, amygdala and prefrontal cortex. To gain a better understanding of these processes, we recorded responses of single neurons in the anterior (APC) and posterior (PPC) piriform cortex to a broad range of structurally varied odorants. Of particular interest was the degree to which responses in these two areas differed, given their differences in anatomical and functional organization. Results indicate that APC cells display selectivity for one or two odors within an odor class ($\mu = 1.37$ odors), as shown previously. Interestingly, single cells displayed such selectivity for multiple classes of odors (e.g., aldehydes and ketones), and such responses were related in complex ways to odorant structures. Results in PPC include evidence for an active, dynamic tuning of odor specificity within an odor trial, and a preferential response by individual cells for multiple distinct odors within an odor class. Neither of these response characteristics were found in APC. Taken together, these results point to separate, complimentary processing of odor information in APC and PPC.

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Poster: Central Olfaction and Chemical Ecology

Experience-Dependent Adaptation of Sensory Synapses in the Olfactory Bulb

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Experience-dependent changes in neural circuits have traditionally been investigated in brain regions several synapses downstream of the sensory organ. Whether sensory experience can alter peripheral sensory synapses remains largely unknown. In many animals, including rodents, synaptic processing of odor information initially occurs in glomeruli of the olfactory bulb. Here, we find that unilateral naris occlusion in neonatal rats results in the strengthening of primary synapses made by olfactory sensory neurons. Sensory information continues to be amplified through the circuit in deprived animals, as second-order excitatory synapses between neurons in the glomerular region were also found to be stronger. The increase in synaptic strength, triggered by sensory deprivation, is mediated by coordinated changes in both pre- and postsynaptic properties. Our observations demonstrate that sensory experience can modify synaptic strength at the very first site of information transfer between the environment and an organism. This modification may possibly serve as a mechanism for homeostatic gain control in odor processing.

Support: NIH and Klingenstein Fund.

Poster: Central Olfaction and Chemical Ecology

Transient Beta-Frequency Spiking Coupling Olfactory and Motor Sites During Olfactory S+ Recognition

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In previous work, we demonstrated that olfactory and motor brain regions display synchronous, transient (<100 ms) high-gamma (40–100 Hz) oscillations during recognition of a reward-associated olfactory stimulus. The fact that we only detected these high-frequency oscillations in analyses of local field potentials, and not unit recordings, raised the possibility that these oscillations were caused by undetected inhibitory interneuron firing or by sub-threshold mechanisms. How, therefore, were these high-gamma oscillations coordinated across widely spatially separated olfactory and motor sites upon recognition of the S+? We tested the hypothesis, stemming from modeling and slice work in other labs, that increased beta-band spiking would be detected in recordings of the posterior piriform cortex, motor cortex, and subcortical red nucleus as animals sniffed the S+ during an olfactory-driven GO/NO-GO task, and therefore could play a role in large-scale network coupling. We found that during S+, but not S-, recognition, (1) more cells fired in the beta range than in other frequency bands, and (2) beta spiking strongly increased upon detection of the S+ relative to the S-, whereas firing in other frequency bands did not. These results are consistent with recent reports of beta oscillations in the piriform cortex, and they suggest that beta-frequency spiking could play a role in coordinating widely separated, task-related areas during olfactory S+ recognition, prior to the onset of voluntary movement.

Poster: Central Olfaction and Chemical Ecology

The Intrinsic Properties and Firing Modes of Two Types of Olfactory Bulb Interneurons

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Previous studies of the olfactory bulb have defined the main features of the synaptology and intrinsic properties of mitral and granule cells, the major cell types in this brain region. However, much less is known about the intrinsic properties and functional connections made by other cell types in the olfactory bulb, including the wide variety of interneuronal subtypes that are known to exist in the granule cell layer. In this study, we compared the intrinsic properties and firing modes of granule cells and Blanes cells, a recently characterized GABAergic interneuron subtype that innervates granule cells. Under normal pharmacological conditions, transient excitation results in a delayed large afterhyperpolarization (AHP) in granule cells. This hyperpolarizing response could be converted into an afterdepolarization (ADP) by bath application of carbachol, a non-selective muscarinic acetylcholine receptor agonist (2 μ M; $n = 34$). Near firing threshold, depolarizing input evoked a transient period of firing in granule cells that corresponded to the time course of the ADP. By contrast, Blanes cells exhibited a large ADP (4.0 ± 0.4 mV at -60 mV) under normal pharmacological conditions and could reliably generate persistent firing (mean duration $n = 16.5 \pm 5.1$ min; $n = 9$). Both the ADP and persistent firing modes of Blanes cells required Ca influx and were blocked by low Ca/high Mg ACSF ($n = 4$) and Cd (200 μ M; $n = 4$). The different firing modes in granule and Blanes cells, and their differential modulation by cholinergic input, are likely to play a significant role in the evolution of activity patterns in the olfactory bulb.

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Poster: Central Olfaction and Chemical Ecology

The Antibody OR-I7 Selectively Affects the Detection of n-Octanal and the Odor Induced C-FOS Expression Pattern in the Rat Olfactory Bulb

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Several pharmacological studies have shown that the rat I7 olfactory receptor (OR-I7) responds preferentially to the n-aliphatic aldehyde n-octanal. However, there are n-octanal related odor compounds—e.g., citral—which also interact with OR-I7. To understand how these two aldehydes interact with the olfactory receptor OR-I7 we performed behavioral experiments and subsequently immunohistochemical (c-fos) studies in which the polyclonal antibody OR-I7: I7-MAP-PEPTIDE (Ab OR-I7) was applied to the rat olfactory epithelium. Rats were trained in an olfactometer by operant conditioning to detect and discriminate low concentrations of n-octanal ($n = 10$) or citral ($n = 5$) from clean air. Ab OR-I7 application reduced the animals' detection performance for n-octanal but not for citral. In the immunohistochemical part of this study we compared the number of c-fos positive periglomerular cells in the olfactory bulb (OB) before ($n = 6$) and after ($n = 6$) antibody application. Ab OR-I7 application resulted in a strong reduction of Fos immunoreactivity in the OB of n-octanal stimulated animals. However, Ab OR-I7 application had no effect on the Fos staining patterns in citral-stimulated animals ($n = 6$). Our data are in agreement with recent results and give strong evidence that n-aliphatic aldehydes evoke overlapping but also significantly different patterns of neuronal activity in the rat OB.

We thank RAM Research Ltd., London, for financial support and Krishna Persaud for the polyclonal antibody OR-I7.

Poster: Central Olfaction and Chemical Ecology

Columnar Organization in the Olfactory Bulb

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Modular organization of glomerular units in the olfactory bulb has been well established. While the olfactory module has been compared to columns previously, more information on the synaptic connectivity is needed to define the nature and extent of column-like architecture. To probe the synaptic organization of the olfactory bulb, we injected a retrograde specific strain (Bartha) of the pseudorabies virus into the olfactory bulb. The viral staining patterns reveal striking columnar organization not only in the known column-like region from the glomerulus to the mitral cell layer, but throughout the whole depth of the granule cell layer as well. This pattern may arise from virus-specific effects, but we hypothesize that the columns represent functional glomerular units. Further, specific patterning is observed suggesting connectivity that is specific to selected glomeruli, rather than strictly distance dependent. These patterns are restricted to either the medial or lateral half of the bulb when the injection is made in the respective area, with the exception of a population on the opposite side assumed to arise from the mirror glomerulus projection. The results provide a new basis for interpreting the synaptic connections between mitral and granule cells within the context of the columnar organization in the olfactory bulb, and have implications for olfactory coding and network organization.

This work was supported by the NIDCD.

Poster: Central Olfaction and Chemical Ecology

Patterned Projections in the Olfactory Bulb from Olfactory Cortex Revealed by Transsynaptic Labeling

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Olfactory information from the olfactory bulb is sent to the olfactory cortex. Genetic labeling has shown that input from a single receptor cell population targets specific clusters of cortical pyramidal cells, and that these clusters overlap to some degree with input from other receptors. The extent of receptor representation and differences in bulb projection pattern from different regions of olfactory cortex is currently unknown. To address this question, we injected retrograde specific pseudorabies virus into five primary areas of olfactory cortex, the posterior piriform cortex, the dorsal anterior piriform cortex, the ventral anterior piriform cortex, the dorsal anterior olfactory nucleus, and the lateral anterior olfactory nucleus. The pseudorabies virus is an alphaherpes virus which is neuron specific, and infects neurons in a transsynaptic manner. Three days after infection, staining patterns in the olfactory bulb show specific patterning of glomerular unit labeling, including columns extending throughout the granule cell layer discussed in another abstract (see Willhite *et al.*, accompanying abstract). The staining from all areas is bilaterally specific within the ipsilateral bulb, with only the anterior olfactory nucleus showing contralateral bulb staining. The degree of bulb staining directly correlates with injection radius

(i.e., volume). A variety of patterns from the various areas is revealed using this approach.

This work was supported by the NIDCD.

Poster: Central Olfaction and Chemical Ecology

Estimating the Number of Modules in Rat Olfactory Bulb by Prediction of Odorant Descriptors

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The intrinsic dimensions of odor space are of great interest for the study of olfactory coding mechanisms. Recent genome studies have estimated the repertoire of olfactory receptors to be around 1,000 in the mouse and 500–750 in humans, which serves as an upper bound of the intrinsic dimensions. An alternative estimate of intrinsic dimensionality may be obtained by considering that glomeruli with similar affinities cluster in close proximity, forming functional modules. The objective of this paper is to extract such intrinsic modules using machine learning techniques. Images of glomerular activity were obtained from the archive by Leon and Johnson at the University of California, Irvine. One hundred and seventy-two images were used to extract modules using a dimensionality-reduction technique known as non-negative matrix factorization. The optimum number of modules was estimated by maximizing the prediction of odorant percepts (ten Flavornet descriptors) from module activity using a support vector machine. The results of the prediction were two-folds. First, the predictive performance (geometric mean of true-positive and true-negative rates: 85%) was much higher than chance level (geometric mean: 50%), supporting a hypothesis that odorant information is represented by spatial activity in the glomerular layer. Second, the highest predictive performance was achieved with 80–100 modules, which serves as an estimate of the intrinsic dimensions of odor space.

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Poster: Central Olfaction and Chemical Ecology

Sexually Differentiated Expression of BDNF and TRKB Associated with the P2 Glomeruli of Mouse Main Olfactory Bulb

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Differential behavioral responses of female and male rodents to various chemosensory signals have been described abundantly in the literature, yet the neural basis mediating those responses is largely unknown. We are testing the hypothesis that sexually dimorphic glomeruli may contribute to sex-differentiated behaviors. The aim of this study was to determine whether expression of BDNF and its receptor, TrkB, have sexually differentiated expression associated with P2 glomeruli previously described as sexually dimorphic. To accomplish this, we bred P2-IRES-tauGFP mice to BDNF-LacZ mice. The former strain expresses GFP in all olfactory

sensory neurons that express the P2 odorant receptor while in the latter strain LacZ replaces one BDNF allele. We show a correlation between the sex differences in the volume of P2 glomeruli and the number of β -galactosidase (β -gal)-immunoreactive (IR) periglomerular (PG) cells surrounding the P2 glomeruli. Specifically, the number of β -gal-IR PG cells surrounding the lateral P2 glomeruli was higher in females compared to males. Consistent with this finding, we show that the intensity of TrkB staining within the lateral P2 glomeruli is higher in females compared to males. These results show that BDNF and TrkB expression associated with the P2 glomeruli is sexually differentiated and suggests that BDNF/TrkB may mediate the sex differences in the volume of the lateral P2 glomeruli.

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Poster: Central Olfaction and Chemical Ecology

The Sense of Smell: Multiple Olfactory Subsystems

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The mammalian olfactory system is not uniformly organized but consists of several subsystems each of which probably serves distinct functions. Not only are the two major nasal chemosensory systems, the vomeronasal organ and the main olfactory epithelium structurally and functionally separate entities, but the latter is further subcompartmentalized into overlapping expression zones and projection-related subzones. Moreover, the populations of OR37 neurons not only express a unique type of olfactory receptors, but also are segregated in a cluster-like manner and generally project to only one glomerulus. The septal organ (SO) is an additional island of sensory epithelium on the nasal septum. SO neurons express a limited repertoire of odorant receptors, including a few which are found in a very high proportion of neurons. One set of SO axons enters glomeruli which are mainly targeted by axons of neurons located in the main olfactory epithelium. Another fraction targets SO-specific glomeruli. Due to its exposed position in the airstream and close to the nasopalatine duct which connects the mouth to the nasal cavity it is considered a “mini-nose” with dual function. A specific chemosensory function of the most recently discovered subsystem, the so-called Grueneberg ganglion, is based on the expression of olfactory marker protein and the axonal projections to defined glomeruli within the olfactory bulb. This complexity of distinct olfactory subsystems may be one of the features determining the enormous chemosensory capacity of the sense of smell.

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Poster: Central Olfaction and Chemical Ecology

Spatially Distinct Sensory Input to Medial Olfactory Bulb Glomeruli and Output Projections into the Habenula and Ventral Thalamus in the Sea Lamprey *Petromyzon marinus*

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In this study of the olfactory system of the sea lamprey, we investigated the spatial origin of olfactory sensory neurons extending into medial olfactory bulb glomeruli, and the projection pathways of second order olfactory bulb neurons. Our previous studies have shown that the medial glomerular territories are biochemically distinct from others in the olfactory bulb. Here, we use double labeling with Dil and DiA to show unique peripheral input to medial glomeruli. Peripheral cells in the accessory olfactory organ, a long ignored tiny structure that is ventro-caudal to the peripheral olfactory organ, as well as olfactory sensory neurons (OSNs) in the olfactory epithelium extended fibers into medial glomeruli. Dye injection into lateral glomeruli filled OSNs in the olfactory epithelium, but failed to label cells in the accessory olfactory organ. Neuronal fibers labeled by microinjection of biotinylated dextran into medial glomerular territories extended through the habenula, ventral thalamus and hypothalamus. Fibers decussating through the dorsal commissure to the contralateral olfactory bulb were also observed. These data suggest the presence of a spatially distinct olfactory sub-system in this ancestral vertebrate.

Poster: Central Olfaction and Chemical Ecology

Functional and Spatial Identity of Mouse Olfactory Glomeruli Innervated by Defined Population of Olfactory Receptor Neurons

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Despite recent progresses in deorphanizing olfactory receptors (ORs), gene-targeting or transgenic approach has been required to characterize odorant-responsiveness of a glomerulus innervated by defined population of olfactory neurons in the olfactory bulb (OB). In this study, we generated transgenic mice to visualize olfactory neurons expressing a eugenol receptor, mOR-EG (MOR174-9). Using these mice, we established an *in vivo* OR identification technique based on glomerulus activity. Several ORs sharing high sequence homology were identified from eugenol-responsive glomeruli. Further, single glomerulus RT-PCR and reconstitution of identified ORs in HEK293 cells recapitulated our results. Spatial characterization of OR-defined glomeruli in the OB across animals revealed that positional relationship of these glomeruli considerably varied between individuals. We also compared odorant-response properties of OR-defined glomeruli with those obtained in HEK293 cells, demonstrating that odorant responsiveness of each glomerulus was not exactly reflected by OR pharmacology observed in heterologous expression system. Our findings point out caveat that odor identity in the OB should be discussed at the OR level, rather than at the level of activity pattern.

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Poster: Central Olfaction and Chemical Ecology

In Vivo Two-Photon Imaging of Mitral Cell Odor Responsiveness

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Recent advances in molecular biology and functional imaging have established that odor information is represented as spatial patterns of activated glomeruli on the olfactory bulb surface. How these glomerular coding patterns are subsequently transformed into the mitral-cell ensemble output has emerged as a next critical question for understanding odor discrimination and recognition. To date, no direct comparison has been made between odor-evoked glomerular activity and its corresponding mitral cell output. In an effort to address this issue, we have carried out *in vivo* imaging of odor responses both from olfactory glomeruli and individual mitral cells. The odorants tested in this study were a homologous series of aliphatic aldehydes. The aldehyde-evoked glomerular activity pattern was imaged with the OMP-synaptophluorin mice originally developed in Peter Mombaerts' laboratory. Mitral cells in these GFP mice were labeled with a calcium-sensitive indicator. By taking advantage of deep-tissue imaging of two-photon microscopy, we were able to trace the glomerular projection of individual mitral cells, and then characterized odor responses of these neurons with known glomerular identity. This approach revealed optically the excitatory molecular receptive range of individual mitral cells in the dorsal olfactory bulb. The odorant receptive range of a mitral cell was found to be similar to that of the corresponding glomerulus. These results suggest a tight functional coupling between a glomerulus and its associated mitral cells. We are currently exploring the conditions under which a mitral cell could have a different odor-response profile from its glomerulus.

Supported by an NIH grant (DC003918).

Poster: Central Olfaction and Chemical Ecology

Neuroanatomical and Functional Characterization of MOR-EG Gene-Targeted Mice: Axon Convergence and Odorant Responses

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We recently identified the odorant-binding site of a mouse eugenol receptor, mOR-EG, providing the structural basis for olfactory receptors (ORs) that recognize broad but selective ligand spectrum. We created three transgenic mouse lines in which olfactory sensory neurons expressing mOR-EG co-expressed gap-EGFP. The zonal distribution of the fluorescent neurons was conserved in all these transgenic lines, whereas aberrant axonal projections to the olfactory bulb were detected in two lines. A gene-targeting approach was also applied to visualize the glomerular convergence of endogenous mOR-EG neurons by X-gal staining, and we compared axon convergence of mOR-EG neurons in gene-targeted mice with that in transgenic lines. To examine odorant responses in these genetically modified mice, we utilized c-Fos as a neuronal activity marker. Eugenol induced c-Fos expression in periglomerular cells and granule cells around the endogenous mOR-EG glomeruli as well as minor mOR-EG glomeruli observed in the transgenic mice. The eugenol-response pattern in the olfactory bulb determined by c-Fos induction correlated well with that obtained by a calcium imaging method.

Supported by PROBRAIN, Japan.

Poster: Central Olfaction and Chemical Ecology**Response Specificity of Olfactory Forebrain Units in the Channel Catfish to Amino Acids**

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We previously described the odotopic maps of both the olfactory bulb (OB) and forebrain (FB) in the channel catfish to amino acids (AA), nucleotides and bile salts. We now report on the specificity of FB units to AA and how it compares to that determined for OB units. All recordings were performed *in vivo* within the AA zone of the FB, and only excitatory responses are reported. As in the OB, FB units of both high (Group I) and lower (Group II) specificities were obtained. Both Group I FB and Group I OB units were excited by only one of three major types of AA: (1) neutral L-amino acids with short side-chains (e.g., Ala or Ser), (2) neutral amino acids with long side-chains (e.g., Met) and (3) basic amino acids (e.g., Arg); responses to acidic AA (e.g., Glu) were scarce at both OB and FB levels. FB units were excited by a lower (~1 log unit) concentration than were OB units, but dose-response functions were similar. The more broadly-tuned Type II FB units showed a broader specificity than the Type II OB units. In addition, complex units were identified in the FB, but not the OB, that were excited by different classes of AA and by nucleotides (feeding stimuli).

Supported by NSF IBN-0314970 and NIH DC-03792.

Poster: Central Olfaction and Chemical Ecology**Selectivity of Bile Salt Responsive Neurons in the Olfactory Bulb of the Channel Catfish**

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An odotopic map of biologically relevant odorants (bile salts, amino acids and nucleotides) exists in the olfactory bulb (OB) of channel catfish, *Ictalurus punctatus*. We previously reported that OB neurons of this region were (1) selectively excited by bile salts that were non-conjugated at carbon 24, (2) selectively excited by bile salts that taurine-conjugated at C24, or (3) generalists that were excited by (1), (2), and glycine-conjugated (C24) bile salts. Previous behavioral studies suggest that bile salts are socially relevant odorants in fishes. The present report indicates that OB neurons are selective for particular combinations of molecular features located at three additional carbon positions (C3, C7, C12) along the perhydrocyclopentanophenanthrene ring. OB neurons previously categorized as (1) and (2) show additional selectivity for hydroxylation (or lack thereof) at C7 and C12 and hydroxylation at C3; other category (2) units require sulfonation at C3. Further, the data suggest that the majority of category (3) neurons respond excitedly to hydroxylation at C7 in combination with hydroxylation at C3 (irrespective of the molecular feature of C24). Olfactory thresholds of OB neurons to conjugated bile salts were lower (0.01–1 μ M) than those to non-conjugated bile salts (1–10 μ M).

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Poster: Central Olfaction and Chemical Ecology**Pattern Recognition for Optical Microbead Arrays with a Neuromorphic Model of the Olfactory Bulb**B. Raman¹, T. Kotseroglou², M. Lebl², L. Clark² and R. Gutierrez-Osuna¹¹Computer Science, Texas A&M University, College Station, TX, USA and ²Illumina, Inc., San Diego, CA, USA

We present a biologically-inspired approach for sensor-based machine olfaction that combines a prototype chemical detection system based on microbead array technology with a computational model of signal processing in the olfactory bulb. The sensor array contains hundreds of microbeads coated with solvatochromic dyes adsorbed in, or covalently attached on, the matrix of various microspheres. When exposed to odors, each bead sensor responds with intensity changes, spectral shifts and time-dependent variations associated with the fluorescent sensors. The microbead array responses are subsequently processed using a computational model that captures two key functions in the early olfactory pathway: chemotopic convergence of receptor neurons onto glomeruli, and center on-off surround lateral interactions mediated by granule cells. The first circuit, based on Kohonen self-organizing maps, is used to perform dimensionality reduction, transforming the high-dimensional microbead array response into an organized spatial pattern (i.e., an odor image). The second circuit, based on Grossberg's additive model, is used to enhance the contrast of these spatial patterns, improving the separability of odors. The model is validated on an experimental dataset containing the response of a large array of microbead sensors to five different analytes. Our results indicate that the model is able to improve the separability between odor patterns compared to that available at the receptor or glomerular levels.

Poster: Central Olfaction and Chemical Ecology**Microelectrode Array Analysis of Odorant-Evoked Spatial Activity Patterns in Piriform Cortex**R. Rennaker¹, A. Ruyle¹, C.F. Chen² and D.A. Wilson²¹Aerospace and Mechanical Engineering, University of Oklahoma, Norman, OK, USA and ²Zoology, University of Oklahoma, Norman, OK, USA

Most sensory cortices incorporate a spatial dimension in the encoding of stimulus identity, resulting for example, in retinotopic, somatotopic and tonotopic patterns of cortical evoked activity. While a form of odotopic patterning exists in the olfactory bulb, the evidence for spatial encoding of odorant identity in the piriform cortex is less clear. Here, we used simultaneous unit recordings across large regions of anterior piriform cortex (aPCX) to further examine cortical spatial odorant coding. Simultaneous recordings of single- and multi-unit activity across large regions of aPCX were performed using fixed microelectrode arrays of 6–8 electrodes nominally spaced at 250–500 μ m apart, and/or extraction of multiple single-units recorded from a single microelectrode in urethane-anesthetized rats. Spontaneous activity, phase locking to respiration, and odorant-evoked activity were analyzed. Odorants included a variety of monomolecular esters, and more complex lemon and peppermint. Analyses of both small (units near a single

electrode) and large scale (1–2 mm) spatial patterns were performed. Initial results suggest the following. (1) An individual odorant can activate widely spaced neurons throughout the aPCX, and conversely nearby neurons may not respond to the same odorants. (2) Highly volatile (more intense) odorants activate larger regions of aPCX, perhaps through caudal expansion. Additional analyses of both spatial and temporal patterning on small and large scales will be presented.

Funded by a grant from NIDCD.

Poster: Central Olfaction and Chemical Ecology

Olfactory Experience Decorrelates Encoding of Mixtures from Components in Rat Piriform Cortex

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Olfactory system encoding of odors has been hypothesized to be similar to visual object encoding. Perceptual odor objects are hypothesized to be synthesized by central circuits through experience. We tested whether experience with an odor mixture would enhance the distinctiveness of cortical coding of the mixture compared to its components. Rats were trained in a Go, No-Go odor discrimination task where the S+ was a mixture of acetic acid, limonene and eugenol. S– odorants were the individual components or clean air. After reaching behavioral performance criterion, rats were urethane-anesthetized and single-unit recordings made from anterior piriform cortex. Odor naive rats served as controls. Responses to the mixture and components, as well as the novel odor isoamyl acetate were analyzed. The proportion of odor responsive cells, odor response magnitude, and correlation analyses of population odor responses were determined. The results suggest that odor experience reduces average aPCX odor-evoked response magnitude to all odorants tested, similar to results reported for the olfactory bulb. Importantly, odor experience produced a significant de-correlation between cortical responses to the mixture and its individual components. Response correlations within pairs of the components were either unchanged or enhanced. These results suggest that cortical encoding of the mixture becomes more distinct from its components through experience, perhaps contributing to reported experiential effects on mixture perception and impaired ability to identify components within mixtures.

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Poster: Central Olfaction and Chemical Ecology

Electrophysiological, Behavioral and Computational Investigation of the Functional Role of Synaptic Adaptation in Olfactory Cortex

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Segmentation of target odorants from background odorants is a fundamental computational requirement for the olfactory system. Recent data from our lab (DAW) have shown that odor specific adaptation in piriform neurons, mediated at least partially by synaptic adaptation between the olfactory bulb outputs and piriform

cortex pyramidal cells, may provide an ideal mechanism for odor-background segmentation. This rapid synaptic adaptation acts as a high-pass filter to enhance cortical responsivity to changing stimuli, while reducing responsivity to static, potentially background stimuli. Interestingly, the adaptation observed at the level of pyramidal cell is very odor specific, while that observed at the synaptic level is specific only to certain odor-features. Using previously developed computational models of the olfactory system (CL), we here show how synaptic plasticity and associative memory function within piriform cortex interacts with synaptic adaptation at the olfactory bulb input to create odor specific adaptation, and in turn contribute to background segmentation. In the computational model, we also test how known physiological effects of acetylcholine in piriform cortex contribute to the cholinergic modulation of this odor specific adaptation.

Supported by NSF grant #0338981 to C.L. and D.A.W.

Poster: Central Olfaction and Chemical Ecology

Temporal Coding of Similar Tastants in the Nucleus of the Solitary Tract of the Rat

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Recent work has shown that spike timing in the first 2 s of response in the nucleus of the solitary tract (NTS) contributes to coding of taste quality. Cells with especially variable response magnitudes across repeated stimulus presentations typically showed the greatest evidence of temporal coding. Responses to repeated trials of tastants that were of similar quality (salty, sour, sweet or bitter) but were of different chemical composition were recorded in the NTS of anesthetized rats. Stimuli were NaCl (0.1 M), LiCl (0.1 M), HCl (0.01 M), citric acid (0.01 M), sucrose (0.5 M), fructose (0.3 M), quinine-HCl (0.01 M) and urea (1.0 M). Temporal coding was assessed using an information-theoretic approach. Response magnitudes evoked by similar-tasting stimuli varied substantially, and often independently from each other, across trials. Spike timing contributed to the information present in taste responses when the relative magnitudes of response to different tastants varied across trials whether the taste responses were evoked by similar or dissimilar tastants. Conversely, when the response to one stimulus of a pair was always greater than the other, rate coding was more likely to account for the information conveyed by the responses. These data suggest that spike timing may contribute to discrimination between tastants in cases where firing rate alone is not sufficient, regardless of whether those tastants evoke the same quality.

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Poster: Central Olfaction and Chemical Ecology

Functional Characterization of Lobster Olfactory Projection Neurons

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Some larger crustaceans have a unique neuropil associated with the olfactory lobe (OL) called the accessory lobe (AL). The AL receives olfactory input from a distinct subset of local interneurons arborizing in the sub-cap region of OL glomeruli, although the role of the AL in processing olfactory information is unknown. Using a perfused nose-brain preparation, we characterized the responses of OL and AL projection neurons (PNs) to olfactory (antennular) input. In contrast to the largely silent OL PNs, AL PNs exhibit spontaneous bursts of action potentials (APs) with a period of 12.3 ± 1.3 s ($n = 6$). Ablation of antennular chemoreceptors abolishes the spontaneous bursting. Odorants evoke APs in both OL and AL PNs in an odorant- and concentration-dependent manner. OL PNs respond with a complex, often multiphasic train of APs. AL PNs respond with a single phasic-tonic train of APs, the duration and latency of which is phase-dependent on the ongoing bursting activity of the cells. AL PNs also respond to visual stimuli with a shorter latency than to odorants (327 ± 48 ms [$n = 37$] vs. 777 ± 171 ms, [$n = 12$]). The ON response to light shortens the subsequent response to odorant, which in turn abolishes the OFF response to light. Our findings raise the interesting possibility that AL PNs are multimodal neurons that process input from a recently discovered subset of inherently oscillatory olfactory receptor neurons that may terminate in the sub-cap region of OL glomeruli.

Poster: Central Olfaction and Chemical Ecology

A Comparison of Ensemble Representations for Natural Plant-Odor Blends and Blend Components in the Antennal Lobe of the Moth *Manduca sexta*

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Although most organisms in nature operate at low odor intensities, many chemosensory studies use olfactory stimuli at non-physiological concentrations. Moreover, clear understanding of how the brain processes complex mixtures as opposed to single odorants has proven elusive owing to the lack of adequate analytical methods. Using the moth, *Manduca sexta*, we first approached an improved understanding of the behavioral importance of complex blends through wind-tunnel experiments to odors from host-plant flowers. Selective removal of key blend components suppressed upwind flight responses in comparison to the entire blend. To compare how the antennal lobe (AL) of *M. sexta* encodes behaviorally relevant floral mixtures and single constituents, a multi-channel neural-ensemble recording array was coupled with a GCMS. Integration of these two technologies allows examination of AL response to natural complex mixtures and provides a means of fractionating those same blends into their single components. Approximately 25% of the units responded specifically to single odorants. Moreover, odor-evoked activity was often spatially restricted to distinct regions of the AL. Spatiotemporal ensemble dynamics were clearly modulated differentially by the blend as compared to the single odorants alone. Together, these results provide new evidence that in moths, upwind orientation to blends is mediated by the precise integration of multiple glomerular pathways, and that blend input transforms the network representations in a manner that is not predicted from responses to single odor compounds.

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Poster: Central Olfaction and Chemical Ecology

Spatial and Temporal Organization of Odor Representation by Uniglomerular Projection Neurons in the Moth Antennal Lobe

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The antennal lobe (AL) is the first relay station for olfactory information in the insect brain and the anatomical equivalent of the olfactory bulb (OB) of mammals. Both systems have common structures called glomeruli in which neurons make synapses. Olfactory receptor neurons expressing the same receptor project to the same glomeruli in the AL. Projection neurons (PNs), the AL output neurons, transmit the processed information into higher order olfactory centers. To investigate spatial and temporal patterning of glomerular activity, we reconstructed olfactory representation by pooled set of single PN recordings. Most of PNs innervated single glomeruli ($n = 126$). PNs showed various slow temporal patterns to odor. PNs innervating the same glomerulus had similar response profiles so that we could reconstruct odor-evoked spatial pattern of PNs firing in the AL. This reconstructed spatial map is highly distributed and dynamic. Different odor elicited different spatial pattern at each time point. The Euclidian distances between odor representations reached maximum at 200 ms after the response onset. There were no clear correlation between physical distance of glomeruli and response similarity of odor-evoked slow temporal patterns. This result is consistent with prior calcium imaging and modeling study. We conclude that olfactory information is encoded by distributed spatial and temporal pattern of PNs firing and there is no clear relationship between the physical distance and response pattern in the moth AL.

Poster: Central Olfaction and Chemical Ecology

Ensemble Coding of Odor-Blend Ratios in the Insect Antennal Lobe

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Foraging insects use complex floral odor blends, consisting of common odorants in specific ratios, to identify host plants. Little is known about how the ratio of the components of a host-flower blend is represented in the antennal lobe, the primary processing center for olfactory information in insects. We investigated whether the response of an ensemble of neurons in the antennal lobe of the moth *Manduca sexta* was tuned to the natural ratio of components in the floral odor of a host plant, sacred datura. Using a synthetic mixture of 14 major components of this blend, we presented mixtures with varying concentrations of single components, from 0.01 to 100 times the natural concentration. The components were also presented alone over the same concentration range. The firing rate of single units and recruitment of additional units both increased with the concentration of the monomolecular odorants alone, consistent with previous results. However, the response to the mixtures did not follow this predicted pattern. Instead, individual units exhibited

an optimal response to the mixture with the natural ratio of components, and deviated symmetrically from this optimum when the concentration of a single component was raised or lowered. The shape of the deviation was furthermore dependent on which component was altered. These results suggest that the representation of a blend in the antennal lobe is not a simple, linear combination of the component representations and that the glomerular circuit may be tuned to natural, behaviorally relevant ratios of the component concentrations.

Supported by NIH grant R01-DC-02751 to J.G.H.

Poster: Central Olfaction and Chemical Ecology

Comparative Functional Morphology of Male-Specific Glomeruli in Two Heliiothine Moth Species, *Helicoverpa zea* and *Heliothis subflexa*

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Two sympatric heliothine species, *Helicoverpa zea* and *Heliothis subflexa*, share a major pheromone component, (Z)-11-hexadecenal (Z11-16:Ald). However, males are not attracted to interspecific females due to the activity of minor components in heterospecific pheromone blends. Action potentials from pheromone-component-sensitive olfactory receptor neurons (ORNs) converge on specific glomeruli for further olfactory discrimination. We examined the morphological and physiological characteristics of male-specific glomeruli with regard to ORN excitation by different pheromone components in male *H. zea* and *H. subflexa* in order to delineate the glomeruli in the antennal lobe to which each type of ORN projects its axons. For this research we used the cut-tip single sensillum recording technique followed by cobalt staining to visualize glomerular targets of physiologically identified ORNs. ORNs specifically responding to each component projected consistently to specific glomeruli in the olfactory lobe. Calcium imaging of component-specific glomerular activities in both species corroborated the cobalt stainings. We also described for the first time a distinct glomerular complex (posterior complex) in both species whose specific glomeruli are the arborization destinations of secondary ORNs compartmentalized with specific pheromone-sensitive ORNs.

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Poster: Central Olfaction and Chemical Ecology

Olfactory Shifts Parallel Superspecialism for Toxic Fruit in a *Drosophila melanogaster* Sibling, *D. Sechellia*

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Olfaction in the fruitfly *Drosophila melanogaster* is increasingly understood, from ligand–receptor–neuron combinations, to their axonal projection patterns into the antennal lobe. *Drosophila* thus offers an excellent opportunity to study the evolutionary and ecological dynamics of olfactory systems. We compared the structure and function of the generalist *D. melanogaster* with that of specialist *D. sechellia*, which oviposits exclusively on morinda fruit. Our anal-

yses show that, whereas the fruit's headspace was dominated by acids, antennae responded most strongly to hexanoates. *Drosophila sechellia* exhibited an extraordinary strong response to methyl hexanoate (MeHex). Behaviorally *D. sechellia* was much more attracted to these morinda fruit volatiles than *D. melanogaster*. The high sensitivity to MeHex was paralleled by a 2.5 to 3× overrepresentation of MeHex neurons on the antenna and a concordant 2.9 × increase in volume of the corresponding glomerulus as compared to *D. melanogaster*. In addition, the MeHex neuron exhibited an extreme sensitivity down to femtograms of its ligand. In contrast, no peripherally-mediated shift was found paralleling *D. sechellia*'s increased attraction to acids. These findings are a demonstration of evolution acting at several levels in the olfactory circuitry in mediating a fruitfly's unique preference for fruit toxic to its sibling species.

Poster: Central Olfaction and Chemical Ecology

Relevance of Age, Sex, and Odor on the Foraging Capabilities of *Manduca sexta*

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Foraging behavior of nectarivorous hawkmoths typically involves brief bouts of floral visitation. During these times, it is possible for the animal to take up relatively large quantities of nectar with respect to body weight. We are interested in the variation between the volume that the animals are capable of imbibing when immobilized versus that which they will freely take up during flight. Additionally, we are interested in the role that odor plays in the quantity of nectar imbibed as well as the relevance of sex and age. Naive *Manduca sexta* of both sexes and from 1 to 5 days post-eclosion in age, were immobilized by clamping the wings and force-fed by manually extending the proboscis into a 25% sucrose solution. The mass of the solution in addition to the masses of the moths was determined before and after each session so as to obtain the total volume ingested. Hand-feedings were conducted with and without the presence of bergamot oil. Another cohort of moths was released, individually, into a flight cage containing mock flowers. Each mock flower contained an eppendorf tube, into which one ml of 25% sucrose solution was pipetted. All moths and tubes containing the solution were weighed before each session. Subsequent to the flight, the moths and the tubes of the flowers visited were weighed. As in the hand-feedings, flights were carried out with and without the presence of bergamot oil. At one day following eclosion, hand-fed males ingested greater volumes on average than females. By the end of the second day quantities imbibed were more uniform between the two sexes.

Poster: Central Olfaction and Chemical Ecology

Chemicals Released by Injured or Disturbed Conspecifics Mediate Defensive Behaviors via the Aesthetasc Pathway in the Spiny Lobster *Panulirus argus*

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Chemicals released by either injured or disturbed individuals induce from conspecifics a repertoire of defensive behaviors similar

to when attacked by predators. This has been demonstrated for many species, including crustaceans such as hermit crabs and crayfish. In this study, we demonstrate that the spiny lobster *Panulirus argus* also has chemically induced alarm responses, and furthermore demonstrate the site of release and site of reception of the alarm cues. Alarm cues of spiny lobsters are present in the blood (hemolymph) of injured animals and in the urine of lobsters under simulated predatory attack (disturbed animals). Hemolymph and urine activate defensive behaviors such as moving away from the odor source, defensive movement of the 2nd antennae, shaking, and moving into a shelter, as well as suppressing food-evoked searching. The alarm cues are species specific: Caribbean spiny lobsters (*Panulirus argus*) show much greater alarm response to hemolymph from *P. argus* than to hemolymph from California spiny lobsters (*Panulirus interruptus*) or blue crabs (*Callinectes sapidus*). Removal of the aesthetascs lead to reversal of behaviors toward hemolymph and urine, switching from defensive to attractive behaviors. In conclusion, we have identified the types of defensive behaviors produced by, the source of, and site of reception of the alarm cues in spiny lobsters. Future goals include identifying the molecules involved, the cellular specificity of the responsive olfactory neurons, and the ecological context of the alarm response.

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Poster: Central Olfaction and Chemical Ecology

Evidence that a Volatile Molecule, 3-Decanol, in Hermit Crab Blood Signals Shell Availability to Conspecifics

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Hermit crabs in poor fitting shells are attracted to and obtain gastropod shells at gastropod and hermit crab predation sites. Hermit crab hemolymph attracts conspecifics within minutes. We hypothesized that aquatic hermit crabs might use volatile molecules in hemolymph as do land hermit crabs. Volatiles from, sea water, hemolymph of hermit crabs *Clibanarius vittatus* and *Pagurus pollicaris* and several brachyuran crabs were purged from a water-hemolymph solution, trapped in seawater and tested for induction of shell-investigation behavior in shell investigation bioassays. Volatiles extracted from crustacean hemolymph by headspace solid-phase microextraction (SPME) were analysed by coupled gas-chromatography mass-spectrometry (GC-MS). Two alcohols, 3-decanol, present in *C. vittatus* hemolymph, and 2-ethyl-1-hexanol, present in hemolymph of all tested crustacean species, were tested in shell investigation bioassays. 3-Decanol stimulated shell-investigation behavior in conspecifics, while 2-ethyl-1-hexanol did not. The response of *C. vittatus* to this volatile water soluble molecules supports the hypothesis that detection and response to volatile cues evolved before the transition of hermit crabs to land.

Poster: Central Olfaction and Chemical Ecology

N-Acetylglucosamino-1,5-Lactone Is a Candidate Sex Pheromone in Female Blue Crabs

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Premolt female blue crabs (*Callinectes sapidus*) release a sex pheromone in their urine. Males detect this pheromone using antennular sensors, resulting in mating behaviors that include precopulatory display and grabbing and guarding females. Male crabs also release a sex pheromone that attracts premolt females. The molecular identity of these pheromones remains unknown. The goal of our study is to identify these molecules using bioassay guided fractionation and analysis of differences in the composition of male and female urine. In search of sex pheromones, we found differences in the chemical composition of male and female urine using NMR spectral analysis. One compound that is specific to premolt females was purified by ultrafiltration and HPLC. NMR spectral analysis led to the characterization of this candidate female sex pheromone as *N*-acetylglucosamino-1,5-lactone (NAGL), which is more abundant in urine of premolt females than in urine of either intermolt, premolt, or postmolt males or intermolt or postmolt females. NAGL, which is an oxidized form of *N*-acetylglucosamine, has never before been reported as a natural product, although synthetic NAGL has been reported to inhibit chitobiase of bacteria and vertebrates, which degrades chitin. Preliminary behavioral experiments demonstrated that male crabs can detect NAGL. Further behavioral testing is planned to evaluate the possible role of NAGL and other minor metabolites as sex pheromones.

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Poster: Central Olfaction and Chemical Ecology

Isolation and Structure Elucidation of the Sea Lamprey Migratory Pheromone

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The sea lamprey (*Petromyzon marinus*) is one of the most ancient vertebrates and has a well-developed olfactory system. It begins life in freshwater streams, which it then leaves to parasitize other fishes before eventually maturing and returning to spawn. Migratory lamprey recognizes suitable spawning streams using a pheromone produced by stream-resident, conspecific larvae. The pheromone contains two new and one known [the lamprey bile acid, petromyzonol sulfate (PS)] active components. Here we report the structure elucidation of the new compounds and aspects of their biological activity. The most potent, petromyzonamine disulfate (PADS), is a novel disulfated aminosterol structurally related to squalamine, an antibiotic produced by dogfish shark. Lampreys were attracted to isolated (or synthetic) PADS at concentrations down to 10⁻¹³ M. The second new component, petromyzosterol disulfate (PSDS, attractive at 10⁻¹² M), was found to be a sulfated ergostadienetriol derivative. The biological activity of various mixtures of PADS, PSDS, and PS confirmed that they synergize one another and comprise the majority of the cue. Use of synthetic pheromone components

is planned for controlling populations in the Great Lakes, where the sea lamprey is an invasive pest. This multi-component cue is the first migratory pheromone to be identified in a fish.

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Poster: Central Olfaction and Chemical Ecology

Release, Detection, Discrimination, and Associative Learning of Conspecific Bile Acids by Migratory Rainbow Trout (*Oncorhynchus mykiss kamloops*)

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Although it is well established that Pacific salmon (*Oncorhynchus* spp.) locate home streams for spawning using odorous cues they learn as juveniles, the identities of these cues are as yet unknown. It has been speculated that odors from both abiotic and biotic stream contents, including the odor of juvenile conspecifics (and perhaps other fishes) could be part of imprinted home stream odor. If so, bile acids, a class of distinctive steroids released by many fishes (and used as a migratory pheromone by the sea lamprey), could be part of the odor salmon recognize. Here we tested this possibility using a migratory strain of rainbow trout (*Oncorhynchus mykiss*). Chemical analysis revealed that trout release a distinctive mixture of three bile acids: taurocholic acid, taurochenodeoxycholic acid, and cyprinol sulfate. EOG and multi-unit olfactory nerve recording showed that the trout peripheral olfactory system detects these and three other bile acids in dose-dependent manners down to concentrations of 10^{-11} M. Binary mixture experiments also showed that these bile acids activate different sets of olfactory receptors. Finally, ongoing classical conditioning is demonstrating that trout can learn to discern and remember individual bile acids for extended periods of time. In conclusion, bile acids appear to have all the characteristics required for them to be part of natural homestream odor. Supported by Minnesota Sea Grant.

Poster: Central Olfaction and Chemical Ecology

Putative Steroidal Pheromones: Synthesis Sites and Olfactory Epithelial Responses in the Round Goby (*Neogobius melanostomus*)

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Pheromone communication may be important for the reproductive success of the round goby (*Neogobius melanostomus*), an invasive fish species in the Great Lakes. The signaling molecules released by reproductive males to attract gravid females may include steroidal compounds synthesized by the testes and accessory reproductive glands. Our previous studies have shown that the testes produce steroids that are potent stimulants of olfactory sensory activity in female round gobies. In this study, we investigate the role of ac-

cessory gonadal glands in the production of steroidal putative pheromones in male round gobies. The base of each testis is attached to a seminal vesicle, and a mesorchial region with a prominent fold is located between the two testes. A separate glandular mass is at the base of the testes, adjacent to the common sperm duct. The accessory reproductive glands with steroidogenic cells were identified, by histological and histochemical procedures. Immunocytochemistry against 11 beta-hydroxysteroid dehydrogenase indicates an abundance of steroidogenic Leydig-like cells interstitial to the columnar epithelium in the mesorchial gland, and sparser immunoreactive cells in the seminal vesicles. Incubation of the seminal vesicles with steroidal metabolic precursors, yielded steroids that stimulated electro-olfactogram responses when presented at picomolar concentrations. These results are indicative of a pheromone producing function for the accessory gonadal glands in male round gobies.

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Poster: Central Olfaction and Chemical Ecology

Crested Auklet Odor Is Indicative of a Feather Ornament

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Crested auklets (*Aethia cristatella*) produce a citrusy, social chemosignal that is linked to a seasonal display (the “ruff-sniff”). To test whether scent exhibited patterns indicative of a secondary sexual ornament, we examined seasonal changes in odor at two locations on the body (the ruff, where displays are focused, and the rump). We made two predictions: 1) Odor of ruff feathers would be greatest early in the summer breeding season, but drop by the end of the season. 2) The ruff would be more scented than the rump early in the season. Three key aldehydes of auklet scent (octanal, decanal, and *cis*-4-decanal) were analyzed via solid phase microextraction (SPME) and gas chromatography/mass spectrometry (GC/MS). All compounds were more concentrated in the ruff at start of the season (June) than at the end (August; median odor loss: octanal = 32%, decanal = 54%; *cis*-4-decanal = 72%; $9 \leq n \leq 17$; $-2.53 \leq Z \leq -1.78$; $0.006 \leq P$ (one-tailed) ≤ 0.04). Rump feathers did not exhibit a striking odor-loss pattern ($7 \leq n \leq 14$; $-1.38 \leq Z \leq 0.48$; $0.09 \leq P$ (one-tailed) ≤ 0.45). Concentrations of two compounds (octanal and decanal) were greater in ruff feathers early in the season than the rump ($6 \leq n \leq 10$; $2.24 \leq t \leq 2.85$; $13.3 \leq df \leq 14.0$, $0.013 \leq P$ (one-tailed) ≤ 0.022). Combined, our results are consistent with odor acting as an olfactory ornament. Odor is greatest early in the season, when ruff-sniff displays are emphasized. At this same time, two of three odor compounds are also concentrated in the ruff, which is the focus of displays.

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Poster: Central Olfaction and Chemical Ecology

Response of a Tangerine-Scented Seabird to Odor and Visual Cues

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Crested Auklets (*Aethia cristatella*) are arctic seabirds that use both seasonally elevated scent and visual ornaments during social communication. We explored the relative importance of odor and visual signals in a non-social context. We trained captive birds to touch the tip of their beaks to a Y-shaped stick for a food reward, a behavioral task known as “targeting.” The Y-stick gave birds a simultaneous choice between an experimental and control stimulus. Experimental stimuli included (1) synthetic Crested Auklet odor, (2) a visual (color) stimulus only, and (3) auklet odor plus the visual stimulus. Birds learned to associate food rewards with each type of experimental stimulus (odor only: $Z = 439.50$, $df = 44$, $P < 0.0001$; visual only: $Z = 105.00$, $df = 19$, $P = 0.0001$; visual + odor: $Z = 76.50$, $df = 16$, $P = 0.0001$). However, the presence of a visual cue greatly enhanced targeting accuracy by 47% to 56% over the odor stimulus alone ($14.0 \leq Z \leq 16.0$; $df = 7$; $0.016 \leq P \leq 0.023$). Our data indicate: (1) crested Auklets are capable of odor learning in non-social contexts, and (2) learning is facilitated with visual stimuli. Such a pattern is consistent with field tests of auklet odor, which indicate an emphasis on visual ornaments. Odor appears to exhibit a synergistic relationship with visual displays. We are currently examining whether variation in odor learning corresponds to an individual’s social rank, and whether sensitivity to odor varies seasonally or by sex.

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Poster: Central Olfaction and Chemical Ecology

Reconstitution of a Chemical Defense Signaling Pathway in a Heterologous System

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Because marine sponges are sessile and cannot physically escape predators, many contain chemical defense compounds that deter predation by reef fishes; however, it is unknown how these fish physiologically detect these chemicals. The objective of this study was to determine if a signaling pathway for chemical defense compounds could be reconstituted in a heterologous expression system. Zebrafish (*Danio rerio*) rejected foods laced with some sponge chemical defense compounds, including scorpionin, previously shown to deter a generalist marine predator (*Thalassoma bifasciatum*). Therefore, clones from a whole zebrafish cDNA library were expressed in *Xenopus* oocytes. The library-expressing oocytes were tested by electrophysiological methods, using the CFTR chloride channel and the endogenous calcium-activated channel as reporters for chemoreceptor activation. Control experiments showed that CFTR activity can be a good indicator of OR-17 receptor activation in oocytes injected with cRNA for that receptor. Octanal and isoproterenol both activated currents in oocytes co-expressing the zebrafish library and CFTR. Furthermore, oocytes expressing library and CFTR, but not uninjected oocytes, showed an electrophysiological response to scorpionin. Therefore, we conclude that zebrafish can detect marine sponge-derived chemical defense compounds, and oocytes can reconstitute the scorpionin-activated signaling pathway.

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Poster: Central Olfaction and Chemical Ecology

Genetic Model of High Responsiveness to Predator Odor

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Risk of predation may significantly affect the behavior of potential prey. Chemosensory detection may be an important aspect of predator avoidance strategy for many mammals. New genetic model has been developed using rats of heterogeneous laboratory population. The phenotype of interest is high responsiveness to predator odor. 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. During 8 years (1997–2005), we selected rats of heterogeneous laboratory population for high embryo resorption rate (over 20%, H-line) and accordingly for low resorption rate (less than 10%, L-line) under predator odor exposures. Currently we have 14th generation of rats of H-line. Rats of H-line have significantly ($P < 0.001$) higher percent of females with 100% resorption rate of embryos under predator odor exposures relative to rats of L-line and rats of heterogeneous population. Also there are significant ($P < 0.001$) differences in litter size between animals of H and L line under predator odor exposures. Developed genetic model may be a useful tool for chemical analysis of predator chemical signals.

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Poster: Central Olfaction and Chemical Ecology

The Role of the Vomeronasal Organ in Alarm Pheromone Perception

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We previously reported that alarm pheromone in male Wistar rats was released from their perianal region and aggravated stress-induced hyperthermia, known as the indices of animal’s anxiety status, in pheromone recipient rats. In addition, we found that this pheromone could be trapped in water. Although alarm pheromone had increased Fos expression in the accessory olfactory bulb of recipients, we still have no clear evidence whether this pheromone is perceived by the main olfactory system or by the vomeronasal system. In the present study, we assessed this issue by exposing alarm pheromone to the three types of the recipients, i.e., intact male, vomeronasal organ excised (VNX) male and VNX-sham male. As was done in our previous studies, alarm pheromone-containing water was prepared by the electrical stimulation to the perianal region of an anesthetized donor rat in a small box containing water droplets on the ceiling. The odor released from neck region of the donor was used as control odor. After the water collection, the filter papers containing one type of the water sample were placed on the

wall of the recipient's home cage, and autonomic responses were monitored for the subsequent 30 min. Alarm pheromone significantly aggravated stress-induced hyperthermia in intact and VNX-sham ($P < 0.05$, ANOVA) recipient as compared to those seen in control odor and vehicle control groups. However, the VNX recipient did not show this autonomic response to alarm pheromone. These results strongly suggest that alarm pheromone in male rats is perceived by the vomeronasal organ.

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Slide: Peripheral Olfaction

Context-Dependent Modulation of Olfactory Epithelial Activity by the Terminal Nerve in Axolotls (*Ambystoma mexicanum*)

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Activity in the vertebrate olfactory epithelium appears to be modulated by peptides released from the terminal nerve, which contains GnRH as well as another peptide that displays NPY-like immunoreactivity. In previous studies we have shown that GnRH modulates odorant responsiveness in the olfactory epithelium and Na^+ and K^+ currents in olfactory receptor neurons. To determine whether NPY also exerts modulatory effects, we obtained synthetic axolotl NPY for use in physiological experiments. NPY is involved in many activities in the central nervous system, but most attention has focused on its role in regulating appetite and hunger. We therefore examined the effects of NPY on the olfactory epithelium in axolotls that were fed either 1 or 10 days prior to testing ("well-fed" and "hungry", respectively). Using electro-olfactogram recordings, we found that bath application of 1 μM NPY increased the magnitude of odorant responses elicited by L-glutamic acid (100 μl at 10 mM) in hungry animals but not in well-fed animals. Using whole-cell recordings from olfactory receptor neurons in epithelial slices, we found that bath application of 0.1–1 μM NPY resulted in an increase in the magnitude of the TTX-sensitive Na^+ current in more than 50% of olfactory receptor cells in hungry axolotls, but none in well-fed animals. Taken together with our previous results indicating that the effects of GnRH vary across the breeding season, these data suggest that modulation of activity in the olfactory epithelium by the terminal nerve depends on the animal's physiological context.

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Slide: Peripheral Olfaction

Roles of TRPM5 in Mouse Olfactory Signal Transduction

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Previously, we have reported responsiveness to putative pheromones in mice defective for the cyclic nucleotide-gated channel (CNGA2) and expression of TRPM5, a transient receptor potential

channel, in olfactory sensory neurons (OSNs). In the present study, we characterized the role of TRPM5 in signal transduction by immunolabeling and electro-olfactogram (EOG) recordings in knockout (KO) mice. In contrast to normal mice, where inhibitors of the cAMP signaling pathway suppressed putative pheromone-evoked EOG responses differentially from responses to other odorants, responses to both types of odorants were similarly inhibited in TRPM5 KO mice. Using Fos protein expression as a measure of odor-elicited activity, we find that putative pheromones and urine activated some but not all glomeruli receiving input from TRPM5-expressing OSNs. Odor-evoked Fos expression in bulbs was reduced significantly in double KO mice lacking both CNGA2 and TRPM5. Unexpectedly, profound abnormalities were observed in the double KOs as compared to CNGA2 or TRPM5 single KOs, which include smaller bulb and glomerular size, depletion of mature OSNs and disappearance of glomeruli in discrete regions of the bulb. These data suggest TRPM5 is important for olfactory signal transduction and for activity-dependent survival of olfactory neurons and maintenance of the glomeruli.

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Slide: Peripheral Olfaction

The Role of the Transcription Factor OAZ in ORN Development

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The generation of mature olfactory receptor neurons (ORNs) requires complex regulation by several classes of transcription factors. Previous studies have implicated the O/E family of transcription factors in the regulation of genes essential for olfactory function (ACIII, Golf, CNGCs, and ORs). The multiple zinc finger transcription factor OAZ (O/E1 associated zinc finger protein) interacts with all of the O/E family members and is preferentially expressed in immature neurons where it is proposed to block O/E function. We have used genetic mouse models to explore the role of OAZ in ORN development. In OAZ-null mice, the projection of ORN axons to the dorsal olfactory bulb was severely impaired. Examination of individual glomeruli showed poor convergence and a ventral shift. To test the hypothesis that OAZ functions as an O/E inhibitor in early ORN differentiation, we created "gain-of-function" mutant mice by overexpressing OAZ using the O/E3 promoter. When expression of OAZ was maintained in the differentiating cells, ORN maturation was arrested at a differentiation stage consistent with the first expression of ORs, and nearly all projections to the olfactory bulb were abolished. This study demonstrates that OAZ functions as a molecular switch in ORN development, mediating the transition from differentiation to maturation phenotype.

Slide: Peripheral Olfaction

The WNT2 and WNT5 Genes Regulate Different Steps in Olfactory Map Development

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The molecular mechanisms regulating the precise arrangement of glomeruli in the olfactory map are poorly understood. Our long-term goals are to identify the molecules and elucidate their functions. We recently found that two members of the Wnt family of secreted proteins are necessary for the precise anatomy of the *Drosophila antennal lobes* (ALs). Mutation in *wnt5* severely disrupts the arrangement of glomeruli in the fly ALs. The derailed (*drl*) transmembrane receptor tyrosine kinase has been proposed to act as Wnt5 receptor. Mutation in *drl* also severely alters glomerular pattern. Loss of *drl* functions leads to enhancement of *wnt5* activity, indicating that *wnt5* and *drl* functions together in patterning the fly olfactory map. In the double mutant, *wnt5* is epistatic over *drl*, indicating that *wnt5* functions downstream of *drl*. Cell-type specific genetic rescue showed that *wnt5* acts in the olfactory sensory neurons while *drl* acts in glial cells. We propose that olfactory neurons express *wnt5* which patterns the olfactory map by regulating the development of glial cells. We recently also observed that *wnt2* is necessary for AL development. Unlike the glomeruli of the *wnt5* mutant, which are relatively normal, those of *wnt2* are indistinct and misshapen, indicating that *wnt2* functions in glomerular establishment rather than patterning. Our preliminary analyses indicate that the dendritic trees of the projection neurons in the *wnt2* mutant fail to coalesce into distinct glomerular structures. In summary, we found that two secreted proteins, Wnt2 and Wnt5, functions at different steps to direct the formation and patterning of glomeruli in the fly ALs.

Slide: Central Olfaction

Glomerular Computations in the Olfactory Bulb Can Normalize Neural Activation Patterns

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Increasing the concentration of most odorants elevates response intensity and activates increasing numbers of glomeruli in the olfactory bulb. Given this, one might predict (1) that the identity of the perceived odor would be altered due to the newly activated glomeruli and (2) that it would be more difficult to discriminate between closely related odorants due to the greater overlap between their responses. In fact, most odorants do not change in quality with increasing concentration, and higher concentration odors are easier, not harder, to discriminate. Notably, when glomerular activation data are normalized with respect to the overall level of activity, odor-specific glomerular activity patterns remain relatively invariant with increasing concentration. Furthermore, the presence of feedforward inhibitory circuits within glomeruli, coupled with the fact that mitral cell responses to increasing odor concentrations do not reflect the monotonic increases in activity observed in glomeruli, suggests that activity normalization does occur between the glomerular and mitral cell responses. We show here that glomerular neural networks in the olfactory bulb can perform the computations necessary to normalize patterns evoked by odorants at different

concentrations. Consequently, activation patterns at the output of the olfactory bulb, conveyed by mitral cell spiking, would be better able to preserve odor quality information across concentrations.

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Slide: Central Olfaction

Olfactory Bulb Glomerular Activity Patterns as a Basis for Odorant Quality Coding: Predicting Perceptual Behavior from 2-DG Functional Maps

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We tested whether odorant-evoked spatial activity patterns at the level of the olfactory bulb could be the neural code underlying odorant quality perception. Using operant techniques, rats were trained to differentially identify the odorants santalol, n-propanol, -pinene, acetone, and pentadecane. The animals were tested using a 5 × 5 odorant confusion matrix task, and the results of the psychophysical tests were used to measure the degree of perceptual dissimilarity between odorant pairs. By way of multidimensional scaling analysis (MDS), the dissimilarity measures yielded a two-dimensional perceptual odorant space for each test animal. Likewise, using 2-DG activity maps of the rat olfactory bulb in response to the same odorants, an MDS analysis of the similarity measures between all possible pairs of glomerular patterns yielded a two-dimensional odorant space based on functional activity. Formal statistical analysis demonstrated a highly significant predictive relationship between the similarity of an odorant's glomerular activity pattern and the perceptual relationship among the odorants ($R^2 = 0.434$; $F_{1,18} = 16.54$; $P = \text{nil}$) that was homogeneous across animals ($F_{5,18} = 0.899$; $P > 0.5$). The successful prediction of the perceptual relationship between odorants based on the similarity in their respective glomerular activity patterns gives strong support to the hypothesis that odorant identity is based on a spatial code at the level of the olfactory bulb.

Slide: Central Olfaction

Development of Functional Odor Maps in the Rodent Olfactory Bulb

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Olfactory sensory axons expressing a particular odorant receptor project to about two glomeruli in each olfactory bulb in adult mice. At birth, the projections are more promiscuous and converge to the adult pattern with a time course that can be affected by sensory experience. To examine whether functional odor maps reflect these anatomical changes during development, we imaged odor-evoked responses in gene-targeted mice expressing the presynaptic reporter synaptopHluorin in olfactory sensory neurons. Using a panel of ~100 odors, we could reliably identify individual glomeruli across different animals based on their functional signatures. Most of the nearly 80 responsive glomeruli per hemisphere were functionally

unique. On average, each functionally unique glomerulus was represented 1.28 ± 0.09 times on each dorsal surface. This matches the number of glomeruli per odorant receptor estimated from gene-targeted mice, supporting the assumption that each functionally unique glomerulus corresponds to an individual receptor. Functional odor maps were present even in 1-week old animals. At 2 weeks, glomerular maps were very similar to those in adults – individual glomeruli did not have more promiscuous responses to odors and individual odors did not evoke responses in more glomeruli. Similar results were obtained in rats using intrinsic optical signals. Our results indicate that precise functional maps develop early in mice and rats. Preliminary experiments have indicated that functional odor maps are relatively unaltered if sensory experience is altered by unilateral naris occlusion.

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Slide: Central Olfaction

Relationship between Sniffing and Odor Representations Imaged from the Olfactory Bulb of Awake Rats

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Rodents actively explore their olfactory environment by sniffing, which modulates the flow of odorants across the nasal epithelium. We are investigating the relationship between sniffing and odor representations in the olfactory bulb using optical imaging of pre-synaptic calcium influx in awake, head-fixed rats. Rats learned a two-odor, go-no-go discrimination task. Olfactory receptor neurons were loaded with calcium-sensitive dye and a chronic imaging window installed over the dorsal olfactory bulb. Odorant-evoked calcium signals were imaged across the bulb during odor discriminations while sniffing was measured. Rats typically discriminated after a single sniff. Sniffing varied in amplitude, waveform, and frequency. Response amplitude was not correlated with sniff amplitude but was highly correlated with sniff interval. Most sniffing occurred at 1–2 Hz, but increased to >4 Hz when rats were presented with a novel odorant. During slow sniffing, glomerular input was tightly synchronized to inhalation, with a latency of ~200 ms and risetime of ~100 ms. During fast sniffing, phasic input following each sniff was attenuated and instead appeared dominated by tonic input. Glomerular response maps were temporally dynamic, changing over the course of a single sniff as well as across sniffs. Surprisingly, one cause of this variation was the failure of some glomeruli to respond to certain sniffs during an odor presentation. These data suggest that the parameters of odor sampling can shape both spatial and temporal representations of odor information in the olfactory bulb.

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Slide: Central Olfaction

Adrenergic Enhancement of GABA Inhibitory Transmission in the Olfactory Bulb

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Noradrenergic modulation of dendrodendritic synapses between the mitral and granule cells in the olfactory bulb is postulated to play a key role in the formation of memory in olfactory mediated behaviors. Current models propose that noradrenaline (NA) increases excitation of mitral/tufted cells (M/TCs) by decreasing the release of GABA from granule cells. Here, in recordings from AOB slices, we show that NA decreases the firing frequency of M/TCs in cell-attached patch and in current-clamp recordings. This effect is due to an increase in the GABA inhibitory input to M/TCs. Application of NA (10 μ M) produced a ~20-fold increase in the frequency of GABA induced miniature inhibitory postsynaptic currents (mIPSCs) without changing their amplitude. A pharmacological analysis indicated that the increase in mIPSCs frequency results from activation of α 1-adrenergic receptors. We have found a similar increase mIPSC frequency in the main olfactory bulb. Taken together, our results suggest that NA increases the release of GABA from granule cells by acting on presynaptic receptors. Thus, the role of the noradrenergic activity in the olfactory bulb may be more complex than previously suggested.

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Symposium: Approaching Taste and Olfaction at the Systems Level

The Integration of Multiple Sensory Modalities and the Creation of Flavor

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The central neural creation of flavor from stimulation originating within the upper airways represents what is arguably the single most profoundly multi-modal sensory integration of which the brain is capable. Inputs from the upper airways reflect taste (salt, sweet, bitter, sour and savory), olfaction (and its myriad qualities), static tactile sensations (touch, pressure, stretch), dynamic tactile sensations (vibration, astringency, creaminess, viscosity, coating), thermal sensations (warm, cool, hot, cold), nociception (stinging, burning, prickling, itching), proprioception (bolus texture, resistance, chewiness, brittleness, crunchiness), and auditory input (via the sounds arising in the oral cavity and bone conduction when foods are manipulated and chewed). Flavor may be conceived of or defined as the congruent integration of all these inputs into a single perceptual gestalt that is projected to originate within the mouth. Some of these different physical inputs may interact at the receptor cell or primary afferent level such as thermal-taste or tactile-taste interactions. Higher in the CNS there are brain areas that appear to process and relay inputs from all of these modalities such as the insula/operculum, orbitofrontal cortex, and amygdala. How these diverse systems are integrated, under what conditions, and the role of attention and learning in these pathways are the focus of an ever-growing and fascinating body of research.

Symposium: Approaching Taste and Olfaction at the Systems Level

Learning to Smell: Cortical Plasticity and Odor Perception

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Olfactory perception involves at least two distinct processes. First, most odors are composed of several to hundreds of volatile molecules. However, under most conditions odor perception is synthetic, with limited access to the underlying features of complex mixtures. Thus, multi-component odorant mixtures can be perceived as unique odor objects through experience-dependent mechanisms hypothesized to be similar to object perception in vision. Second, odors are almost invariably experienced against odorous backgrounds from which the foreground odor must be extracted from the background through an analytical (as opposed to synthetic) process (figure-ground separation). Work in our lab has been examining piriform cortical contributions to both of these processes. Here I will focus on the process of olfactory figure-ground separation. Neurons within the piriform cortex show rapid, odor-specific adaptation, despite relatively maintained input from olfactory bulb mitral/tufted cells. This cortical adaptation is mediated by pre-synaptic metabotropic glutamate receptors that induce an activity-dependent depression of afferent synapses. Pharmacological manipulations show that cortical adaptation contributes to background odor adaptation and short-term behavioral odor habituation. Behaviorally, rats are able to filter background odors and identify a target odor presented against that background. Similarly, piriform cortical neurons adapt to background odors, and respond to novel target odors presented against that background as if the target odors were presented alone. These findings present a specific cortical mechanism to allow perception of odors in odorous backgrounds.

Supported by NIH and NSF.

Symposium: Approaching Taste and Olfaction at the Systems Level

Neural Population Coding of Satiety States

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Voluntary feeding involves behavioral states associated with meal initiation (hunger) and termination (satiety). In this activity multiple brain regions act in concert to regulate the onset of these behaviors. Previous electrophysiological investigations revealed that single neurons located in primate brain areas such as lateral hypothalamus (LH) and orbital frontal cortex (OFC) decrease their firing rate levels as animals transition from hunger to satiety. Similar responses were observed in human functional neuroimaging studies. We will present recent data obtained from hungry rats that have bundles of microelectrodes implanted in their LH, OFC, insular cortex (IC) and amygdala (AM) that freely lick to satiety. These data show that single units mostly encode for specific hunger states within a feeding cycle (hunger-satiety-hunger), while neuronal population activity reflects the overall motivational (hunger/satiety) state across several cycles by combining information from its constituent units. This population code seems to be distributed across LH-IC-OFC-AM circuits of both lean and obese/diabetic rats. We suggest that this distributed code underlies the control of voluntary feeding behavior under different metabolic states.

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Symposium: Approaching Taste and Olfaction at the Systems Level

Hedonic Aspects of Chemical Stimuli: Corticolimbic Circuits that Mediate Reward and Choice

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That chemical stimuli can exert powerful effects on behavior is due both to evolutionary pressures and to learning; i.e., the formation of associations with biologically potent events such as nutrients, fluids, illness and so on. Associations of this kind modify the affective valence and, hence, the preference for specific flavors and tastes but they are also the basis for changes in the hedonic response to these stimuli. Current evidence suggests that this latter aspect is a product of contiguous emotion feedback elicited by the stimulus through a system of sensory-motivational and affective connections, and that it determines the assignment of reward value to a particular stimulus. Thus, for example, shifts in motivational state do not reduce the reward value that animals' assign to taste stimuli until the effect of the shift in state is experienced through direct consummatory contact with the taste. Sensory-specific satiety is a particularly potent means of producing selective changes in the reward value of stimulus events as indexed by changes in the performance of actions that gain access to those events. Evidence from rodents will be presented suggesting that these changes are mediated by a corticolimbic circuits involving particularly connections between gustatory insular cortex, basolateral amygdala and the nucleus accumbens.

Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Regulation of the VNO by a Molecular Clock

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Behavior and physiology coordinated to the 24-h day are maintained through an internal timing mechanism. Time is maintained by a molecular clock driven by recurrent rhythms in RNA and protein levels through transcriptional feedback loops of "clock genes". Rhythmic expression of clock genes in peripheral tissues drives local rhythms. The rhythmic expression of clock genes and their role in the vomeronasal organ (VNO) are examined in this study. Initially we identified the expression profile of *Per1* and *Per2* in the VNO and found that these clock proteins are expressed by vomeronasal sensory neurons. We examined their temporal expression profile, as well as the clock gene *Bmal1*, using real time PCR. The rhythmic expression of *Bmal1* mRNA occurred with a nadir in the early evening or subjective night and a peak in the early morning or subjective day. In an opposing rhythm the expression of *Per1* and *Per2* mRNA occurred with a nadir in the early morning or subjective day and a peak in the early evening or subjective night. The rhythmic expression of clock genes identifies the VNO as the locus of a peripheral clock. We further evaluated the effect of a peripheral clock on pheromone sensitivity. A urine preference assay revealed a diurnal

rhythm in pheromone sensitivity. These results indicate the importance of VNO oscillators in mediating daily rhythms in pheromone detection and in behavior.

Support contributed by: NIDCD.

Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Attenuation of the Production of Inositol 1,4,5 Trisphosphate in the Vomeronasal Organ by Antibodies Against the α Q/11 Subfamily of G-Proteins

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The social and reproductive behaviors of most mammals are modulated by pheromones, which are perceived by the vomeronasal organ. Vomeronasal transduction in vertebrates is activated through G protein coupled receptors, which in turn leads to the generation of IP₃ and DAG by the activity of phospholipase C. DAG has been shown to gate the transient receptor potential channel 2 (TRPC2) which allows the subsequent increase of calcium. The role of IP₃ in this transduction cascade and the G protein that is activated in the process has yet to be determined. To investigate the role of the alpha subunits of G_o, G_{i2} and G_{q/11} in the transduction process, microvillar membranes from female VNO were pre-incubated with selective C-terminal peptide antibodies and then stimulated with adult male urine. Incubation of VNO membranes with antibodies against G_{q/11} blocked the production of IP₃ in a dose dependent manner. Furthermore pre-incubation with antibodies against G_o also significantly impaired the production of IP₃ whereas the use of G_{i2} antibodies did not impair the production of IP₃. Thus our observations indicate that the alpha subunits of G_{q/11} and G_o play a role in pheromonal signaling in the VNO.

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Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Arachidonic Acid Plays a Role in the Odor Responses of Mouse Vomeronasal Neurons

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Mouse vomeronasal neurons detect pheromones, as well as some general odorants. The odor responses appear to be transduced through G protein coupled receptors linked to PLC cascade. The activation of PLC causes a Ca²⁺ influx that depolarizes the cells. It was recently proposed that DAG activates a Ca²⁺-permeable channel, TRPC2. However, DAG can be hydrolyzed to arachidonic acid (AA) by DAG lipase. We found that AA also plays a role in odor responses. We used a mixture of male and female urine to stimulate odor responses in isolated vomeronasal neurons. With perforated patch clamp recordings (gramicidin), application of diluted urine induced an inward Ca²⁺ current (-80 mV). Only part of the current was decreased by a DAG lipase inhibitor, indicating that DAG is not the only second messenger that activates odor responses and AA might play a role. To test this, TRPC2 inhibitors were used.

The inhibitors did not completely eliminate the odor-induced inward current, supporting a role for AA in odor responses. Direct stimulation with AA also induced a Ca²⁺ transient, which was sensitive to Cd²⁺, but not to TRPC2 inhibitors, suggesting AA activates a channel different from TRPC2. Moreover, the effect of AA could be mimicked by a non-metabolizable AA analogue, oleic acid (OA). Inhibition of an AA metabolic pathway by 12-LOX (increased [AA]_i) activated an inward current. These data suggest the Ca²⁺ channel activation was not caused by AA metabolites. Together, our data indicate that both DAG and AA mediate the excitatory odor responses of vomeronasal neurons, by activating different Ca²⁺ channels.

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Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Female Snake Sex Pheromone Induces Membrane Responses in Vomeronasal Sensory Neurons of Male Snakes

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The vomeronasal organ (VNO) is important for activating accessory olfactory pathways that are involved in sexually dimorphic mating behavior. The vomeronasal system of male garter snakes is critically important for detection of, and response to, female sex pheromones. In the present study, under voltage-clamp conditions, male snake VNO neurons were stimulated with female sexual attractiveness pheromone. Thirty-nine of 139 neurons exhibited inward current responses (reversal potential: 10.6 ± 2.8 mV). The amplitude of the inward current was dose-dependent, and the relationship could be fitted by the Hill equation. Under current-clamp conditions, application of pheromone produced membrane depolarizing responses and increases in firing frequency. These results suggest that the female pheromone directly affects male snake VNO neurons and results in opening of ion channels, thereby converting the pheromone signal to an electrical signal. The response to female pheromone is sexually dimorphic, i.e., the pheromone does not evoke responses in VNO neurons of female snakes. An associated finding of the present study is that the female sex pheromone which is insoluble in aqueous solutions became soluble in the presence of Harderian gland homogenate.

Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Evidence for a Perireceptor Role for Harderian Gland Secretions in Garter Snakes: Delivery of Pheromone Molecules to the Vomeronasal Organ

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One of the few vertebrate pheromones that have been isolated, purified and characterized is the sex pheromone of the female red-sided garter snake (*Thamnophis sirtalis parietalis*). This pheromone, a blend of 13 long-chain (C29–C37) saturated and monounsaturated methyl ketones, is expressed during the mating season and responded to by males with stereotyped courtship behaviors including chin-rubbing, rapid tongue-flicks and caudocephalic body undulations. Due to the high molecular weight and the aliphatic chain length, this nonpolar female sex pheromone is insoluble in aqueous solution. Perception of the pheromone is mediated by receptor neurons of the vomeronasal organ (VNO). These receptor cells are bathed in a fluid originating in the Harderian gland (HG). The question arose: How does this nonpolar lipophilic pheromone access VNO receptor cells? We found that homogenates of HG were effective in solubilizing the pheromone, which allowed us to demonstrate that the pheromone incubated with HG homogenate increased IP3 production in VN sensory epithelial homogenates. Furthermore, HG removal in reproductively active males resulted in a significant reduction in courtship behavior. Following the breeding season, feeding Harderianectomized males were impaired in earthworm attack. These results indicate that HG secretions possess solubilizing and/or pheromone binding properties that are critical for delivery of chemosignals (pheromones and prey cues) to the VNO.

Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Gonadotropin Releasing Hormone Increases Vomeronasal Neuron Response to Male Salamander Pheromone

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Electrophysiological studies have shown that gonadotropin releasing hormone (GnRH) influences chemosensory neurons responses to odors. In the present study we used agmatine uptake as a relative measure of the effects of GnRH on pheromone-induced neural activation of vomeronasal neurons in *Plethodon shermani* salamanders. Whole male pheromone extract containing 3 mM agmatine with or without 10 μ M GnRH was applied to the nasolabial groove of female salamanders. Immunocytochemical procedures, using diaminobenzidine as the chromogen, were conducted to visualize and quantify relative labeling density of activated vomeronasal neurons in 20 micron thick sections of vomeronasal organ. Tissue sections from each experimental group were processed on the same slide to ensure identical labeling procedures. Densitometry data were collected from individual vomeronasal neurons that showed clear labeling of the entire cell. Each value was standardized by dividing the cell body density by background density. Multiple density values from each of three specimens from each group were used to determine inter-group differences. Groups were compared using Student's *t*-test. Vomeronasal neurons exposed to pheromone and

GnRH demonstrated higher density values (average density = 1.79) than neurons exposed to pheromone alone (average density = 1.64; $t = -2.79$, $df = 226$, $P < 0.003$). This result suggests that GnRH increases the response of female vomeronasal neurons to male pheromone.

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Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Synthesized Male Sea Lamprey Pheromone Summons Conspecific Females to Traps

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The sea lamprey (*Petromyzon marinus*) is an ecologically destructive invader of the Laurentian Great Lakes. Our previous studies indicate that sexually mature male sea lampreys release through gills 7 \tilde{N} , 12 \tilde{N} , 24-trihydroxy-5 \tilde{N} -cholan-3-one 24-sulfate (3kPZS), which induces robust and predictable upstream movement in ovulatory female conspecifics. This compound offers a possible system for developing a pheromone-based sea lamprey control, even though vertebrate animals have not been expected to “fly up” the concentration gradient of pheromones like insects. In the present study, synthesized 3kPZS was introduced into a lamprey spawning stream where tagged ovulatory females were acclimated and released. At subpicomolar concentrations, 3kPZS lured more than 50% of females into traps. This compound was effective in guiding females to the exact site of traps over 600 m. Temperature fluctuation appeared to influence the trapping efficiency. We conclude 3kPZS can be developed into a potent pest control agent.

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Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Reflections among Asian Elephants: Chirality Counts

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The two enantiomers or molecular mirror images of the male elephant-temporal gland released pheromone, frontalin (1,5-dimethyl-6,8-dioxabicyclo[3.2.1]octane) are emitted in specific proportions, dependent on the male elephants age and phase of musth. Musth and male maturation are linked, progressively culminating in overall sexual, social, physiological and physical maturity, resulting in a lengthy annual period of musth characterized by heightened sexual activity and intensified aggression. We demonstrate that, compared with young males, older males show a consistent, repeatable pattern of enantiomeric proportions during individual musth episodes and that behavioural reactions by conspecifics are more influenced by the ratio of enantiomers than total frontalin. In addition, frontalin enantiomers, as tightly stable molecules, can be traced as far as a source origin in the blood of musth

elephants and importantly, with their precursor are found in other mammalian tissues, including whales. This argues for a link to fundamental metabolism via the mevalonate shunt, perhaps influenced by steroid hormones. Such is one of the foci of our continuing investigations.

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Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Infusions of Lidocaine in the Accessory Olfactory Bulb (AOB) Reduce Sexual Incentive Motivation in Male Rats

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Olfaction is important for the correct display of sexual behavior. The aim of the present study was to determine if a temporary inactivation of the accessory olfactory bulb (AOB) inhibits sexual incentive motivation and copulatory behavior in male rats. Sexual incentive motivation was evaluated in an arena where incentive animals (a sexually receptive female and a stud male) were located at diagonally opposing corners and confined behind a wire mesh. The parameters registered were: time in and frequency of visits to an area adjacent to the incentives. Subjects were also tested for sexual behavior with receptive females. Subjects were tested twice before being bilaterally implanted with a guide cannulae aimed above the AOB. One week after surgery, the animals were tested again three times in both tests. First without any manipulation then with saline or lidocaine (1 µl of lidocaine at 10%) infused into the AOB in random order. Cannula placements were verified using standard histological procedures. Injections of lidocaine prolonged mount and intromission latencies and reduce the time spent near the receptive female. These results suggest that reduced preference for a receptive female after AOB inactivation can be explained as a consequence of reduced incentive value of the female. However detailed studies are necessary to dissociate between reduce sexual motivation and lack of olfactory integration.

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Poster: Chemosensory Molecular Genetics and VNO/Pheromone

VNO/AOB Function in the Absence of MOB Input from Olfactory Epithelium

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In separate experiments with male mice we determined that: 1. Transport of HRP*WGA from OE to MOB is completely blocked for 5 or more days after syringing nasal epithelium with 50 µl of 5% ZnSO₄. This treatment had no apparent effect on anterograde transport from VNO to AOB. 2. Treatment had little or no effect

on EVOG response to liquid application of mouse urine or 2-heptanone to the VNO. 3. Application of mouse urine to the naris of awake mice induced strong Fos protein expression in glomeruli as well as mitral and granule cell layers in the AOB. 4. Despite this evidence for a functional VNO/AOB system in the absence of OE input to the MOB, treated mice given extensive pre-training in an olfactometer to detect the vapor of ethyl acetate, methyl benzoate, mouse urine and 2-heptanone were anosmic to these odors. 5. Nevertheless, some mice given extensive fighting experience continued to engage in vigorous aggressive behavior after treatment. This is in contrast to the total absence of aggression in olfactory bulbectomized mice. Thus, in the absence of MOB activation, VNO sampling may occur in the presence of appropriate species-specific social signals but does not occur to (or does not support detection of) olfactometric presentations of vapors from known pheromonal and non-pheromonal stimuli.

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Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Volatile, Sex-Specific Urinary Odors Detected by the Main Olfactory Epithelium Augment FOS Expression in the Accessory Olfactory Bulb of Female Mice

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Volatile male urinary odorants elicit distinct patterns of glomerular activation in the main olfactory bulb (MOB) of female mice whereas non-volatile body odorants most reliably activate the mouse accessory olfactory bulb (AOB). We asked whether volatile urinary odors from male vs. female mice differentially activate MOB glomeruli as well as AOB mitral and/or granule cells of female subjects. Using increased Fos immunoreactivity (IR) in juxtglomerular cells as an index of activation, we found that volatile urinary odors from male vs. female mice activated distinct clusters of glomeruli in the ventral portion of the female's MOB. Surprisingly, exposure to volatile urinary odors from male, but not from female, conspecifics also augmented the number of Fos-IR mitral and granule cells in the female's AOB compared with exposure only to clean air. Bilateral lesions of the main olfactory epithelium induced by ZnSO₄ irrigation of the nares eliminated the ability of volatile male urinary odors to stimulate Fos expression in the AOB and the MOB, suggesting that the ability of volatile male odors to activate the female's AOB normally depends on their detection by the main olfactory epithelium as opposed to the vomeronasal organ. Our results suggest that centrifugal inputs from the main olfactory system to the AOB may selectively convey information about opposite-sex conspecifics that facilitates mate recognition and successful reproduction.

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Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Response of Opossum Accessory Olfactory Bulb Neurons to Urine

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In many mammalian species, urine contains pheromones that stimulate investigatory behaviors. Previous work in this laboratory demonstrated that, whereas female opossums do not respond to male opossum urine, male opossums vigorously investigate urine of diestrous females. In this study, we examined the response of accessory olfactory bulb (AOB) mitral cells to urine delivered to the vomeronasal organ (VNO) of male and female opossums using extracellular single unit recordings. Mitral cells of male opossums responded to diestrous female urine with two distinct patterns: excitation followed by inhibition or inhibition. Either pattern could be mimicked by application of GTPγS and blocked by GDP-β-S, indicating that the response of neurons in this pathway is through a G-protein-coupled receptor mechanism. Male urine was ineffective as a stimulus for mitral cells in the AOB of male or female opossums. These results indicate that urine of diestrous females contains a pheromone (or pheromones) that directly stimulates vomeronasal neurons through a G-protein-coupled receptor mechanism and that the response to the urine is sexually dimorphic.

Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Noradrenergic Modulation of Synaptic Transmission from Mitral to Granule Cells in the Accessory Olfactory Bulb

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We have shown that the mitral to granule cell synapse in the accessory olfactory bulb (AOB) is critical site for olfactory learning in mice, in which a female forms a memory to the pheromonal signal of the male that mates with her. The formation of this memory depends on mating-induced release of noradrenaline (NA) in the AOB. In support of this, we have also shown in slice preparations that NA gates long-term potentiation at the mitral to granule cell synapse via the activation of alpha-2 adrenoceptors. Therefore, we investigated the action of NA on synaptic transmission from mitral to granule cells with the use of whole-cell patch-clamp recordings. There are five main observations: (1) NA depressed stimulus-evoked excitatory postsynaptic currents (eEPSCs) recorded from granule cells without affecting the decay of the synaptic currents; (2) NA depressed the high-threshold calcium currents in mitral cells and the effect of NA was mimicked by the alpha-2-adrenoceptor agonist clonidine; (3) in mitral cells treated with pertussis toxin the effect of clonidine on calcium currents was abolished; (4) clonidine failed to affect outward potassium currents in mitral cells; and (5) clonidine reduced the frequency of miniature EPSCs recorded from granule cells without affecting the amplitude of the events. Taken together, our results indicate that alpha 2-adrenoreceptor activation depresses glutamate release from mitral cells by a G-protein-mediated inhibition of calcium channels and a direct modulation of vesicle exocytosis.

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Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Medial Amygdala Responses to Chemosensory Stimuli from Same and Different Species

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In the medial amygdala of both hamsters and mice, immediate early gene (IEG = Fos/FRAs) expression shows a response to pheromone containing chemosensory signals originating from both the animal's own species (conspecific) and other species (heterospecific). In male hamsters, conspecific stimuli, regardless of gender, activate both anterior and posterior medial amygdala (MeA, MeP). With heterospecific stimuli, MeA is activated but (dorsal) MeP (MePd) appears to be suppressed. Stimuli conspecific for hamsters included female hamster vaginal fluid and male or female flank-gland secretion. In male mice, initial results suggested the same pattern of response but later data suggest that responses in MePd to urine stimuli from other male mice may be less than the response to urine stimuli from female mice. Others have found a more dramatic difference, with male stimuli producing no significant response in medial amygdala of other male mice, and have interpreted these results in terms of a reproductive response to female stimuli and a defensive response to male stimuli, and to heterospecific stimuli. The division of the mouse medial amygdala into putative reproductive and defensive regions may not account for all responses to pheromone containing chemosignals in mice, or in other species. Ongoing experiments examine amygdala responses in male and female mice and hamsters to additional conspecific and heterospecific stimuli likely to evoke defensive responses, to address these questions. In male hamsters and mice, the largely GABAergic intercalated nucleus (ICN) of the amygdala was activated when MeP was suppressed by heterospecific stimuli, suggesting inhibition of MeP by ICN.

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Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Volatile MHC Odortypes

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Major histocompatibility complex (MHC) genes influence urinary odors (odortypes) of mice. That volatile odorants are involved is supported by the observation that odortype identity can be detected from a distance. Furthermore, chemical analyses of urines have revealed numerous volatile odorants that differ in relative abundance between mice that differ only in MHC genotypes. In addition, urines from MHC-different mice evoke distinct odor-induced activity maps in the main olfactory bulbs. Recent studies reported that non-volatile MHC class I peptides may directly act as MHC-associated signals. These studies raise the question of whether the behavioral evidence for volatile MHC signals needs further verification. To accomplish this, we designed a procedure to collect peptide-free urinary volatiles and we tested these volatiles for their ability to

mediate chemosensory discrimination of mice differing only in their MHC genotype. The headspace volatiles from urines of C57BL/6 congenic mice (haplotypes H-2b and H-2k) were collected by solid phase microextraction. These volatiles were then desorbed into a gas chromatograph and the entire chromatographic eluate was collected into a buffer solution. Our results show that mice trained to discriminate between unadulterated urinary signals of the congenic mice generalize the discrimination, without reward or training, to the buffer solution containing the peptide-free urinary volatiles. Thus volatile signals, perhaps along with non-volatile ones, mediate behavioral discriminations of mice of different MHC genotypes.

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Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Fetal Odortypes: Contributions of MHC and Background Genetic Variation

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It has long been known that individual animals of many species are distinguished by unique odors. We and others have repeatedly demonstrated that these genetically determined individual odors, which we have called odortypes, are influenced by genes of the major histocompatibility complex (MHC). Other genetic loci (collectively termed "background" here) are also involved in provisioning individual mice with unique odors. Among these background loci are ones on the X and Y chromosomes and loci coding for major urinary proteins (MUPs). We have recently found that MHC-determined odortypes produced by fetuses are expressed in the urinary odors of the pregnant female and these fetal odortypes remain in the mother's circulation days or longer after the pups are born and have been removed from the mother. This phenomenon may be related to microchimerism, the finding that fetal cells remain functional in the mother long after the infant has been born. In the current studies we asked whether, in addition to MHC genetic variation, background genes also contribute to the fetal odortypes. Additionally, we investigated the interaction between MHC and background variation on a recently parturient female's odortype. Using our standard Y-maze training paradigm we found that (1) background genetic differences influence fetal odortypes remaining in the mother and (2) in some cases background genotype and MHC interact to obscure the independent contributions of each. Individual volatile body odors are thus influenced in complex ways by genetic variation at several genetic loci.

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Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Background Strain Dependence of MHC-Related Odorants

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Genes of the major histocompatibility complex (MHC) influence the urinary odors of mice. Mice can discriminate between urinary odors of mice differing only at the MHC. Notably, mice trained to discriminate between MHC types for a particular mouse strain can generalize the discrimination to other strains, suggesting that some odorants have an MHC association that is independent of background strain. To characterize the effects of MHC and background genotypes on specific odorants, we analyzed the urines of congenic B6 and Balb strains that are MHC homozygous with haplotypes H-2b or H-2k by SPME-GC/MS. A number of compounds have an MHC association that is independent of background strain; some of these compounds might contribute to the generalized discrimination. However, a surprisingly large number of compounds (109 out of 277) have an MHC association that is moderated by the background genotype (i.e., there is a MHC × background interaction). The strong background effects suggest a complex, yet unknown, mechanism of MHC-related odor expression.

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Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Detection of the Same Social Cues by the Main and Accessory Olfactory Systems: Direct Comparison of Their Functions

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Recent work from several laboratories has shown that the traditional view that common odors are perceived by the main olfactory system and pheromones by the vomeronasal system needs to be revised. We have found that the mouse main and accessory olfactory systems detect, in part, overlapping sets of molecular cues that regulate social behaviors (e.g., urinary pheromones, MHC peptide ligands). This finding enables us to directly compare the functional properties of both systems. Several surprising results are emerging. (1) Both systems can detect and process social chemosignals of volatile and nonvolatile nature. (2) Both systems are highly sensitive with detection thresholds in the subnanomolar range. (3) Coding strategies and tuning properties of individual sensory neurons in MOE and VNO differ significantly, suggesting that different receptors are employed in both systems for the detection of the same ligands. (4) System-specific signal transduction pathways are used for the detection of these cues, indicating that diverse olfactory mechanisms have evolved to assess the structural diversity of social chemosignals. (5) In behavioral tests using mice with genetic and surgical lesions, stimulation of each system by the same social cues can lead to distinct behavioral outcomes. Therefore, the same chemosignals might mediate different sexual and social behaviors through differential activation of each system.

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Poster: Chemosensory Molecular Genetics and VNO/Pheromone

A Role for Major Histocompatibility Molecules in the Main Olfactory Bulb

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The mechanisms involved in establishing and maintaining the exquisite organization of synaptic connections found at the glomerular layer of the main olfactory bulb (MOB) remain to be resolved. Recent findings have linked major histocompatibility class I (MHCI) molecules and the MHCI signaling pathway to neuronal restructuring and refinement. In this study, we have identified a putative role for MHCI molecules in the MOB. We demonstrate histochemically that MHCI molecules are expressed in the MOB in control animals. Additionally, we show that mice deficient in the expression of MHCI molecules display defects in the targeting of olfactory sensory neurons to the MOB. Tap1 gene targeted mice lack a critical component for the expression of MHCI molecules on cell surfaces. In these mice, we find an increased number of P2 labeled glomeruli per bulb as compared to the control littermates. Additionally, the location of these glomeruli is shifted along the rostral-caudal axis of the bulb as compared to the control mice. Although severely deficient in the expression of MHCI molecules, Tap1 mice do express MHCI molecules. Therefore, we are currently characterizing mice which also lack another component involved in presenting MHCI molecules on cell surfaces: beta-2 microglobulin.

Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Gene Profiling of Aging in the Murine Olfactory System: Immune Signatures

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Gene expression levels in the olfactory epithelium (OE) and bulb (OB) of young and old mice were evaluated to identify characteristic signatures of aging in expression profiles. Total RNA was isolated from OEs and OBs of 3 young (1.5 months) and 3 old (20 months) C57BL/6 male mice. cDNA from each tissue of each mouse was hybridized on an Affymetrix MG U74Av2 GeneChip (12 chips total). After data scrubbing and ANOVA, known genes with significant differences in expression levels ($P < 0.05$) between old vs. young mice were further characterized by EASE analysis to identify functional categories based on the Gene Ontology database. For genes up-regulated in the OE, 13/33 categories with significant EASE scores (<0.05) indicated immune system involvement; for down-regulated genes, 0/44 immune system-related categories had significant EASE scores. For genes up-regulated in the OB,

10/21 categories with significant EASE scores were related to immune activity; for down-regulated genes, 2/17 categories with significant EASE scores were immune-related. Up-regulated immune system genes in both OE and OB were predominantly related to autoimmunity and MHC antigen presentation. Down-regulated genes in the OB were associated with chemokines and T cell regulation. These results identify immune system activity as a characteristic signature of aging in the murine OE and OB.

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Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Probing Functionality of the Human VNO

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Mammalian pheromones influence behavior and/or hormonal state in conspecifics, often through the vomeronasal organ (VNO). Human putative pheromones have been identified, but whether human adults have a functional VNO remains controversial. Here we test whether the putative human VNO mediates the effects of smelling the putative pheromone 4,16-androstadien-3-one (AND). Smelling AND influences autonomic nervous system activity, endocrine state, and mood in women. We set out to quantify these effects, and then ask whether we could negate them by physically and chemically blocking the putative VNO (VNOblock). 30 women are scheduled for three 2.5-h long sessions separated by 28 days. In a double-blind study, VNOblock was applied selectively before exposure to either AND or CONTROL. Session order was counter balanced, and AND/CONTROL were matched for intensity/pleasantness. This design allowed us to assess the differential impact of VNOblock on the physiological, endocrine, and psychological response to AND and CONTROL. To date, 31 women have completed the first day of study, 12 have completed 2 days. A limited pilot analysis of 7 subjects who were counterbalanced for days ONE and TWO suggests a greater physiological response to AND than CONTROL ($F(1,69) = 10.3$, $P < 0.002$), but no interaction with VNOblock ($F(1,69) = 0.8$, $P = 0.36$). Although this points against functionality of the human VNO, the power of this comparison is minimal ($n = 7$). By Achems meeting time, all participants will have completed all days, and we expect to be able to determine whether blocking the putative human VNO influences the response to putative human pheromones.

Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Functional Neuronal Processing of Body Odors Differs from that of Common Odors

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Our ability to identify related individuals based solely on their body odor is remarkably high also in the absence of conscious awareness of our performance. By means of positron emission tomography (PET), we sought to elucidate the neuronal substrate behind body odor perception to answer the question of whether central processing of body odors differs from that of common, non-body odors. To date, six participants, of a total of twelve, were scanned while smelling either body odors, a mixture of perceptually similar common odors, or clean air. Initial analyses indicate that smelling the mixture of common odors activated regions commonly associated with olfactory processing such as the piriform cortex and the orbitofrontal cortex. In contrast, body odors uniquely activated areas seldom seen in olfactory processing such as superior frontal cortex, superior temporal cortex, and occipital cortical areas. Taken together, these preliminary results suggest that body odors are processed differently from common odors, with a larger involvement of multimodal processing mechanisms.

Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Different Cerebral Activation Produced by a Putative Social Chemosignal and Perceptually Similar Odorants

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Aim of this study was to compare cerebral activation produced by a putative social chemosignal and perceptually similar odorants, namely androstadienone, androstenone, and butanol. A computer-controlled olfactometer was used for stimulus presentation (OM6b, Burghart Instruments, Germany); 12 healthy female volunteers (mean age 24 years; 20–30 years) participated. The three odors were rated as equally intense, and received similar hedonic ratings (n.s.). Subjects were right-handed, normosmic and had no nasal pathology. They were examined in a 1.5 Tesla MRI scanner (Siemens, Germany). Stimuli were presented in blocks (30 s odorless air, 30 s odorant; 1 s stimulus duration, 3 s ISI, randomized). Preliminary investigations showed differences in cortical representation, pointing to a higher involvement of limbic structures (cingulate cortex, amygdala) and orbitofrontal cortex for androstadienone. The data suggest that a putative social chemosignal produces different patterns of activation compared to “normal” odors.

Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Culture, Olfaction and Cognition: Multidimensionality of ‘Culturally Scented Knowledge’

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Olfactory experiences and odor meanings are multidimensional: They are individual and simultaneously impacted by collective

representations (cultural norm). The dimension of the individual perceiver is influenced by evolutionary and environmental factors, stimulus property, and odorant/receptor interactions as well as by a variety of upstream processes, e.g., psychophysical, psychogenetic and cognitive mechanisms. These top-down and bottom-up dimensions interact simultaneously when it comes to experiencing and ‘making scents of’ odor. One of the major debates concerning the current scientific discourse revolves around the origin of odor hedonics. While some odor meanings may be derived from our evolutionary legacy, the majority is learned in the pre-natal stage as well as afterwards, suggesting THAT our socio-cultural milieu is impacting in the process. The simultaneity of the processes of odor learning and the acquisition of cultural knowledge is suggested by (1) the location in the CNS where both odor meaning and rational information is processed, namely the left frontal cortex, (2) the diversity of odor schemata employed and generated across space and time as to what odor is deemed socially acceptable or unacceptable, and (3) observations about how individuals manipulate olfactory experiences, that is, how humans negotiate the boundaries between nature and culture and between the individual and the collective. An investigation of the interactions between these dimensions furthers our understanding as to what odor indeed ‘means’. By introducing the cultural dimension to our current cognitive paradigm this presentation proposes a synthesized model of what is involved when generating ‘culturally scented knowledge’.

Poster: Chemosensory Molecular Genetics and VNO/Pheromone

A Possible Genetic Basis for General Hyperosmia

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Specific anosmia has been amply described as the far end of a genetically determined bell-shaped distribution of individual sensitivities towards a given odorant. We suggest that innate general anosmia similarly represents an extreme of a general olfactory threshold distribution, which also includes general hyposmia, normosmia and hyperosmia. Our preliminary experiments have demonstrated significant concordance among human detection thresholds to six odorants (Menashe *et al.*, this volume), thus supporting the existence of a common general olfactory sensitivity factor. We are exploring the genetic basis of the entire general olfactory sensitivity curve, which may stem from genetic polymorphisms in olfactory signaling pathway components, such as Golf and adenylyl cyclase III. Our study is particularly focused on general hyperosmia, since it has a lower probability of arising from non-genetic factors. Currently, whole genome scans with Affymetrix 100k single nucleotide polymorphism (SNP) arrays are performed on hyperosmic subjects in comparison to hyposmic and normosmic controls. This will help to identify genomic regions which differ significantly in their SNP patterns between the groups. In addition, we attempt to discover similar regions in the murine genome. This can be done utilizing automated olfactometry, and the recently described *in silico* mapping method, based on a dense SNP map in 48 inbred mouse strains. Species comparisons, fine mapping and sequencing should allow the identification of specific genes, which underlie human olfactory variability.

Poster: Chemosensory Molecular Genetics and VNO/Pheromone

A New Structural Subclass of Olfactory Receptors

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Recognition of the mechanism of olfaction depends upon understanding the sequence-structure-function relationships of olfactory receptors. We used bioinformatics methods to identify a new structural sub-class of olfactory receptors and GPCRs. We used available statistical methods to predict trans-membrane helical domains in olfactory receptor hOR17-210, a receptor that has been shown to be variably functional and pseudogenic in humans. TM domain identification was undertaken as a prelude to modeling this olfactory receptor in order to understand its interaction with ligands that have been experimentally shown to bind to this receptor. Our analyses revealed that the N-terminus of this protein is intracellular and the C-terminus is extra-cellular. This reversed polarity in the termini does not disrupt the positions of typical OR-motifs that initiate the signal transduction process at the membrane. Our observations are contrary to conventional structural knowledge about ORs and GPCRs. Preliminary sequence analysis studies have shown that such a structure is observed in a limited number of olfactory receptors distributed across different mammalian species. We present the results of the survey of all known olfactory receptors and more than 15,000 proteins identified as GPCRs. We also present a methodology for modeling such olfactory receptors. When combined with experimental data, we believe that this information will further our understanding of olfaction.

Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Characterization of a Novel Human Testicular Odorant Receptor

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In addition to their conventional role in nasal chemodetection, members of the odorant receptor (OR) family have been attributed a potential function as molecular mediators of mammalian sperm behavior. Recently, we identified and characterized a human testicular OR, OR1D2 (alternate name: hOR17-4) that triggers chemotactic and chemokinetic responses in navigating sperm. Whether other putative sperm ORs control similar behavioral responses and whether the popular 'one cell-one receptor' concept also applies to OR expression in male gametes is currently unknown. Here, we

report functional description of a novel human testicular OR. Comparing the activation profiles of both receptors in a recombinant expression system revealed distinct non-overlapping receptive fields. Our findings thus provide a tool to investigate individual OR-activated signaling mechanisms in mature sperm and determine their specific behavioral effects. Using a combination of calcium imaging and video motion analysis, on-going studies show that individual human sperm cells might express several functional ORs which differentially trigger distinct signaling cascades and behavioral responses. These results provide new insight into the physiological principles that underlie OR-mediated sperm behaviors.

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Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Highthroughput Screening System for Olfactory Receptors

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In human, more than 380 olfactory receptor (OR) genes might be required to account for the perception of several thousands of odorant molecules. Exhaustive identification of ligands for each of these OR would not only allow the understanding of odour recognition and discrimination but their use will also represent an essential industrial tool. It will help to discover and design new molecules, and optimize, refine or replace existing molecules by more potent, more appropriate and better protected ones. The design of specific antagonists might also allow to mask undesirable smells and achieve a better product profile. Screening programs and the construction of the human olfactory map can hardly be considered without a robust and reliable assay technology. Chemcom has set up screening procedures making use of a proprietary cell line allowing functional expression of ORs and containing an adapted reporter system. The assay was designed to run on a robotized platform, allowing the screening of large chemical libraries on multiple OR's. An automated single cell calcium imaging system has also been developed. This assay can be used to validate ligand-receptor couples but also to screen numerous OR's against individual ligands. Results obtained with both approaches are presented.

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Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Functional Analysis of the Mouse Odorant Receptor MOR42 Subfamily

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Phylogenetic analysis groups mammalian odorant receptors (ORs) into two broad classes and numerous subfamilies, which may reflect functional organization. To investigate the receptive ranges of members of OR subfamilies, we are using *Xenopus* oocytes as a heterologous expression system. A variety of Class I and Class II mouse ORs (MORs) can be expressed in *Xenopus* oocytes and co-expression

with G α olf and the cystic fibrosis transmembrane regulator allows measurement of odorant responses using electrophysiological methods. All receptor constructs included the N-terminal 20 amino acid residues of human rhodopsin and for 7 out of the 9 MORs tested, this was sufficient for functional expression. Co-expression of accessory proteins (RTP1, RTP2 and REEP1) allowed functional expression of the remaining 2 MORs. We screened the members of the MOR42 subfamily against a panel of 31 aliphatic odorants varying in chemical group and carbon chain length. MOR42-1 responded to dicarboxylic acids (C9–C12). MOR42-2 responded to monocarboxylic acids (C7–C10). MOR42-3 responded to dicarboxylic acids (C8–C10) and monocarboxylic acids (C10–C12). Thus, the receptive range of each receptor was unique. However, overlap between the individual receptive ranges shows that the members of this subfamily are contributing to one contiguous subfamily receptive range, supporting the idea that OR subfamilies constitute functional units.

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Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Exploring the Molecular Receptive Ranges of Mammalian Odorant Receptors

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Each odorant receptor (OR) is thought to recognize a particular array of odorant molecules, termed the molecular receptive range (MRR) of the receptor. Several mouse ORs (MORs) have been shown respond to linear aliphatic compounds. MOR 23-1, 31-4, 32-11, 40-1 and 40-4 each respond to octanoic and nonanoic acid. In addition to these common ligands, additional compounds activate one or more of these receptors suggesting that each MRR is unique. However, the extent to which the MRRs of these receptors differ is unclear. We have expressed each OR in *Xenopus* oocytes, along with G α olf and the cystic fibrosis transmembrane regulator (CFTR), allowing measurement of OR function using two-electrode voltage clamp. MOR23-1 required an accessory protein (RTP1) for functional expression, while the other receptors did not. We screened each MOR against a panel of 39 odorant compounds that varied in chemical group and carbon chain length. Each MOR displayed a distinct MRR. For example, MOR40-4 had a narrow MRR, responding well only to 9-, 10- and 11-carbon monocarboxylic acids and the 11-carbon aldehyde. In contrast, MOR23-1 had a broad MRR, responding well to alcohols, aldehydes, monocarboxylic acids, and bromocarboxylic acids with carbon lengths ranging from 6 to 9. Thus, while the MRRs for these receptors show some overlap, the MRR for each receptor is unique.

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Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Deciphering the Molecular Basis of Human Olfactory Threshold Variations

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Humans are highly variable in their olfactory thresholds. Specific anosmia/hyposmia and specific hyperosmia, the two extremes of this phenotypic spectrum are documented for dozens of odorants and suggested to have a genetic basis. Olfactory receptor (OR) segregating pseudogenes, having both functional and non-functional forms in the human population, are excellent candidates to underlie these phenotypes. To examine this hypothesis, we assessed the olfactory thresholds for 6 diverse odorants (androstenone, isoamyl acetate, isovaleric acid, l-carvone, pentadecalactone and cineol) in 377 individuals, and genotyped the segregating single nucleotide polymorphism in 30 ORs in this cohort. A strong association was seen between three OR genes on chromosome 14 and sensitivity to isovaleric acid, suggesting a locus related to specific anosmia. We also examined the average individual sensitivity across all odorants. These were found to span a wide range of >4 orders of magnitude, with different individuals having disparate values of average threshold. Such result, which stems from a concordance between odorant thresholds in each individual, implies a “general olfactory factor” that governs olfactory thresholds, in parallel to the odorant-specific determinants. Thus, we have demonstrated that the extensive threshold variation among humans towards particular odorants is a complex trait, contributed to both by olfactory-receptor-specific variations as well as by potential inter-individual differences in downstream components in the olfactory signaling pathway.

Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Identification of Amino Acids Involved in G Protein Activation by a Mouse Eugenol Receptor, MOR-EG

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Thousands of odorants are recognized and discriminated by olfactory receptors (ORs) that belong to seven-transmembrane (TM) G protein-coupled receptors (GPCRs). Once activated, GPCRs undergo conformational changes that trigger an intracellular signal transduction cascade by activating heterotrimeric G proteins. The aim of this study is to elucidate molecular mechanisms underlying G protein activation by an OR. We have previously shown that a eugenol receptor, mOR-EG, is functionally expressed in HEK293 cells such that odorant responsiveness is determined by intracellular Ca²⁺ increase via co-transfected G α 15 or by an increase in cAMP level via endogenous G α s. We introduced site-directed mutations within highly-conserved residues in the third intracellular loop and the C-terminal tail of mOR-EG. Each mutant was characterized by assays of Ca²⁺ and cAMP levels in HEK293 cells. Some mutations resulted in dramatic decreases in cAMP production, whereas no effect was observed on Ca²⁺ responses. The results suggest that these amino acid residues appear to be involved in coupling to G α s but not to G α 15. Next, we introduced mutations within the cytosolic half of TM6 to investigate a role of TM6 in receptor dynamics upon odorant activation. One of mutations exhibited a dramatic increase in cAMP responsiveness, suggesting that TM6 is involved in regulation of G protein-coupling efficiency. The present study is the first to provide insight into the interaction of an OR with G proteins at the molecular level.

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Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Changes in Olfactory Receptor Expression in Aging Mice

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Olfactory perception deteriorates in mammals with age, which is accompanied by structural and molecular changes along the olfactory pathway including the olfactory epithelium, the olfactory bulb, and the olfactory cortex. Previous studies have shown that the main pathological change in the nose during aging is the reduction of the olfactory epithelial surface (area and thickness), and a net loss of mature olfactory sensory neurons. Odorant receptors (ORs) expressed in the epithelium are critical for smell, but it is not known how their expression levels change with age. In this study, we investigated changes in olfactory receptor expression by performing *in situ* hybridization for representative ORs selected from all four zones. The density of olfactory sensory neurons expressing particular ORs was compared at ages of 1, 12, and 18 months. The expression of some receptors decreased, while others increased. For example, the density of MOR267-16 neurons decreased from $4,120 \pm 781$ cells/mm³ at 1 month to $2,784 \pm 601$ cells/mm³ at 18 months. In contrast, the density of MOR235-1 neurons increased from $8,836 \pm 1501$ cells/mm³ at 1 month to $12,954 \pm 273$ cells/mm³ at 18 months. The results indicate that age-related olfactory deterioration is more complex than the simple loss of olfactory receptors.

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Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Signatures of Aging: Profiles of Gene Regulation in the Murine Olfactory Epithelium

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We are investigating age-related changes in gene regulation in the murine olfactory system at 3 representative ontogenic stages: the 1.5 month (mo) young adult, the 6.0 mo adult, and the 20 mo old adult. Following total RNA isolation from the olfactory epithelium (OE) of 3 age-matched male mice at each stage, cDNAs from each mouse were hybridized on 9 Affymetrix 430 2.0 whole mouse genome GeneChips. After data scrubbing and ANOVA, 3,840 known genes had significant differences ($P < 0.01$) in their mean hybridization signals between at least 2 stages. We used 2 methods of informatics analysis to identify signatures of gene regulation associated with aging. First, the Expression Analysis Systematic Explorer (EASE) program identified several Gene Ontology categories associated with cellular proliferation as being over-represented (EASE scores of < 0.05) and down-regulated. Second, post-hoc statistical comparisons identified 136 significantly regulated genes ($P < 0.01$) that exhibited 1 of 3 characteristic temporal profiles. There were 15 genes

that were up-regulated with increasing age, 30 genes that were down-regulated with increasing age, and 91 genes that had mixed patterns. The results of our study have identified specific molecular and pattern profiles of gene regulation that establish signatures of aging in the murine olfactory epithelium.

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An Integrated Olfactory Receptor Microarray Gene Expression Database

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Aims: The olfactory receptor gene expression pattern is an important component of signal encoding mechanisms in the mammalian olfactory system. We have developed the Olfactory Receptor Microarray Database (ORMD; <http://neurolab.med.yale.edu/ormd/>) to house OR gene expression data and have integrated the database with the SenseLab system, in particular, ORDB (<http://senselab.med.yale.edu/senselab/ORDB/>). **Methods:** ORMD is a Web-based database, built in Java and Oracle. This is a secure database, which requires an authenticated login to access private data. Data contributors may define the public availability of individual datasets. **Results:** ORMD allows users to manage projects/experiments and to deposit related gene expression data. For each experiment, raw data files can be downloaded and analyzed gene expression data can be viewed or exported. For each probe set, a hyperlink is provided to directly access the related olfactory receptor in ORDB. On the other hand, hyperlinks are provided in ORDB for individual receptors, allowing Web visitors to access the related microarray data in ORMD. **Conclusions:** (1) ORMD will facilitate microarray study of olfactory receptor gene expression (2) together with ORDB in SenseLab, ORMD integrates gene expression data with genomics data for the olfactory receptors, providing resources for database users as well as the public.

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Chromatin Structure at Odorant Receptor Loci

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The mammalian olfactory system is able to detect and distinguish among tens of thousands of odorants in the environment. This ability is dependent on an organizing principle in which each sensory neuron in the nose expresses only one olfactory receptor (OR) allele from among >1000 OR genes in the genome. We are interested in understanding the regulatory mechanisms that govern this mutually exclusive expression. Previous genomic and genetic studies suggest

that OR genes reside in repressed regions of the genome, and we are exploring the hypothesis that mutually exclusive transcription of only one OR allele is facilitated by limiting transcriptional access by chromatin modifications. We have used chromatin immunoprecipitation (ChIP) assays to generate preliminary results on the histone modifications at an OR locus that we have previously shown becomes active in a differentiated olfactory sensory cell line. For all six modifications tested, histones around the tested OR locus in undifferentiated cells show acetylation and methylation patterns consistent with inactive euchromatin. This result suggests that the ground state for OR loci in premature sensory neurons is “closed”. We are now assaying histone acetylation and methylation patterns at multiple OR loci to test whether all OR genes are similarly “closed” in undifferentiated cells, and investigating how chromatin patterns change during the differentiation process. We anticipate having preliminary results for these studies by the date of this meeting.

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Potentiation and Inhibition among Odorants Acting on Human VN1-Type Receptors

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The human nose is exposed to odours that are shaped in many cases rather by mixtures of odorants than by a single odorant. For example, the key aroma compounds from food or human skin emanations are quite diverse, but also include homologous series of aliphatic odorants, that vary over size and functional groups. When tested with individually applied odorants, olfactory receptors (ORs) display specific odorant recognition profiles. At the receptor level, related odorants can antagonize each other's effect. The complex actions of odorant mixtures on OR, however, are poorly understood. In 96-well Ca^{2+} imaging FLIPR experiments, we identified specific C7-C11 aliphatics as best agonists for all five human VN1-type receptors (VN1Rs), when expressed in HeLa/Olf cells. In binary mixtures with agonists, related odorants with the same functional group, but with a carbon chain length <C7, or >C11 acted as antagonists. Related odorants with a different functional group but similar length had no effect by themselves, but potentiated the effect of the agonist. For example, VN1R1 responded specifically to aldehydes with decanal as best agonist. Hexanal and undecanal concentration-dependently inhibited the decanal responses, while decanal had no effect by itself, but potentiated the decanal response about 2-fold. Similar effects were observed with the other VN1Rs. Antagonism and potentiating effects may thus account for the dominating or masking of odorants in complex mixtures. Our observations with binary mixtures anticipate an even higher level of complexity of odorant coding at the level of ORs in nose.

Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Identification of a Mouse V2R Receptor Expressed in Vomeronasal Sensory Neurons Stimulated by a Male-Specific Peptide ESP1

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The vomeronasal organ (VNO) is thought to be responsible for mediating pheromone information in mice. We recently identified a sex-specific peptide, named ESP1, in tears of male mice. The secreted ESP1 appears to be transferred to the female VNO wherein it elicits an electrical response in vomeronasal sensory neurons (VSNs), and therefore, ESP1 is a candidate sex-pheromone in mice. The ESP1 gene was a member of a novel multigene family (ESP family) that composed of ~30 homologous genes in mouse genome. Some of orthologous genes were found in rat genome, albeit the number was smaller than that in mice, whereas no apparent ESP gene was found in human genome, suggesting that the ESP family has rapidly evolved during the evolutionary process. ESP1 induced c-Fos expression in V2R-expressing VSNs that were stained with a V2Rp probe potentially hybridizing with eight homologous V2Rs. To identify which V2R in the V2Rp subfamily is expressed in c-Fos-induced VSNs, we designed a set of probes from different regions of V2Rp and performed high-stringency *in situ* hybridization. One probe that hybridized with only a V2Rp5 gene product recognized 100% of c-Fos-positive VSNs. These results suggest that a ligand spectrum of a pheromone receptor is narrowly tuned and specific, not like in main olfactory system wherein each odorant is recognized by a different set of olfactory receptors.

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Poster: Chemosensory Molecular Genetics and VNO/Pheromone

An Earlier Origin for the Vomeronasal System: TRP2 in Sea Lamprey (*Petromyzon marinus*)

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The vomeronasal system (VNS) is one of two nasal chemosensory systems found in tetrapods. Currently, the VNS is defined by two morphological components, the vomeronasal organ and the accessory olfactory bulb. These components are only found in tetrapods (amphibians, reptiles, and mammals). Alternatively, the VNS could be defined by system-specific genetic components. Compared with a purely morphological view, this view gives a different picture of VNS evolution. In mammals, VNS-specific genetic components have been well characterized, and all three mammalian VNS-specific genes (V1Rs, V2Rs, and Trp2) have been identified in teleost fish with tissue-specific expression, indicating that they are involved in chemosensory detection. Additionally, studies revealed that these teleost genes are co-expressed and function together. Thus, the VNS-specific signal transduction pathway predates the morphologically defined VNS. Here, we suggest an even earlier origin of the VNS by describing the partial sequence of Trp2, in a jawless vertebrate, the sea lamprey (*Petromyzon marinus*). Expression studies could confirm that this VNS-specific gene in lampreys indicates that the VNS was present in the common ancestor of jawless fish and jawed vertebrates at least 550–650 million years ago.

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Two Families of Candidate Taste Receptors in Fishes

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Vertebrates receive tastants, such as sugars, amino acids, and nucleotides, *via* taste bud cells in epithelial tissues. In mammals, two families of G protein-coupled receptors for tastants are expressed in taste bud cells—T1Rs for sweet tastants and umami tastants (L-amino acids) and T2Rs for bitter tastants. Here, we report two families of candidate taste receptors in fish species, fish T1Rs and T2Rs, which show significant identity to mammalian T1Rs and T2Rs, respectively. Fish T1Rs consist of three types: fish T1R1 and T1R3 that show the highest degrees of identity to mammalian T1R1 and T1R3, respectively, and fish T1R2 that shows almost equivalent identity to both mammalian T1R1 and T1R2. Unlike mammalian T1R2, fish T1R2 consists of two or three members in each species. We also identified two fish T2Rs that show low degrees of identity to mammalian T2Rs. *In situ* hybridization experiments revealed that fish T1R and T2R genes were expressed specifically in taste bud cells, but not in olfactory receptor cells. Fish T1R1 and T1R2 genes were expressed in different subsets of taste bud cells, and fish T1R3 gene was co-expressed with either fish T1R1 or T1R2 gene as in the case of mammals. There were also a significant number of cells expressing fish T1R2 genes only. Fish T2R genes were expressed in different cells from those expressing fish T1R genes. These results suggest that vertebrates commonly have two kinds of taste signaling pathways that are defined by the types of taste receptors expressed in taste receptor cells.

Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Expansion of the Honey Bee Odorant Receptor Family Supports the 1 Neuron/1 Or/1 Glomerulus Model of Insect Olfaction

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We have built 170 odorant receptor (Or) gene models in the draft honey bee genome sequence, of which about 10 are pseudogenes. These include a massive subfamily expansion of 157 receptors, including 60 in a perfect tandem array. This number of roughly 160 functional Or genes matches well the number of glomeruli in the bee antennal lobe at roughly 160–165. This match supports the 1 neuron/1 Or/1 glomerulus model of insect olfaction. In stark contrast, bees encode just 10 gustatory receptors (Grs), which represent most of the major Gr lineages in insects, but with no subfamily expansions. Thus bees have expanded their Or repertoire significantly relative to flies (60–80 Ors), perhaps to meet their needs for floral odor recognition in addition to their use of several pheromones in social communication and chemicals for kin recognition. The lack of Gr

family expansion relative to the flies (68–76 Grs) might reflect their mutualistic relationship with plants, thus not needing to detect toxic plant chemicals, as well as their nursing of larval bees which do not need to forage. Bees might also employ Ors as gustatory receptors when antennating each other and other objects.

Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Diversity and Conservation of Lepidopteran Olfactory Receptors

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The ability of organisms to detect and discriminate between many odours is pivotal to their survival and primarily due to the olfactory system. In vertebrates, *C. elegans* and *Drosophila*, odorant receptors (OR's) provide the molecular basis for odor coding and belong to the large super family of G-protein coupled receptors. In insects, OR's are extremely diverse across orders and species with the exception of the Or83b homologues. The Or83b receptor exhibits a high level of sequence conservation across four orders and appears to be required for localizing other OR proteins to the dendrites of olfactory neurons. The genomes of *Drosophila*, *Anopheles gambiae* and *Bombyx mori*, the silk moth, are now available for sequence mining. We are mining the genome of *Bombyx* for homologues of the known ORs of *Drosophila* and *Heliothis virescens* and also for homologues of novel ORs isolated experimentally from other lepidopteran species. We are particularly interested in the extent to which specific OR sequences are conserved within an order or other taxon and the functional significance of such sequence conservation. We are using degenerate RT-PCR to probe for conservation of OR sequences in six lepidopteran species, besides *Bombyx*, representing another five lepidopteran families. RNA *in situ* hybridisation and functional studies will help us to elucidate the functions of the ORs we have identified.

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Female Specific Odorant Receptors Expressed in the Adult Antennae of the Silkworm, *Bombyx mori*

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Olfaction plays an important role in the life history of insects, including key pest behaviors such as host selection and oviposition by female moths. We identified 54 novel scaffolds encoding complete or partial odorant receptors (Or) from the recently sequenced silkworm (*Bombyx mori*, Bm) genome. This brings the total number of known BmOrs to 60, a number that corresponds well to the approximate 61 glomeruli in the silkworm antennal lobe, supporting the one odorant

receptor—one sensory neuron—one glomerulus model of insect olfaction. Each Or was screened for female biased expression patterns in adult moth antennae using quantitative PCR. Several Ors were moderately female biased, 3–10 times more abundant in female as compared to male antennae. Three were of particular interest since their relative abundance in female antennae was much greater, 40–800 times that of male antennae. The complete cDNA sequences of the three female specific Ors are currently being cloned and attempts to characterize their ligand binding specificity are underway.

This work represents an important step towards elucidating female specific olfactory pathways that are involved in key female specific behaviors in lepidopteran moths.

Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Shifts in the Use of Two Aldehydes and the Evolution of Olfactory Communication in Heliothine Moths

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In heliothine moths, a cosmopolitan group with extant species on 6 continents, divergence in olfactory communication is often manifested by shifts in the use of two secondary, but essential compounds in the pheromone blend: (Z)-9-tetradecenal (Z9-14:Ald) and (Z)-9-hexadecenal (Z9-16:Ald). In several species, Z9-14:Ald has become antagonistic to male behavior and in two *Helicoverpa* species the blends have shifted to the extreme inasmuch as Z9-16:Ald has become the primary pheromone component. We seek to understand the genetic complexity underlying the shift in male preference for either of these two odorants in the *Heliothis virescens*/*Heliothis subflexa* system where males have diverged to utilize Z9-14:Ald or Z9-16:Ald, respectively. Through behavioral and olfactory studies of hybrid and backcross males of interbred *H. virescens* and *H. subflexa*, we have shown that the preference for either Z9-14:Ald or Z9-16:Ald is associated with a change in the specificity of peripheral olfactory receptor neurons. QTL analysis from behaviorally phenotyped backcross males indicated that most variation in this character was explained by a single chromosome. Subsequent mapping revealed that the candidate pheromone receptor gene, HR14 also mapped to this same chromosome. In the current studies we report on the behavioral and olfactory phenotypes and genotypes of males generated by recurrent backcrossing to *H. virescens*. In these males a single *H. subflexa* chromosome was isolated in an *H. virescens* background. The results indicate that a major gene may play a fundamental role in male olfactory preference and pheromone blend evolution in heliothine moth species.

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Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Pheromone Receptor Mediates Behavior in *Drosophila*

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Insect pheromones elicit stereotypic behaviors critical to survival and reproduction. The only identified volatile pheromone in *Drosophila* is 11-*cis* vaccenyl acetate (VA), a male-specific lipid that mediates aggregation behavior. VA is detected by a few dozen olfactory neurons located on the antenna in a subset of trichoid sensilla (T1 sensilla) in both male and female flies. We previously showed that sensitivity to VA requires LUSH, a non-neuronal secreted protein present in the sensillum lymph bathing the trichoid olfactory neuron dendrites. Here we identify a neuronal receptor that mediates VA sensitivity expressed exclusively by the T1, VA-sensitive neurons. We report two mutants lacking T1 sensilla and demonstrate that expression of the VA receptor is reduced or eliminated in these mutants. Importantly, we show mis-expression of this receptor in olfactory neurons that are normally insensitive to VA confers pheromone sensitivity in a LUSH-dependent manner. These data provide new insight into the molecular components and neuronal basis of volatile pheromone perception in *Drosophila*.

Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Characterization of a *Drosophila melanogaster* Chemosensory Specific SNMP

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Sensory neuron membrane protein (SNMP) is an antennal 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. Studies of the temporal and spatial patterns of this protein suggests CG7000 expresses in subsets of chemosensory (olfactory and gustatory) and mechanosensory neurons of adults, and chemosensory neurons of larvae. We have also conducted *in situ* hybridization experiments confirming the validity of the *cd8::GFP* expression pattern. We have generated double stranded RNA strains for this gene to knockdown normal expression of CG7000. Behavioral studies will allow us to see whether CG7000 is a suitable candidate for studying SNMP function as it relates to insect olfaction as well as studying the roles of diverse SNMP/CD36 homologues in a single species.

Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Evolution of the SNMP Gene Family in the Diptera *Drosophila melanogaster*, *D. pseudoobscura*, and *Anopheles gambiae*

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SNMPs are membrane bound proteins that associate with olfactory neurons in lepidoptera and are thought to play some central role in odor detection. SNMPs belong to a larger gene family characterized by human CD36. In this study, we have iteratively blasted the genomes of *Drosophila melanogaster*, *D. pseudoobscura*, and *Anopheles gambiae* to identify SNMP/CD36 homologs, finding 12–18 candidates in each species. As the distance between these species differs by an order of magnitude (25–50 million years divergence for the two *Drosophila* species, 220–250 million years divergence from *Drosophila* to *A. gambiae*), we have two comparative time scales with which to examine the evolution of the SNMP family. A neighbor joining tree constructed from aligned amino acid sequences suggests that each of the SNMP homologs found in *D. melanogaster* has a likely ortholog in *D. pseudoobscura* with the exception of *D. melanogaster* CG12789. Since CG12789 does have a likely ortholog in the *A. gambiae* SNMP homologs, we suggest that the related gene was lost in *D. pseudoobscura* after the divergence of the two *Drosophila* species. Fewer of the *A. gambiae* sequences align as orthologs with candidates from the two *Drosophila* species, unsurprising considering the relative time since divergence. To further characterize these relationships, a map was constructed of intron locations within the aligned amino acid sequences, and a Dollo parsimony tree made by assigning intron locations a binary value for characters. Both trees have similar structure, providing additional assurance that the phylogeny is reasonable and suggesting that parsimony trees constructed based in intron locations are valid.

Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Drosophila Sugar Receptors

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The sense of taste provides valuable information about the nutritional quality of food. Like mammals, the fruit fly *Drosophila* can taste both attractive and aversive compounds, which are detected via taste neurons on the legs as well as the mouthparts. Gustatory receptor (*Gr*) genes, which are expressed in these neurons, are members of a large, divergent gene family. Only one of these *Gr* proteins has been assigned a ligand—previously we have shown that *Gr5a* is a receptor for the disaccharide sugar trehalose. *Gr5a*-positive neurons do respond to various other sugars revealing that additional taste receptors are also expressed in them. Such co-expression of receptors raises intriguing possibilities about the mechanisms of sugar detection and discrimination. To explore these questions, we sought to identify taste receptors for other sugars. We reasoned that seven *Gr* genes that are closely related to *Gr5a* might encode receptors for other sugars. Consistent with this idea, we find that at least two of these receptors are expressed in *Gr5a* neurons. Using various genetic tools we have generated deletion mutations that result in the loss of multiple receptor genes of the *Gr5a* subfamily. Analysis of mutant flies shows that they have electrophysiological or behavioral defects in their responses to several sugars, including maltose and sucrose, as compared to control flies. Transgenic experiments are underway to determine the response profiles of individual receptors. Interestingly, we find that some of these deletions have a dramatic effect on lifespan as well as starvation resistance of mutant flies. Currently, we are investigating further the link between sugar reception, feeding behavior and longevity.

Poster: Chemosensory Molecular Genetics and VNO/Pheromone

The G Encoding Gene Family of the Malaria Vector Mosquito *Anopheles gambiae*: Expression Analysis and Immunolocalization of AGGQ and AGGO in Female Antennae

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To initiate a comprehensive investigation of chemosensory signal transduction downstream of odorant receptors, we identify and characterize the complete set of genes that encode G-protein subunits in the genome of the malaria vector mosquito *An. gambiae*. Data is provided on the tissue-specific expression patterns of 10 corresponding *aga*-transcripts in adult mosquitoes and pre-imaginal developmental stages. Specific immunoreactivity in chemosensory hairs of female antennae provides evidence in support of the participation of a subset of *AgGaQ* isoforms in olfactory signal transduction in this mosquito. In contrast, *AgGao* is localized along the flagellar axon bundle but is absent from chemosensory sensilla, which suggests this G-protein subunit does not participate in olfactory signal transduction.

Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Genetic Analysis of Tongue Size and Tongue Weight in Recombinant Inbred Strains of Mice

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Little is known about the genetic factors underlying variability of tongue morphology. Quantitative trait locus (QTL) analysis is an important methodology for mapping genes underlying differences in morphology. QTL detection methods use a forward genetics approach, where a well-characterized phenotype is quantified in an effort to identify a set of genes that are responsible for the variability of the phenotype. In the present study, the morphology of the mouse tongue was examined in 18 recombinant inbred BXD strains. We measured four tongue dimensions that included apex to vallate (ApV), apex to median eminence (ApM), tongue width and tongue weight. A correlation analysis revealed that none of these measurements more than modestly correlated with body weight, suggesting that the genetic factors are largely independent of body size. Tongue weight correlated with tongue lengths of ApV and ApM at $r^2 = 0.7$ and $r^2 = 0.8$, respectively, and only modestly correlated with tongue width ($r^2 = 0.5$). Interestingly, the detected QTLs observed from residual regression analysis for ApV and ApM were different from those of tongue weight. We detected a QTL on chromosome 7 (LOD > 3.8) for both ApV and ApM, while tongue weight showed two suggestive QTLs on chromosomes 9 (LOD = 4.0) and 16 (LOD = 3.8). Pair-scan analysis revealed that the two suggestive QTLs affecting tongue weight were purely additive in effect. These results are a first characterization of genetic variability in tongue morphology among BXD strains of mice.

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Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Expression of GPR4, a Proton Sensing GPCR, in Human Fungiform Papillae

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The molecular mechanisms underlying sourness remain controversial. Most proposed mechanisms postulate the involvement of various ion channels. Recently, a proton sensing molecule named GPR4 has been characterized which is a GPCR rather than an ion channel. Here we summarize our studies on the expression of GPR4 in human taste tissue. Human fungiform papillae (HFP) were obtained from two subjects who identified 15 mM citric acid as sour in both whole mouth and tongue tip tests. RTPCR of the pooled papillae confirmed the expression of GPR4. Subsequently, the entire coding sequence of GPR4 (1086 bp) was amplified from the pooled papillae. An open reading frame of 362 amino acids was identified, only two of which differed from the GenBank sequence. RTPCR of individual cells isolated from HFP showed that GPR4 was expressed in a subset of taste cells. Two sour-abnormal subjects were studied, one of whom (ID # 62) identified the sourness of 15 mM citric acid in a whole mouth test but not at the tongue tip. RTPCR of HFP from Subject 62 failed to detect the coding sequence of GPR4 after 50 cycles. The other sour-abnormal subject (ID # W) was unable to identify the sourness of citric acid, either at the tongue tip or in a whole mouth test, at concentrations up to 18 mM. RTPCR of HFP from Subject W failed to detect the coding sequence of GPR4 after 50 cycles. Taken together, these initial data raise the possibility of a role for GPR4 in human sour perception.

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Poster: Chemosensory Molecular Genetics and VNO/Pheromone

A Newly Identified Neohesperidine Dihydrochalcone Binding Site in the Human Sweet Taste Receptor Overlaps with Allosteric Modulator Binding Sites for Class 3 GPCRS

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The sweet inhibitor lactisole and the sweetener cyclamate share an overlapping binding site in the seven-transmembrane section of the TAS1R3 subunit of the human sweet receptor. Using heterologous expression and functional analysis of rat/human receptor chimeras we recently showed that the seven-transmembrane section of hTAS1R3 is crucial for the activation by the sweetener neohesperidine dihydrochalcone (NHDC). Interestingly, lactisole competitively inhibits NHDC and cyclamate activation. We therefore

assumed that NHDC may share some residues with the lactisole and cyclamate binding site. To test this hypothesis we tested 13 point mutants involved in lactisole and cyclamate binding towards NHDC. Indeed, 7 of them increased the EC50 of NHDC > 10 fold. Modelling of the NHDC pharmacophore revealed 12 additional possible interaction sites. Mutational analysis showed that 10 of them clearly affected NHDC activation. Moreover, sequence alignments of the TAS1R3 seven-transmembrane section with other members of the class 3 GPCRs revealed that 40% of the amino acids involved in NHDC activation overlap with known binding positions of allosteric modulators in other class 3 GPCRs. This allows us to predict additional residues in the TAS1R3 seven-transmembrane section that may be involved in the binding of other sweeteners.

Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Taste Receptors for Glutamate in Human Fungiform Papillae

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Molecular and behavioural experiments in rodents suggest that several candidate receptors might be involved in glutamate taste detection. Truncated isoforms of metabotropic glutamate receptors, namely Taste-mGluR4 and Taste-mGluR1, have been found in taste buds together with a heterodimer receptor (TAS1R1 and TAS1R3). Interindividual variability in the sensitivity to MSG in humans led us to study candidate receptor expression in human fungiform papillae, both by RT-PCR and immuno. Our data indicate that mGluR4, TAS1R1 and TAS1R3 are present in human fungiform taste buds. Potential variations of the sequences in the genes coding for TAS1R1 and TAS1R3 were examined in 215 subjects. Sequencing of the 6 exons encompassing the coding region of TAS1R1 and TAS1R3, respectively, uncovered three single-nucleotide polymorphisms (SNPs) in TAS1R1 and five SNPs distributed throughout the coding sequence in TAS1R3. Two of 3 SNPs in TAS1R1 and 4 of 5 SNPs in TAS1R3 lead to an amino acid substitution. The prevalence of each SNP was evaluated and will be presented. Additionally, Mendelian transmission of each SNP leading to an amino acid substitution was studied in 25 families. These results represent a first step towards understanding the genetic factors underlying interindividual variability of sensitivity for MSG in humans.

Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Positional Cloning Approach to Identification of the Sucrose Octaacetate Aversion (SOA) Locus

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Sucrose octaacetate (SOA) tastes bitter to humans and has an aversive taste to some mice and other animals. While some mice avoid SOA, others do not, which depends on allelic variation of a single locus, *Soa*. A dominant *Soa*^a allele produces the taster phenotype (i.e., SOA avoidance); recessive alleles *Soa*^b and *Soa*^c produce the nontaster and demitaster phenotypes, respectively. We use a positional cloning approach to identify a gene corresponding to the *Soa* locus. Our previous studies have shown that the *Soa* locus resides within approximately 5-Mb region on chromosome 6. This region contains a number of genes encoding G protein-coupled receptors from the T2Rs family that are proposed to be bitter receptors and therefore are candidate genes for the *Soa* locus. Currently, we conduct a high-resolution mapping of the *Soa* locus. We created a dense set of markers throughout the *Soa* region, produced a large (>1,000 mice) cross between strains with different *Soa* alleles, and are genotyping these mice to find recombinations that shorten the critical *Soa* interval. We expect to reduce the genomic segment encompassing *Soa* to <100 kb and thus exclude many of the T2R genes from the list of candidates for *Soa*.

Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Molecular Modeling of Sweet Taste Receptors

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The heterodimer of T1R2 and T1R3 is a broadly acting sweet taste receptor responsive to natural sugars, artificial sweeteners, D-amino acids, and sweet-tasting proteins. T1Rs are characterized by a large extracellular Venus flytrap model (VFTM), which is linked by a cysteine rich domain (CRD) to the 7-TM-domain (TMD). Although crystal structures are not available for the sweet taste receptor, useful homology models can be developed based on appropriate templates. The VFTM, CRD and TMD 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.

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Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Probing the Aspartame Binding Site of Human T1R2

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The heterodimer of T1R2 + T1R3 is a broadly acting sweet taste receptor responsive to natural sugars, small molecule artificial

sweeteners and sweet tasting proteins. Certain compounds are sweet to humans but not rodents; this species-specificity can be replicated *in vitro* by expressing the human or mouse T1R2 + T1R3 heterodimers. We had previously used human/mouse mismatched and chimeric receptors and directed mutagenesis to map the sweet receptor's sites of interaction with the sweet protein brazzein (interacts with the cysteine rich domain of T1R2), the sweetener cyclamate and the inverse agonist lactisole (both bind within the transmembrane domain of T1R3). Li and colleagues had determined that the extracellular "Venus Fly Trap Domain" (VFTM) of human T1R2 was required for a human-type response to the dipeptide sweetener aspartame. Based on models of T1R2 with aspartame docked to the receptor we have predicted residues of the VFTM likely to be critical for the interaction with aspartame and other dipeptide sweeteners. We have used chimeric receptors and mutants to analyze the interaction of aspartame, neotame and other dipeptide sweeteners with the canonical ligand binding site in the VFTM of T1R2. From this functional analysis we have tested and refined our molecular models of the aspartame-T1R2 VFTM interaction. Our validated model provides a biophysical explanation for why neotame is a much more potent sweetener than aspartame.

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Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Properties of the Seven Transmembrane Core Domains of the Human T1RS

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The human T1R family of taste receptors consists of three family C G-protein-coupled receptors that act as heterodimers to detect sweet-tasting compounds and amino acids. Although recent experiments have examined the ligand binding properties of these receptors, little is known about their signaling properties. Using a baculoviral system, we expressed truncated forms of the T1R receptors, which have intact seven transmembrane core domains but lack extracellular domains. Receptor-enriched membranes were purified from infected insect cells and used in *in vitro* reconstitution assays. We demonstrate that the core domains of T1R1 and T1R2 when expressed alone displayed significant constitutive activity, catalyzing by several fold the exchange of GDP for GTP on transducin, a close homolog of gustducin. The constitutive activity of either the T1R1 or T1R2 core domain was unaffected by the addition of lactisole or cyclamate and did not require dimer formation with their common partner, T1R3. These results indicate that the *in vitro* reconstitution assay can be used to determine the G protein selectivities of the T1Rs. Furthermore, the results are consistent with the suggestion that in their native heterodimer configurations (T1R1/T1R3 or T1R2/T1R3), T1R1 and T1R2 are the signaling components, and the T1R3 core domain contributes the allosteric ligand binding site.

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Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Effect of Maillard Peptides (MPS) on TRPV1 Variant Salt Taste Receptor (TRPV1T)

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Proteolysis occurs during ripening of protein-rich foods. The resulting protein hydrolysate plays an important role in enhancing the flavour and taste of food. We investigated the effect of the naturally occurring MPs fractionated by ultrafiltration (500–10,000 Da) from mature Korean soy sauce on human salt taste perception. MPs (0.05%) presented a significant salt-masking activity. To test if MPs modulate salt taste by interacting with TRPV1t, we monitored benzamil (Bz)-insensitive chorda tympani (CT) taste nerve responses in rats and in wild type and TRPV1 knockout (KO) mice. In 100 mM NaCl + 5 μM Bz, varying the concentration of MPs (0–1.5%) produced biphasic CT responses in rat and wild type mouse. Between 0.1% and 0.5% concentration, MPs increased the Bz-insensitive NaCl CT response. Above 0.5%, MPs inhibited the response, and at 1.5% the response was decreased to baseline. SB-366791 (1 μM), a specific inhibitor of TRPV1t, eliminated the constitutive Bz-insensitive NaCl CT response and inhibited the response to 0.4% MPs by 60%. In the presence of 0.4% MPs, raising the temperature from 23° to 42° increased the CT response by 116.6%. TRPV1 KO mice demonstrated no Bz-insensitive NaCl CT response and no response to MPs above baseline. We conclude that MPs modulate the Bz-insensitive NaCl CT response and human salt taste by interacting with the TRPV1t cation channel in fungiform taste receptor cells.

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Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Effect of N-Geranyl Cyclopropylcarboxamide (NGCC) on TRPV1 Variant Salt Taste Receptor (TRPV1t)

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A compound synthesized by IFF, NGCC (1 μM), significantly enhanced salt taste perception in human psychophysical studies. To test if NGCC modulates salt taste by interacting with TRPV1t, we monitored benzamil (Bz)-insensitive chorda tympani (CT) taste nerve responses in rats. In 100 mM NaCl + 5 μM Bz, varying the concentration of NGCC (0–50 μM) produced biphasic CT responses. Between 0.25 μM and 2.5 μM, NGCC increased the Bz-insensitive NaCl CT response and above 2.5 μM it was inhibitory. At 50 μM NGCC, the CT response was not different from baseline. SB-366791 (1 μM), a specific inhibitor of TRPV1t, elim-

inated the constitutive Bz-insensitive NaCl CT response and inhibited the entire tonic response at NGCC concentrations between 0.25 μM and 50 μM. Increasing the temperature between 23° and 55.5° produced a biphasic increase in the CT response with a maximum response around 43°. NGCC (2 μM) increased the CT response at all temperatures without a shift in the temperature threshold of the response. In contrast, N-cyclopropyl E2 Z6-nonadienamide, a chemically related compound, did not affect human salt taste and also demonstrated no effect on the Bz-insensitive NaCl CT response at concentrations between 0.25 μM to 50 μM. We conclude that NGCC produces its effect on human salt taste by specifically interacting with TRPV1t in fungiform taste receptor cells and by modulating the Bz-insensitive NaCl responses.

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Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Expression of Fatty Acid-Activated G Protein Coupled Receptors in Chemosensory Cells

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Fatty acids (FA) have been implicated as chemosensory cues that convey the taste and texture of fat, where they have been shown to interact with either delayed rectifying K⁺ (DRK) channels and/or the FA transporter CD36 in taste receptor cells. Recently, a number of orphan G protein coupled receptors (GPCRs) have been shown to be activated by free FA in a variety of cell types. Using RT-PCR on mRNA isolated from several chemosensory cell types including taste buds, trigeminal neurons and an enteroendocrine cell line, we have probed for expression of DRK channels, CD36, and the fatty acid-activated GPCRs: GPR40, GPR41, GPR43 and GPR120. While FA-sensitive DRKs are found in all three rat lingual taste buds, expression of CD36 appears limited to the foliate and vallate papillae, which also expresses GPR40, GPR41 and GPR43, but not GPR120. We are currently exploring the subtypes of cells within the taste bud which express each receptor type by single cell PCR. Enteroendocrine cells, which also respond to dietary fat, express FA-sensitive DRK channels, GPR120, GPR40, GPR41, GPR43, but not CD36. Trigeminal neurons, which contribute to the textural properties of fats, also express a variety of putative fat receptors. In addition to a variety of FA-sensitive DRK channels and CD36, trigeminal neurons express several of the aforementioned FA-activated GPCRs. Our laboratory is continuing to explore the functional consequences of the expression of this variety of different fatty acid-responsive proteins.

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Poster: Chemosensory Molecular Genetics and VNO/Pheromone

Replication of Linkage and Association of PROP Perception to Chromosome 7 and Suggestion of Novel Loci on Chromosome 6

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This study was conducted to replicate linkage to chromosome 7, association with known TAS2R38 haplotypes and to search for other chromosomal regions that may influence PROP perception. The perceived bitterness of a 6.0×10^{-4} M propylthiouracil (PROP) solution and a dry PROP strip (from a saturated solution) was examined in a genetically informative sample of 62 MZ and 131 DZ twin pairs and 237 sib pairs. Multipoint linkage analysis (761 microsatellites) and association analyses (rs713598, rs1726866) were performed using the software packages MERLIN and Mx, respectively. Linkage analysis generated a LOD score of 4.9 on chromosome 7 (D7S684) for the PROP solution with no evidence of linkage to this region for the PROP strip. The highest peak for the PROP strip was on chromosome 6 (D6S1017) with a LOD score of 2.0 with the PROP solution having a LOD score of 1.7 at the previous marker (D6S2427). Association with the three TAS2R38 haplotypes (PAV, AAV and AVI) indicated that TAS2R38 accounts for 66% and 24% of the variation in the perceived intensity of the PROP solution and strip, respectively. Given that the heritability of both the solution and strip measures is 0.75, there are additional genes responsible for variation in perceived intensity of PROP, especially the strip, and we suggest that these may reside on chromosome 6.

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Symposium: Olfactory Bulb Computations

Mechanisms that Generate Precise Synchrony in Olfactory Bulb Neurons

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Synchronized oscillatory activity at the gamma frequency (30–70 Hz) is thought to be important for information processing in many sensory systems, including olfaction. Here, I used patch-clamp recordings in neuron-pairs to assess mechanisms underlying such “gamma” activity in rat olfactory bulb slices. During recordings from pairs of excitatory mitral cells, patterned electrical stimulation of afferents that mimicked a natural odor stimulus elicited rapidly synchronized spikes (lag ≤ 5 ms), along with ~ 50 Hz gamma frequency activity. Analysis of coupling potentials, combined with dendritic sectioning, indicated that mitral cell synchrony was driven by precisely timed inhibitory post-synaptic potentials (IPSPs) imposed by GABAergic granule cells at dendrodendritic synapses. Recordings in granule cell pairs, done to determine the mechanisms underlying the synchronized IPSPs, revealed that granule cells were themselves synchronized following afferent stimulation. Granule cell synchrony, which would enhance mitral cell synchrony by coordinating GABA release, was due to divergent excitatory inputs imposed by mitral cells, in combination with rapid spike response-times. Significantly, both mitral/mitral and granule/granule cell synchrony occurred in the absence of electrical coupling. Taken together, these results indicate that rapid gamma frequency activity

in the olfactory bulb is a consequence of the precise back-and-forth synaptic interplay between populations of mitral cells and granule cells, rather than direct cell-to-cell coupling.

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Symposium: Olfactory Bulb Computations

Optical Studies of Active Properties in Dendrites of Olfactory Bulb Neurons

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The majority of neurons in the olfactory bulb release neurotransmitter from dendrites so the modulation of dendritic Ca^{2+} influx directly controls synaptic transmission within the bulb. We use a combination of electrophysiological recording and imaging of fluorescent Ca^{2+} indicators to examine activity dependent Ca^{2+} influx into dendrites with particular attention to conditions that alter the spatial distribution of influx along or between different branches. In mitral cells the extent of action potential (AP) propagation into secondary dendrites, and thus the amount of voltage-dependent Ca influx that results along the length of these processes, is under the control of fast inactivating K^+ (Ia) channels. Activation of D2 receptors reduces Ca^{2+} influx primarily through hyperpolarization induced removal of inactivation of Ia with a distinct proximal to distal gradient of effectiveness. Ca^{2+} influx is reduced in distal dendrite more than proximal due to progressive loss of AP amplitude. Granule cell dendrites readily support antidromic and orthodromically propagating Na^+ dependent APs. However, APs initiated in a distal dendrite may or may not fully invade the soma/proximal dendritic segment. If distally generated APs do not initiate APs in the soma/proximal dendrite then subsequent propagation out into other dendrites does not occur with the result that Ca^{2+} influx, and thus transmitter release, can be restricted to a subset of presynaptic release sites on specific branches.

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Symposium: Olfactory Bulb Computations

Persistent Activity in Inhibitory Local Circuits in the Olfactory Bulb

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Inhibitory local circuits in the olfactory bulb play a critical role in determining the firing patterns of output neurons following sensory stimulation. Recent studies have identified several local circuit interactions that affect the sensory input stage of olfactory processing. By contrast, relatively little is known about the synaptic circuitry in the major plexiform layers of the olfactory bulb except for the reciprocal dendrodendritic synapse formed between mitral and axonless granule cells. We have recently identified Blanes cells, large stellate-shaped interneurons located in the granule cell layer, as a local circuit source of inhibitory input to granule cells.

We found that Blanes cells are GABAergic and, unlike granule cells, generate large ICAN-mediated afterdepolarizations following bursts of action potentials under normal pharmacological conditions. Using paired 2-photon guided intracellular recordings, we found that Blanes cells have a presumptive axon and monosynaptically inhibit granule cells. Blanes cells receive excitatory input following sensory neuron stimulation that can trigger long epochs of persistent spiking that can be reset by hyperpolarizing membrane potential steps. Persistent firing in Blanes cells may represent a novel mechanism for representing short-term olfactory information though by modulation of the tonic inhibitory synaptic input to bulbar neurons.

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Symposium: Olfactory Bulb Computations

An Account of Odor Discrimination Times in the Mammalian Olfactory System

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Recent behavioral experiments in rodents indicate that odor discrimination is rapid, occurring within a single sampling bout but that the overall time taken depends on stimulus similarity. *In vivo* electrophysiological and population imaging data in rodents show that the activity of mitral cells in the olfactory bulb (OB) is modulated by a sniff-coupled subthreshold oscillation. This paper addresses the question of whether measured OB action potential (AP) latencies could account for the speed and similarity-dependence of odor discrimination. Using measured mitral cell input resistances, AP thresholds, odor-evoked AP rates and latencies, we constructed an OB model of realistic size (50,000 mitral cells [MCs]) that showed all the hallmarks of published OB properties. Stimulus separation times and their similarity-dependence were assessed using a template-matching scheme reliant on AP latencies. In this scheme very different stimuli were accurately separated within 10 ms after odor onset (100% accuracy 10 ms after MC firing onset; A vs. B). However successful separation of binary mixtures required substantially longer ($87.5 \pm 5.8\%$ accuracy, 80 ms after onset of MC firing for 0.4A/0.6B vs. 0.6A/0.4B). The separation time was critically dependent of mixture similarity (52.8 ± 3.0 ms/log unit stimulus similarity; 90 ms longer for most similar odor mixtures that were still discriminated [$>85\%$]). These data indicate that the temporal properties of the onset patterns of MC activity may account for the stimulus dependence and overall time course of olfactory processing.

Slide: Clinical Chemosensory

Smell Testing and Datscan Imaging in Diagnosing Idiopathic Parkinson's Disease

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Objective: To compare the value of smell testing versus dopamine transporter SPECT (DaTScan) in the diagnosis of early Idiopathic Parkinson's Disease (IPD). Background: There are no specific tests for IPD and errors of diagnosis occur in 10–25%. DaTScan has high

sensitivity for IPD but cannot distinguish parkinsonian syndromes. Olfactory identification is impaired in more than 80% of patients with IPD but likewise has low specificity. Method: We recruited 30 newly diagnosed, drug naive IPD patients from the neurology clinic with mean disease duration of 2.2 years (1–7 years) and mean Hoehn and Yahr stage of 1.7 (1–3 years). All conformed to the UK PD Brain Bank Criteria step1 and scored a minimum of 27/30 on Minimal State Examination. The following tests were used. (1) UPSIT-item 40. (2) Olfactory event related potentials (OERP) to H₂S using the Burghart OM2 Olfactometer. (3) (123 I)-FP-CIT DaTscan. For controls, we used 245 subjects for UPSIT and 70 of these for OERP. A value exceeding 2 SD, age-adjusted was considered abnormal. Results: Abnormal DaTScan was found in 26/30 IPD (86%). UPSIT results were abnormal in 20/30 IPD (66%). OERP was recordable in 21/30 of whom 2 had abnormal latency. Conclusion: This preliminary analysis suggests that both DaTScan and UPSIT are of diagnostic value in early IPD but they measure different aspects of the disease. OERP has low sensitivity in early IPD. UPSIT may provide a simple, inexpensive but possibly less sensitive screening test in an early case.

Slide: Clinical Chemosensory

Altered Chemosenses in Parkinson's Disease

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Background: According to Braak *et al.* (2003) the first lesions in Idiopathic Parkinson's Disease (IPD) appear in the olfactory bulb and the dorsal nuclear complex of IX and X. Aim: To evaluate the olfactory and taste function in patients with early IPD. Methods: 51 patients with a clinical diagnosis of early-stage IPD (mean Hoehn and Yahr stage = 1.6), conforming to the UK PD Brain Bank Diagnostic Criteria step 1 and scoring at least 27/30 on Mini Mental State Examination. All received the UPSIT-item 40 and taste threshold assessment with the Rion TR-06 Electrogustometer. The same tests were performed on 46 healthy controls (HC). Results: Mean UPSIT scores were 32.9/40 for HC vs. 19.8/40 for IPD group ($P < 0.001$). Taste threshold for the chorda tympani area was 11.06 dB for HC vs. 20.08 dB for IPD ($P < 0.001$). For vallate papillae area 13.5 dB in HC vs. 19.9 dB in IPD group ($P < 0.001$). Conclusion: We confirmed that smell function is impaired severely and early in IPD and showed for the first time that taste sense is also damaged in the early phase of disease. Both procedures could be used as diagnostic clues in the early stages of the disease. The combined deficits have implications for appetite, food consumption and weight control in these patients.

Slide: Clinical Chemosensory

San Francisco/Oakland Bay Bridge Welder Study: Olfactory Function

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The sense of smell is vulnerable to damage from xenobiotic agents, given the rather direct exposure of its receptors to the outside environment. Industrial vapors and dusts, particularly those of heavy metals such as cadmium and manganese, are known to adversely alter smell function. In this study, we evaluated olfactory function in 43 professional welders with over 15 years of welding experience who had worked on a regular basis from 1 to 2 years on the San Francisco/Oakland Bay Bridge. These individuals were part of the multi-center San Francisco/Oakland Bay Bridge Welder Study. Nearly half reported having problems smelling and tasting. Relative to age-, gender-, race-, education-, and smoking behavior-matched controls, the welders exhibited marked olfactory dysfunction, as measured by the 40-item University of Pennsylvania Smell Identification Test (UPSIT); respective means (SEMs) = 36.55 (0.88) and 23.06 (0.90); $P = 0.000$. Thirty-eight of the 43 welders (88%) scored below their individually matched controls. Although no correlation was present between the UPSIT scores and blood levels of Mn, Pb, Fe, Cu or Zn, those in the top UPSIT tertile had higher Mn levels than those in the bottom UPSIT tertile ($P = 0.038$), a paradoxical finding that has been observed by others. Mn blood levels were positively correlated with the number of months a welder had worked on the bridge ($r = 0.35$, $P = 0.02$).

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Slide: Clinical Chemosensory

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

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Mechanical obstruction of odorant flow to olfactory receptor sites is likely one of the primary causes of olfactory loss in chronic rhinosinusitis (CRS) patients, in addition to other possible pathological mechanisms. However, quantifying the functional impact of various nasal obstructions and the subsequent treatment outcomes using acoustic rhinometry (AR), rhinomanometry (RM) or CT scans is uninformative: numerous studies have shown a poor correlation between conventional measurements and patients' subjective symptoms or measures of psychophysical performance. In this preliminary study, 8 CRS patients were assessed using AR, RM and CT scans, and their odor identification and olfactory thresholds (to carvone, D-limonene and phenethyl alcohol) were also obtained. Using computational fluid dynamics techniques, we converted each patient's CT scans into an anatomically accurate 3-D numerical nasal model that was used to simulate nasal airflow and predict odorant delivery rates to the olfactory epithelium. The accuracy of the models were verified by comparing model predictions with measured nasal resistance, airflow rates, pressure drops, area-distance function, etc. Variations in measured olfactory sensitivity were then correlated with differences of nasal airflow and odorant delivery rates to olfactory neuroepithelium. Our ultimate goal is to quantitatively reveal the underlying conductive mechanisms contributing to olfactory loss in CRS that cannot be determined using

the existing tools. In the future, such modeling techniques may provide quantitative evaluation of treatment for CRS and an important pre-treatment guide to optimize airflow and odorant delivery in human nose.

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Symposium: Presidential: Why Have Neurogenesis in Adult Olfactory Systems?

Adult Neurogenesis in the Mammalian Brain

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The hippocampus undergoes substantial neurogenesis in adulthood—in the young adult rat many thousands of new neurons are produced everyday. Adult neurogenesis in the hippocampus has been observed in many mammalian species, including humans. The formation of new neurons in this brain region is affected by hormones and experience. Numerous forms of stress, including exposure to predator odor, have been shown to exert a negative influence on cell proliferation in the hippocampus. On the other hand, enriched environments, learning and physical activity have a positive effect on the number of new neurons. These findings suggest potential roles for adult neurogenesis. Since the hippocampus is important for certain types of learning and memory, as well as for anxiety and stress regulation, new neurons may participate in these functions.

Symposium: Presidential: Why Have Neurogenesis in Adult Olfactory Systems?

The Olfactory Pathway of Decapod Crustaceans—A Model for Life-Long Neurogenesis

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Decapod crustaceans are similar to vertebrates in having life-long neurogenesis in both the peripheral and central olfactory pathways. Work from various labs over the last decade has revealed that in crustaceans adult neurogenesis in the olfactory organ occurs as a slow “longitudinal” turnover and net addition of receptor units whereas in the central olfactory pathway it mainly consists of a slow, continuous addition of neurons. Compared to the situation in the vertebrate olfactory bulb, adult neurogenesis in the central olfactory pathway of crustaceans shows similarities but also distinct differences. Most notable among the similar features is the susceptibility of crustacean adult neurogenesis to a host of external and internal parameters, such as afferent input, serotonin level, season of the year, environmental richness, and social status. In contrast to the vertebrate situation, the predominant neuron type produced in adult crustacean brains are projection neurons, while new local interneurons are generated in lower number and only in some species. Furthermore, recent studies indicate that in crustaceans very few neuroblasts serve as neuronal stem cells whereas in the vertebrate olfactory bulb new neurons originate from more numerous astroglia-like precursors. Further analysis of adult neurogenesis in decapod crustaceans will reveal more about its underlying cellular mechanisms and its functions in olfactory information processing. Comparing these data with the situation in vertebrates might ease

identification of fundamental principles in the layout of the olfactory pathway that are served by and thus require adult neurogenesis.

Symposium: Presidential: Why Have Neurogenesis in Adult Olfactory Systems?

Sensory Enrichment, Neurogenesis and Olfactory Learning in an Adult Insect

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In the adult cricket brain, new neurons are being produced throughout the insect's life. This neurogenesis occurs in the main integrative centers of the insect brain, the mushroom bodies (MB), where the neuroblasts responsible for their formation persist after the imaginal moult. The rate of production of new neurons is controlled not only by internal cues such as morphogenetic hormones (juvenile hormone and ecdysone) but also by environmental cues. Crickets reared in a sensory enriched environment presented an increase in neuroblast proliferation as compared to crickets reared in an impoverished environment. Conversely, unilateral sensory deprivation led to reduced neurogenesis in the mushroom body ipsilateral to the lesion. In search of a functional role for the new cells, we specifically destroyed mushroom body neuroblasts in young adults by gamma-ray irradiation of the head. We developed a learning paradigm adapted to the cricket, that we called "escape paradigm." Using this operant associative learning test, we showed that crickets lacking neurogenesis exhibited delayed learning and reduced memory retention of the task, especially when olfactory cues were used. Our results suggest that environmental cues are able to influence adult neurogenesis and that in turn, newly generated neurons participate to olfactory integration, optimizing learning abilities of the animal, and thus its adaptation to its environment. However, we have to consider and discuss that learning in insects cannot always be attributed to new-born brain neurons because in many insect species neurogenesis is completed during preimaginal stages.

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Symposium: Presidential: Why Have Neurogenesis in Adult Olfactory Systems?

Integrating New Neurons into the Adult Olfactory System

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In the adult olfactory bulb, newly born neurons are constitutively generated throughout life and form an integral part of normal functional circuitry. This process of late neurogenesis is subject, at various stages, to modulation and control by external influences, suggesting strongly that it represents a plastic mechanism by which the brain's performance can be optimized according to the environment in which it finds itself. But optimized how? And why? This presentation will concentrate on such functional questions regarding neurogenic plasticity. After outlining the processes of adult neurogenesis in the olfactory system, and after discussing their reg-

ulation by internal and environmental influences, we shall ask how existing neuronal circuits can continue to work in the face of constant cell arrivals and departures, and explore the possible functional roles that newborn neurons might subserve in the adult olfactory system. In particular, we shall report the degree of sensitivity of the bulbar neurogenesis to the level of sensory inputs and, in turn, how the adult neurogenesis adjusts the neural network functioning to optimize sensory information processing. We will bring together recently described properties and emerging principles of adult neurogenesis that support a much more complex role for the adult-generated cells than just providers of replaceable units. Throughout, and concentrating exclusively on mammalian systems, we shall stress that adult neurogenesis constitutes another weapon in the brain's armory for dealing with a constantly changing world.

Poster: Central Taste and Chemosensory Behavior

Chorda Tympani (CT), Greater Superficial Petrosal (GSP) and IXth Nerve Terminal Fields in Hamster Solitary Nucleus (NTS)

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In mammals, GSP afferents innervate taste cells in palatal and nasoincisor mucosae, while CT and IXth nerves (IX) innervate taste cells of fungiform and circumvallate papillae, respectively. The nerves terminate centrally in the rostral pole of the NTS. In this study, we quantify anatomical overlap among terminal fields of these taste nerves in adult hamster NTS. We used triple fluorescent nerve labeling to visualize CT, GSP, and LT-IX terminal fields in NTS. CT, GSP, and IX were isolated, cut, and labeled with unique dextran amine conjugates. After 3–7 days survival, animals were sacrificed and perfused. Fixed brains were sectioned horizontally and sections examined by confocal microscopy. Serial optical sections through physical sections of complete terminal fields were analyzed offline. Data so far show that IX terminal field appears ~100 μm dorsal to GSP and CT, which enter the rostral NTS nearly coincidentally in the dorsal-ventral plane. GSP terminal field extends ~100 μm ventral to that of IX, while CT terminal field extends ~50 μm ventral to that of GSP. IX and GSP terminal field volumes are roughly similar and appear to be greater in volume than CT field. This difference is attributable to the restricted caudal extent of CT terminal field. All three terminal fields overlap extensively throughout the dorsal-ventral plane. Nearly the entire volume of GSP field overlaps with IX or CT terminal fields, while IX and CT fields enjoy ~50 μm of unique territory in dorsal and ventral NTS, respectively. These results provide normative data for studies of development in the hamster central taste system.

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Poster: Central Taste and Chemosensory Behavior

Ca²⁺ Imaging of Primary Gustatory Afferents in the Vagal Lobe of Goldfish

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The primary gustatory nucleus in goldfish has a characterized anatomy whereby gustatory afferents terminate in distinct laminae. We injected Ca²⁺ green dextran in the vagus nerve and allowed 3-days recovery to allow filling of the primary nerve terminals in the vagal lobe. Vagal lobe slices were prepared for *in vitro* recording. The primary afferent fibers were electrically stimulated with pairs of 2.0 ms pulses separated by 30 ms while optically recording Ca²⁺ signals from the layers containing primary afferent terminals. Hence the Ca²⁺ signal arose only from the primary afferent fibers. Results showed paired pulse facilitation in that significantly more Ca²⁺ was detected after the second pulse than the first. This indicates more primary afferent terminals are activated by the second pulse than the first. In addition, the area of increase in Ca²⁺ signal after the second stimulating pulse was broader than that observed after the first pulse, suggesting that the first pulse activates the internal circuitry of the sensory layer of the vagal lobe. Since the recorded signal arises entirely from primary afferents, these findings suggest that the primary afferent terminals may be under tonic inhibition which is released by the initial stimulus pulse.

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Poster: Central Taste and Chemosensory Behavior

Temporal Patterns of Neural Activity in the Nucleus of the Solitary Tract of C57BL/6BYJ Mice

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The spontaneous and evoked activity of taste-responsive neurons can be characterized in terms of mean response rates over periods with specific durations, but the temporal patterns of activity within those periods may also contain important information. The goal of this work was to examine temporal patterns of single-unit activity in the rostral nucleus of the solitary tract (NST) of C57BL/6ByJ mice. The extracellular activity of 39 NST cells was measured in anesthetized animals. The spontaneous firing patterns of neurons were investigated by plotting the distributions of interspike intervals (ISIs). ISIs less than 10 ms were especially common in some neurons, which gave evidence of a preferred firing interval. In general, the presence of a preferred interval was a characteristic of a cell and was not changed by application of taste stimuli, and preferred intervals were more likely to be found in neurons with salt- or acid-oriented response profiles than in those with sugar-oriented profiles. Temporal patterns of taste-evoked responses were also examined and were found to vary across compounds that are thought to taste sweet to mice. This variation meant that the sweeteners evoked similar across-neuron patterns of activity across a 5-s evoked period, but did not when evoked periods less than 1 s were used. Given that perceptions of sweetness are likely to occur in less than a second, these results suggest that factors besides across-neuron patterning make a substantial contribution to taste quality perception.

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Central Taste and Chemosensory Behavior

Computational Models of Temporal Firing Properties of Single Neurons in the Nucleus of the Solitary Tract

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Electrophysiological responses to taste in the brain stem have most often been characterized by their mean firing rate rather than by the temporal structure of the spike train. However, previous data from our lab have shown that firing rate across stimulus repetitions can vary widely in some NTS cells and further, that those cells which showed most variable firing rates nevertheless conveyed information about taste stimuli via spike timing. In the present project, we tested the hypothesis that taste-sensitive neurons could generate non-random spike trains given a random spike train as input. Numerous computational models of taste-sensitive neurons were built based on physiological properties obtained from *in vitro* recordings reported in the literature. Strength of input and various morphological parameters were systematically varied and the results were assessed by metric-space analysis and by statistical tests for randomness. Results suggest that taste-sensitive neurons can generate more reliable temporal patterns of response with higher frequency or stronger inputs. However, the length of the dendrite, the number of dendritic branching points and the distribution of the synapses do not play a significant role in determining a neuron's temporal firing properties. These simulation results suggest that precise spike timing can be determined by intracellular biophysical parameters and the distribution and strength of the excitatory inputs, without the need for inhibitory feedback.

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Poster: Central Taste and Chemosensory Behavior

Presynaptic Nicotinic Receptors Regulate Glutamate Release in the Nucleus of the Solitary Tract of the Rat

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The nucleus of the solitary tract (NST) is the first relay in the processing of gustatory and sensory visceral information. We have shown previously that NST somata express nicotinic (nAChRs) and muscarinic receptors that may shape the information processing in the NST. Here, we report that in rat brainstem slices, activation of presynaptic nAChRs by picospritzer applications of nicotine (500 μ M, 70 ms) to NST somata facilitates spontaneous release of glutamate. The effect of presynaptic facilitation lasted for \sim 1 min, upon a single picospritzer application; and it could be evoked as often as every 3 min. Analysis has shown that the effect was characterized by a significant increase in the mean miniature postsynaptic current (mPSC) frequency ($P < 0.004$, paired one-tailed) and an insignificant increase in the mPSC amplitude ($P < 0.07$, paired two-tailed). The effect was resistant to tetrodotoxin (0.5 μ M), a blocker of sodium action potentials, and 20 nM methylcaconitine, a blocker of $\alpha 7$ nAChRs; but it was blocked by 10 μ M mecamylamine, a broad spectrum nAChR blocker. The effect was

Ca²⁺-dependent, because it was eliminated when 2 mM Ca²⁺ in the extracellular solution was replaced with 0 mM Ca²⁺+5 mM EGTA; but it was resistant to 200 μM Cd²⁺+200 μM Ni²⁺, blockers of voltage-gated Ca²⁺ channels. Intriguingly, the effect was observed in only ~20% of NST neurons, suggesting that it defines a subpopulation of NST neurons confined to a certain, unknown at this point, function. We conclude that the observed effect of presynaptic facilitation results from elevations in [Ca²⁺]_i in presynaptic glutamatergic terminals due to a direct Ca²⁺ influx through non-α7 nAChRs.

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Poster: Central Taste and Chemosensory Behavior

Characteristics of Inhibitory Postsynaptic Activity of Rat Inferior Salivatory Nucleus Neurons

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Neural information derived from stimulating taste buds initiates reflex salivary secretion. The efferent limb of this reflex is composed of secretomotor neurons contained in the salivatory nucleus situated along the medial border of the nucleus of the solitary tract (NST). We have investigated synaptic activity of neurons of the inferior salivatory nucleus (ISN) that control the parotid and von Ebner salivary glands. Stimulation of the NST evokes mixed excitatory and inhibitors postsynaptic potentials in the ISN neurons. To characterize the inhibitory synaptic activity whole-cell recordings and immunocytochemical staining for GABA and glycine was performed on identified ISN neurons in rat brainstem slices. ISN neurons responded to both GABA and glycine with membrane hyperpolarization and a decrease in input resistance in the presence of 2 μM tetrodotoxin (*n* = 7) indicating that ISN neurons have both GABA and glycine receptors. Immunocytochemical labeling also revealed that about a 50% of the ISN neurons were positive for GABA and glycine. Inhibitory postsynaptic potentials (IPSP) evoked by electrical stimulation of the NST were studied under glutamate receptor block. The amplitude of the IPSPs was not significantly altered by the glycine receptor antagonist strychnine (2 μM, *n* = 9, *P* > 0.05), but was eliminated by the GABA_A receptor antagonist bicuculline (10 μM, *n* = 8). This result indicates that inhibition of ISN neurons is mediated by GABA_A receptors driven via synaptic input from the NST, but glycine receptors receive input from the other brain regions.

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Poster: Central Taste and Chemosensory Behavior

Excitatory Postsynaptic Activity of the Rat Inferior Salivatory Nucleus Neurons

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Stimulation of taste buds results in a number of reflex activities organized at the brainstem level. Important to taste transduction is the reflex secretion of saliva. The output limb of this reflex arc originates in a column of parasympathetic motor neurons (the salivatory nucleus) closely associated with the brainstem taste relay nucleus—the nucleus of the solitary tract (NST). To characterize this reflex we have focused on the inferior salivatory nucleus (ISN) responsible for the control of the parotid and von Ebner salivary glands. Stimulation of the NST evokes postsynaptic potentials (PSP) in the ISN neurons which have both an excitatory and inhibitory components. To characterize the excitatory component we have used whole-cell recordings and immunocytochemical staining for ionotropic glutamate receptor subtypes on identified ISN neurons in rat brainstem slices. The inhibitory component of the PSPs was blocked by superfusion of the GABA_A receptor antagonist, bicuculline. ISN neurons were strongly positive for all the glutamate receptor subtypes including NMDA (NR1, NR2A, NR2B), AMPA (GluR1, GluR2, GluR3, GluR4), and kainate (GluR5-7, KA2). In whole cell recordings the NMDA receptor antagonist APV (50 μM) and the AMPA/kainate receptor antagonist CNQX (10 μM) both decreased the amplitude of the EPSPs. Mixtures of CNQX and APV eliminated the EPSPs. These results suggest that excitatory postsynaptic activity of ISN neurons induced by synaptic input from the NST is mediated by NMDA, AMPA and kainate receptors.

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Poster: Central Taste and Chemosensory Behavior

Solitary Nucleus–Reticular Formation Projections in a Neonatal Slice Preparation

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Data obtained from *in vivo* studies suggest that projections from the rostral nucleus of the solitary tract (rNST) to the subjacent reticular formation (RF) constitute a pathway through which taste stimuli influence oromotor responses of ingestion and rejection. To further investigate the underlying neural mechanisms, we conducted anatomical, behavioral and physiological studies to determine if this substrate could be studied *in vitro*. Injections of fluorescent microspheres into the hypoglossal nucleus of neonatal rats retrogradely labeled pre-oromotor neurons in the intermediate zone of the RF (IRt) in a distribution identical to adult rats. Neurons in the RF became opaque to iDIC microscopy after P14 however, thus limiting the ability to record from identified neurons in a slice. Although RF neurons in younger animals were visible under iDIC, it was unclear whether these younger animals also produce “adult-like” oromotor behavior. One study suggested that neonatal rats gaped in response to QHCl, however another study was more equivocal. Thus, we re-evaluated the capacity of rats from age P2–P14 to gape in response to QHCl (0.01 M). Although not all rats gaped, the likelihood of gaping as well as the magnitude of the gape response increased in a graded manner over time. Lastly, we determined from extracellular and patch clamp recordings, that neurons in the IRt could be excited and suppressed by electrical stimulation of the rNST. Because electrical stimulation can elicit both licks and gapes *in vivo*, this may provide an approach to studying taste-oromotor pathways in a slice preparation.

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Poster: Central Taste and Chemosensory Behavior**The Organization of the Gustatory Neural Network in the Hamster Brainstem**Y.K. Cho¹ and C. Li²¹Kangnung National University, Kangnung, Kangwondo, South Korea and ²Anatomy, Southern Illinois University, Carbondale, IL, USA

Taste information elicited from the anterior tongue is first carried to the nucleus of the solitary tract (NST) and then to the parabrachial nuclei (PbN), from which taste information is further transferred to forebrain gustatory nuclei. In the present study we examined the gustatory neural connectivity among gustatory nuclei in the brainstem. Three recording/stimulating electrode assemblies were used first to record taste neurons and then electrically stimulate the nuclei in order: left PbN, right PbN and left NST. A fourth electrode, a micro glass capillary was used to record taste cells in the right NST. A total of 45 taste cells were isolated in the PbN and the responsiveness of each cell to electrical stimulation of the contralateral PbN was examined: 5 neurons (11%) were antidromically invaded and 9 neurons (20%) responded orthodromically. In the NST, 123 taste cells were isolated and responses of each NST cell to the stimulation of bilateral PbN were tested. Eighty-one percent of NST taste cells were ipsilateral PbN-projection cells. The same proportion of NST taste neurons received descending input from the ipsilateral PbN. In contrast, 3 cells sent axons to the contralateral PbN and 47 NST neurons received descending influence from the contralateral PbN. The influence of the NST taste neurons to the contralateral NST stimulation was examined from 100 NST taste neurons: 58 neurons were activated orthodromically and 11 antidromically. These data demonstrate the intricate interconnection among the four gustatory nuclei in the brainstem.

Supported by NIDCD006623.

Poster: Central Taste and Chemosensory Behavior**Vagal Gustatory Reflex Systems in Goldfish**

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In goldfish, the primary sensory nucleus for vagally mediated taste is part of a complex laminated lobe. The sensory layers of this lobe are equivalent to the n. solitarius while the motor layers contain motoneurons equivalent to the n. ambiguus. The sensory layers are coupled to the motor layers via a simple reflex arc homologous to the solitario-ambiguo reflex system of mammals. To detail the morphology of neurons that form this reflex system in goldfish, the retrograde tracer, biocytin, was injected into the motor zone of *in vitro* slices. Diverse neurons were retrogradely labeled in the sensory zone, from the surface (layer II–III) to the deepest portion (XI). Most labeled neurons had a monopolar or bipolar soma with radially-directed dendrites branching in layer IV, VI, and IX—the layers of termination of primary vagal gustatory inputs. These projection neurons were organized topographically along the dorso-ventral axis, projecting only to the immediately subjacent motoneurons. In functional imaging experiments, motor neurons were retrogradely labeled by injections of calcium green dextran

(Ca⁺⁺ indicator) into the vagus nerve. Increases in Ca⁺⁺ followed electrical stimulation in the sensory zone of *in vitro* slices. These Ca⁺⁺ responses were enhanced by application of the GABA_A receptor antagonist, bicuculin, suggesting tonic inhibition of the reflex pathways by GABAergic systems. Finally, reflex activation of the motoneurons was blocked by application of the glutamate antagonist DNQX suggesting that this gustatory reflex system utilizes glutamate acting on AMPA/kainate receptors as the principal neurotransmitter.

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Poster: Central Taste and Chemosensory Behavior**Tastant-Induced C-Fos Expression in the NST of Mice that Don't Taste**

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ATP is an essential neurotransmitter coupling taste buds to gustatory nerves. Genetic deletion of the ionotropic purinergic receptor subunits P2X2 and P2X3 eliminates neural responses to all taste stimuli. However, these P2X2/P2X3 KO mice still avoid citric acid and caffeine, as well as high concentrations of quinine hydrochloride (QHCL). We hypothesize that the P2X2/P2X3 KO mice detect some noxious substances via laryngeal and/or pharyngeal/esophageal solitary chemoreceptor cells. We examined cFos-like immunoreactivity (c-FLI) in the nucleus of the solitary tract (NST) of P2X2/P2X3 KO mice after stimulation with 1 mM QHCL, 150 mM monosodium glutamate (MSG), or water. Water-induced c-FLI did not differ between P2X2/P2X3 KO mice and wild-type controls. MSG-induced c-FLI was moderately reduced throughout the NST in P2X2/P2X3 KO mice compared to wild-type controls. QHCL-induced c-FLI was elevated compared to water-induced c-FLI, within the caudal NST of P2X2/P2X3 KO mice, where afferents from the larynx and pharynx terminate. These preliminary results suggest that chemosensory input reaches the caudal NST in P2X2/P2X3 KO mice, probably arising from the laryngeal or pharyngeal/esophageal nerves. This input may be sufficient to allow the P2X2/P2X3 KO mice to avoid certain tastants. Future directions include intra-oral cannulation to control the volume washed across the tongue and superior laryngeal nerve transactions in both P2X2/P2X3 KO mice and controls.

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Poster: Central Taste and Chemosensory Behavior**Taste-Induced C-Fos Expression in the Rostral Portion of the Solitary Tract Nucleus of Neonatal Rats**

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Taste-induction of Fos expression in the rostral portion of the solitary tract (NTSr) was previously examined in adults showing that Fos-immunoreactive (FI) cells were prominent in the NTSr, for quinine monohydrochloride (QHCl) in the medial zone of the nucleus

while the sucrose (S) elicited FI concentrated in the lateral area, little is known about taste stimuli-induced activation of brainstem neurons in neonatal rats. The aim of this study was to compare the distribution of FI following intraoral stimulation with, QHCl, S and NaCl in rats of 5, 15, and 25 days of age. Subjects were isolated from the mother 3 h before the stimulation and later on the pups were stimulated with some of the following solutions: H₂O, QHCl 0.03, 0.003 M, S 0.1 M and NaCl 0.1 M and 90 min after subjects were anesthetized the brain was removed and processed for Fos immunostaining. Data showed that FI was increased in this nucleus in QHCl stimulation at all ages compared with S, NaCl and non-stimulated (ANOVA, $P < 0.05$). No differences were found in the FI between H₂O and NaCl. In the NTSr, FI cells were distributed mainly in the medial region after QHCl and in the lateral region after S at all ages. The number of FI cells in the NTSr after QHCl stimuli peaked on P15 and then decreased on day P25. Data show that taste-specific responses distribution, are already present at birth and may change during NTSr maturation. The number of FI in the NTSr between neonates and adults might partly depend on the reorganization of the neuronal circuitry occurring early in life as a result of dietary experiences.

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Poster: Central Taste and Chemosensory Behavior

Differential Effects of Cross-regeneration of the Lingual Gustatory Nerves on Quinine-Stimulated Gaping and fos-like Immunoreactivity in the Nucleus of the Solitary Tract

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An intact glossopharyngeal nerve (GL) is essential for normal unconditioned quinine-stimulated gaping behavior and fos-like immunoreactivity (FLI) in the gustatory nucleus of the solitary tract (gNST), especially in the medial-dorsal subfield (MD). Transection of the GL, but not the chorda tympani nerve (CT), attenuates gaping behavior and MD-FLI in response to quinine, which is restored upon GL nerve regeneration. In this study, the GL and CT were cross-regenerated. Some rats had the central CT-stump sutured to the peripheral GL-stump (CT → PosteriorT); other rats received the converse surgery (GL → AnteriorT). Histological analysis of taste buds confirmed nerve regeneration. Numbers of gapes elicited by 3 mM quinine in CT → PosteriorT ($n = 5$) and sham-operated (SHAM-Q, $n = 5$) rats were similar and significantly higher than those observed in water-stimulated controls (SHAM-W, $n = 6$), while the number of quinine-stimulated gapes in GL → AnteriorT rats ($n = 6$) was comparable to that observed in SHAM-W rats. Likewise, quinine-stimulated MD-FLI in CT → PosteriorT and SHAM-Q rats was comparable and significantly higher than MD-FLI in both SHAM-W and quinine-stimulated GL → AnteriorT rats at the most rostral level of the gNST. These findings suggest that unconditioned quinine-induced gaping and MD-FLI in the rostral gNST are more dependent on the taste receptor field stimulated than on the nerve that transmits the signal.

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Poster: Central Taste and Chemosensory Behavior

Licking and Gaping Elicited by NST Microstimulation

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Bitter compounds evoke a distinct distribution of Fos-like immunoreactive cells (FLI) concentrated in dorsomedial rNST. IXth nerve section, but not decerebration disrupts this pattern, paralleling the consequences of these manipulations on oral rejection behavior (gaping), thus suggesting that the region of Fos expression defines an afferent trigger zone for bitter-elicited protective reflexes. We tested this using rNST microstimulation. Microelectrodes and intraoral cannulae were implanted under electrophysiological guidance. Rats were tested with taste (0.3 M sucrose and 3 mM quinine) and electrical stimulation (0.2 ms biphasic pulses, 100 Hz) at varying intensities (5–40 μ A) and durations (0.1–24.3 s), and then 30 mM quinine was infused to elicit FLI. NST microstimulation was effective for eliciting licking and gaping, and the amount of oral behavior was a positive function of current intensity and duration. Licking was elicited in most (7/8) animals with placements in rNST, but gaping was observed in half (4/8). 2/4 rats had a lower threshold for licking than gaping but the reverse was never true. Correlations between the number of gapes and distance from the densest FLI failed to reveal a systematic relationship ($r = -.25$, $P > 0.1$). Interestingly, however, only one subject had a placement centered in the FLI, and this was the single instance where gaping was the dominant behavior. These results suggest that the substrate for gaping involves neurons with a more limited anatomical extent and perhaps a higher threshold than for licking, but defining the critical topography requires further investigation.

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Poster: Central Taste and Chemosensory Behavior

Melanin Concentrating Hormone Increases Brief-Access Licking for Sucrose and Water but Not Quinine Hydrochloride

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Previously we showed that 3V melanin concentrating hormone (MCH) injections (5 μ g) increased sucrose intake by increasing lick rate early in the meal and the mean lick-burst size, suggesting enhanced gustatory evaluation. Therefore, we evaluated brief-access (20 s) licking for water, sucrose and quinine hydrochloride (QHCl) solutions after MCH/vehicle treatment. Under vehicle, licking for sucrose increased monotonically with concentration (0.015 M to 1 M). Licking for weak concentrations of QHCl (0.001–0.03 mM) was comparable to that for water, but declined exponentially across the three strongest concentrations (0.1–1 mM). MCH uniformly increased licking for all concentrations of sucrose, and water ($P < 0.001$). When sucrose responses were standardized to water, the effect was completely lost, indicating that although MCH increased

avidity for the tastants, it not did modify the concentration response function. These results are consistent with the effects of food deprivation on licking for sucrose and water in brief access tests. MCH also increased responding for water and weak concentrations of QHCl, but it had no effect on licking for the three strongest concentrations of QHCl. Therefore, MCH did not produce non-specific increases in oromotor activity, and it did not change the perceived intensity of the tastants. We conclude that MCH enhances the gain of responses to normally-accepted stimuli at a phase of processing that occurs after the initial gustatory appraisal and after the decision to accept or reject the taste stimulus.

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Poster: Central Taste and Chemosensory Behavior

Injection of Cholecystinin into the Waist Area of the Parabrachial Nucleus Increases Taste Reactivity Responses to Intra-oral Infusion of Quinine in Rats

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Neurons in the waist area of the parabrachial nucleus (W) process taste input and project to medullary regions that generate taste reactivity responses. Cholecystinin (CCK) is a gut-brain peptide that has been implicated in satiation. Since CCK and its receptors are located in W, we hypothesized that this peptide influences the processing of taste information thereby altering the oromotor responses to taste input. To test this hypothesis, 10 mM CCK (400 nl) was injected into the pons through implanted guide cannula (Plastics One) in 12 male Wistar rats immediately before intra-oral infusion of 0.01 M NaCl, 0.01 M sucrose and 0.003 M quinine HCl. The injection of CCK into W increased the number of gapes and tongue protrusions performed following intra-oral infusion of quinine as compared to when vehicle was injected on the following day ($n = 5$, $P < 0.05$). Although there was a trend for fewer ingestive behaviors following intra-oral infusion of NaCl when CCK was present, this effect was not statistically significant ($P = 0.098$). Taste reactivity responses to sucrose were not altered by CCK injections. Indicating that these effects of CCK were due to action within or near W, injections just dorsal, medial and rostral to W did not alter oromotor responses to the intra-oral infusion of any tastant used. These preliminary results suggest that CCK may act within the waist area of the parabrachial nucleus to alter oromotor behaviors to taste input.

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Poster: Central Taste and Chemosensory Behavior

Altered Parabrachial Taste Processing in Obese OLETF Rats

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Otsuka-Long-Evans-Tokushima-Fatty rats (OLETF) lack functional CCK-1 receptors, are hyperphagic, and gradually develop obesity and diabetes during their life span. Recently we have reported a greater preference for sucrose in prediabetic OLETF compared to age-matched, lean controls (LETO). This study investigated sucrose taste processing in the pontine parabrachial nucleus using a semi-chronic preparation that allowed data collection over several recording sessions (e.g., 12, 13, 15, 16, 19, and 20 weeks of age). Forty-four taste neurons were tested and, using cluster analysis, categorized based on response profile to 0.1 M NaCl, 0.01 M citric acid, 0.003 M QHCl, and six sucrose concentrations (0.01, 0.03, 0.1, 0.3, 1.0, and 1.5 M). These neurons were further divided into Block 1 (weeks 12 and 13) and Block 2 (weeks 15–20) representing different stages of glucose tolerance. For NaCl-best cells, the Block 2 response rates to 0.1, 0.3, 1.0, and 1.5 M sucrose were 21–47% smaller relative to Block 1 sessions in OLETF, but not LETO rats. Sucrose-best cells (S-best), on the other hand, were 15–69% more responsive to these concentrations of sucrose during Block 2 sessions relative to Block 1 sessions both for OLETF and LETO rats. The net effect was to alter the across-neuron pattern evoked by sucrose. That is, advancing age in OLETF rats, but not LETO rats, increased the percentage of sucrose information carried by S-best cells. This effect may contribute to the increased behavioral sensitivity to palatable meals in this strain.

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Poster: Central Taste and Chemosensory Behavior

The Properties of Inhibitory Taste Neurons in the Parabrachial Nucleus of Rats

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In rodents, the parabrachial nucleus (PBN) is the second relay of the taste system, that receives projection from the gustatory portion in the nucleus solitary. Our earlier study found that there was some taste neurons with inhibitory response to taste stimuli in addition to the majority of taste neurons with excitatory response in PBN. The aim of the present study was to characterize the spontaneous and evoked activities of the inhibitory gustatory neurons of PBN. The single PBN neurons were recorded extracellularly and identified by the responses of the neurons to taste stimuli including 0.3 M NaCl, 0.01 M HCl 0.003 M QHCl and 0.5 M sucrose in the anesthetized rat. A total of 19 inhibitory neurons were studied. The spontaneous firing rates of these neurons were in the range of 0.5–30 Hz and were depressed remarkably and quickly, from 3.89 ± 1.85 Hz to 0.46 ± 0.09 Hz, by application of taste stimuli. The inhibition lasted about 5–80 s. Most of them responded to more than one of the tastants. On the basis of their largest inhibitory responses to the four basic stimuli. These inhibitory taste neurons were classified as follows: NaCl-best (56.3%); HCl-best (12.5%); QHCl-best (12.5%) Sucrose-best (18.7%). These findings suggest that there are inhibitory taste neurons in PBN that may play role in modulation of gustatory information.

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Poster: Central Taste and Chemosensory Behavior**Efferent Projection from the Bed Nucleus of the Stria Terminalis Suppresses Activity of Taste-Responsive Neurons in the Hamster Parabrachial Nuclei**C. Li¹ and Y.K. Cho²¹Anatomy, Southern Illinois University, Carbondale, IL, USA and ²Physiology & Neuroscience, Kangnung National University College of Dentistry, Kangnung, Kangwon-do, South Korea

Although the reciprocal projections between the bed nucleus of the stria terminalis (BNST) and the gustatory parabrachial nuclei (PbN) have been demonstrated neuroanatomically, there is no direct evidence showing that the projections from the PbN to the BNST carry taste information or that descending inputs from the BNST to the PbN modulate the activity of PbN gustatory neurons. In the present study, we recorded from 105 taste-responsive neurons in the PbN and examined their responsiveness to electrical stimulation of the BNST bilaterally. Twelve neurons (11.4%) were antidromically invaded from the BNST, mostly from the ipsilateral side (11 cells), indicating that a subset of taste neurons in the PbN project their axons to the BNST. The BNST stimulation induced orthodromic responses on most of the PbN neurons: 103 out of 105 (98.0%), including all projection units that were modulated by BNST stimulation. This descending modulation on the PbN gustatory neurons was exclusively inhibitory. We also confirmed that activation of this efferent inhibitory projection from the BNST reduces taste responses of PbN neurons in all units tested. The BNST is known to be involved in sodium appetite and taste aversion learning. The BNST is also participates in the neural circuits that involve stress-associated feeding behavior. Therefore, this neural substrate may be important in taste aversion learning and sodium appetite as well as the stress-elicited alteration in ingestive behavior.

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Poster: Central Taste and Chemosensory Behavior**Ensemble Responses of Gustatory Cortical Neurons Accurately Predict Tastant Identity**

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Gustatory neurons respond to tastants with patterns of spikes that are highly variable—both throughout the time course of a single response and across repeated deliveries of the same stimulus. Yet current analyses of coding in the gustatory system rely heavily on time and trial averaging. Here we investigate cortical processing of taste stimuli using Hidden Markov Modeling (HMM), an analysis method that does not average over time or trial, but instead classifies responses in individual trials as progressions through different neural states. We recorded extra-cellular activity from small ensembles of well isolated neurons (6–12 simultaneously) in the gustatory cortex of awake rats while the 4 basic tastes were delivered via intra-oral cannulae. We found that the ensembles reliably transition through a taste-specific series of states (defined by the firing rates of each neuron). This allowed us to predict tastant iden-

tity from single trial responses and compare these predictions to those computed using a method based on averaged firing rates. HMM predictions were consistently more accurate than the average-based method (10 of 12 sessions). Examination of HMM solutions revealed the source of their high-quality performance: the exact timing of the progression through a taste-specific set of states varied from trial to trial, such that some of the information available in single trials using HMM was lost in the across-trial averages. This indicates that the gustatory cortex may utilize specifically timed changes in firing patterns of neural ensembles to process taste information.

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Poster: Central Taste and Chemosensory Behavior**Gustatory Cortex Encodes Multiple Features During an Intensity Discrimination Task**C.J. MacDonald¹, M.A. Nicolelis² and S.A. Simon²¹Psychological and Brain Sciences, Duke University, Durham, NC, USA and ²Neurobiology, Duke University, Durham, NC, USA

Previous work on taste intensity processing in gustatory cortex (GC) of awake animals relied on integrated neural activity without taking into account the behavior of the animal. To this end, we used chronic multi-electrode recordings in the behaving rat to characterize GC activity during a NaCl intensity-discrimination task. In this task, the rat licked on a “sample” lick spout positioned in the center of the chamber wall and could receive 20 μ L of 30 or 120 mM NaCl (perceptual anchors) during a trial after each of two successive licks. These anchors were rewarded with water after correctly licking on one of two “choice” lick spouts positioned to the left or right of the sample spout, which categorizes the concentration as “High” (120 mM) or “Low” (30 mM). Intermediate NaCl concentrations were also delivered during the experiment but were not rewarded. The probability of categorizing NaCl as “High” increased with NaCl concentration. We identified two sub-populations of neurons whose firing rate monotonically increased or decreased within one lick in response to concentration. We also identified several additional neuron “types” including licking and water sensitive neurons in addition to neurons that changed activity at defined points during the behavioral sequence that makes up a single trial. In summary, these data show that the GC contains many different neuronal types that mediate different aspects of taste-guided behavior.

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Poster: Central Taste and Chemosensory Behavior**Anticipatory Cortical Activity in a Taste Discrimination Task**R.M. Gutierrez¹, M.A. Nicolelis¹ and S.A. Simon²¹Neurobiology, Duke University, Durham, NC, USA and ²Anesthesiology, Duke University, Durham, NC, USA

Previously we have shown that when freely licking rats know what tastant will be delivered, their anticipatory cortical activity can

distinguish among them. Here we explore whether such discrimination would be present when the animal does not know which tastant will be delivered. This was accomplished by recording the activity of neuronal ensembles in the OFC, insula and nucleus accumbens while rats performed a taste discrimination task. For this task, rats were trained to lick an empty tube (10 times) in order to randomly receive NaCl (positive cue) that signaled the availability of a reward (sucrose) or MSG (negative cue) that signaled the delivery of quinine. Importantly, taste cues (NaCl, MSG) and outcomes (sucrose, quinine) were delivered in identical, but independent compartments and rats initiated licking in a similar way in both compartments. The anticipatory activity in the “cue” compartment was similar, meaning that when rats do not know what tastant will be delivered, there is not any anticipatory activity related to the identity of the tastant. However, in the “outcome” compartment, we found differences in the anticipatory activity of the expectation of sucrose or quinine. Of the three cortical areas explored, we found a greater percentage of neurons in the nucleus accumbens that increased firing rate in the “outcome” compared to “cue” compartment. In summary a distributed number of cells in the brain anticipate behavioral outcomes only when the rat knows what reward is coming.

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Poster: Central Taste and Chemosensory Behavior

Gender Differences in Activation Within the OFC in Response to Taste Stimuli with Positive or Negative Valence are Related to Hunger and Satiety

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Previous research supports the hypothesis that valence specific brain activation related to positive and negative taste stimuli involves the orbital frontal cortex (OFC). The physiological states of hunger or satiety influence behavior relating to the reward value of taste stimuli. This study employed fMRI to investigate cortical activation in response to taste stimuli that are high and low in reward value when the subject was hungry or sated. A region of interest (ROI) analysis was conducted to test the hypothesis that gender differences in activation within the OFC to positive (sucrose) and negative (caffeine) stimuli are affected by hunger and satiety. Subjects rated the pleasantness of stimuli using the gLMS, while stimuli were presented to the mouth as 0.3ml boluses in distilled water. Imaging was conducted on a 3T GE scanner. Image analysis was conducted using AFNI. Fit coefficients were subjected to ANOVA to compare the degree of activation in ROIs. Activation within the OFC in response to sucrose in the hunger condition is greater for females than males. In contrast, activation in response to caffeine is greater in the sated condition for females than for males. These findings suggest that gender differences in activation within the OFC in response to valence specific stimuli are related to hunger and satiety.

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Poster: Central Taste and Chemosensory Behavior

Differential Interactions between Amygdala, Insula, Caudomedial and Caudolateral Orbitofrontal Cortex Depend upon the Nature of Stimulus Perceived or Task Performed

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Previously we used fMRI and identified a region of anterior insula/frontal operculum (AIFO) that responded during perception of a taste but not a tasteless solution, irrespective of the nature of the task (detecting its presence, judging its pleasantness or its quality, or passively tasting). In contrast, a region of caudolateral orbitofrontal cortex (OFC) responded preferentially when Ss judged pleasantness, irrespective of whether they received a taste or a tasteless solution. Here we used effective connectivity analyses (ECA) to assess potential differential connectivity between regions depending on the nature of the stimulus or the task. ECA showed greater connectivity between the AIFO and bilateral amygdala when the Ss tasted passively compared to when they evaluated a taste, suggesting that even though BOLD response in the AIFO is not affected by task, information transfer between it and the amygdala differs depending on whether an evaluation is made or not. We also found greater connectivity between caudolateral and caudomedial OFC when Ss received a taste versus a tasteless solution, indicating that connectivity between these OFC taste regions is dependent on sensory stimulation. This finding is in accordance with work by Pritchard *et al.* (2005) highlighting the importance of the caudomedial OFC in gustatory processing.

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Poster: Central Taste and Chemosensory Behavior

Change of Serum and Leptin Receptor Immunoreactivity in the Amygdala of Rats Following Intraoral Infusion of Sweet Tastant

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To determine whether the level of the leptin and the leptin receptor (OB-R) expression in amygdala changes following sweet taste stimuli, the serum leptin concentration was measured by using rat leptin RIA kit, long form of leptin receptor (OB-Rb) mRNA in the brain sections was examined by *in situ* hybridisation (ISH) and the expression of OB-R was assessed by immunohistochemistry ABC method with a highly specific goat anti-OB-R antibody. Comparing with the control group (intraoral infusion of distilled water), the level of serum leptin increased in the sweet group (sucrose and saccharin) ($P < 0.05$). Many neuronal bodies and dendritic processes in amygdala, which have intensive relationship with taste and feeding, showed leptin receptor immunoreactivity (LR-IR). But the count of positive-stained cells in amygdala showed no significant difference between the taste stimulation group and the control group. The level

of OB-Rb mRNA expression increased in the basolateral part of the amygdala (BLA), while no different expression was found in the central nucleus of amygdala (CeA) after sweet tastant intraoral infusion. These findings lead us to study possible effects of leptin on taste responses. Probably, leptin influences food intake by means of the sense of taste.

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Poster: Central Taste and Chemosensory Behavior

Taste Guided Behavior in *Drosophila melanogaster* to Traditional Psychophysical Test Solutions

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The ability to perceive the taste quality of a food source is critical for the survival of an organism. Flies are omnivores and have very similar taste sensitivities as humans with regard to attractants and repellents. Like humans, they are attracted to and ingest many sugars and dilute salts and avoid ingesting toxins and acids. We have tested flies from the wildtype laboratory strain Canton-S (CS) for their taste responses to a variety of compounds considered sweet or bitter by humans. Many of these compounds have never before been tested in *Drosophila*. Flies responded to and rejected many stimuli that humans find bitter. Flies reject quinine, caffeine, denatonium, and MgSO₄. On the other hand, flies appear insensitive to PROP, PTC, SOA, limonin, cyclo-LeuTrp, epicatichin, and naringin at the concentrations tested. When testing artificial sweeteners flies have, so far, found all tested compounds to be appetitive. The intensive sweeteners tested thus far included sodium cyclamate, sodium saccharin, sucralose, aspartame, neohesperidin dihydrochalcone (NHDC), glycyrrhizic acid, and thaumatin. Some of these sweeteners do not elicit a positive sweet response from rodents; sodium cyclamate, aspartame, NHDC and thaumatin taste sweet only to humans and old world monkeys. New world monkeys and rodents do not respond to these sweeteners. We conclude that *Drosophila* taste responses are often more similar to human responses than are rodents and those of many primates. The strength of *Drosophila* as a behavioral genetic model make these data especially useful for taste gene discovery purposes.

Poster: Central Taste and Chemosensory Behavior

Does Learning Shape Olfactory Acuity in the Moth *Manduca sexta*?

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Learning changes the neural processing of odor in primary olfactory centers. These changes are manifest as evolving spatial and temporal representations. The functional role of these changes, however, remains a mystery. One model predicts that olfactory acuity is learning-dependent and sharpens with experience. To test this prediction, we first conditioned *M. sexta* moths to respond to 1-octanol using a Pavlovian olfactory-learning paradigm. Moths were placed into one of 4 groups receiving 1, 2, 4 or 8 conditioning trials. Twenty-four hours after training, moths were tested with a homologous series of alcohols in a pseudo-randomized sequence. Results

indicated that there was a sharpening of the generalization slope only between groups receiving one versus two conditioning trials. However, as the number of conditioning trials increased, only a general increase in responsiveness was observed, suggesting higher acquisition and not acuity *per se*. To further explore this result we performed two more experiments. First, we replicated the above methods but tested with molecularly distinct odorants to assess whether changing gradients are related to task difficulty. In a final experiment, moths received 2 blocks of 8 conditioning trials, each block separated by 24 h. Following each block and again 24 h after the last block, moths were tested with the homologous series of alcohols. This procedure assessed whether acuity might be short-term and whether additional conditioning trials enhances acuity. Results of these experiments will be discussed.

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Poster: Central Taste and Chemosensory Behavior

Correlating Behavioral and Physiological Measures of Odor Detection in the Moth *Manduca sexta*

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Olfactory systems must detect and discriminate odors while maintaining a degree of perceptual invariance of identity in a highly variable environment. The goals of this study were to measure conditioned response (CR) probabilities to odors as a function of odor concentration and to correlate these measures to physiological measures of antennal input. Using Pavlovian olfactory-learning, we characterized detection thresholds in the moth *M. sexta*. Moths were conditioned to respond to an odor (CS) at neat concentration and then post tested at 24 and 48 h, first with a blank and then with the CS across a 5 log step mineral oil dilution series. Stimuli were presented from low to high (starting at 5 µg/µl) to prevent extinction. Detection thresholds were defined as the lowest concentration at which the CR probability was significantly greater than that elicited by the blank. Results indicated odor specific thresholds. Analysis of variance indicated that CR probability varied by; sex, test day, conditioning odor and concentration. Furthermore, there was a significant interaction between concentration and odor indicating odor specific dose response functions. Next, we measured electroantennogram responses to the same odorants across the same concentration series in order to correlate our behavioral measures with physiological evidence of detection. Results of these experiments indicate a close correspondence between the two measures suggesting that behavioral measures accurately reflect underlying physiological evidence of sensory detection.

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Poster: Central Taste and Chemosensory Behavior

The Effect of Intensity on Discrimination Learning and Performance in the Moth *Manduca sexta*

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Underlying the ability to discriminate olfactory stimuli are neural responses with both spatial and temporal components. Our goals were to behaviorally characterize the effect of stimulus concentration on both discrimination learning and discrimination performance. Groups of *M. sexta* moths were differentially conditioned to respond to one odorant (CS+) but not another (CS-) using a Pavlovian paradigm. Moths were conditioned with neat odorants to control for salience-dependent learning effects. At 24 and 48 h post conditioning, moths were tested with a blank then the CS+ and CS- (pseudo-randomly) across a 5 log step series of increasing concentration (starting at 5 µg/µl) to avoid extinction. ANOVA indicated that differential conditioned responses to the CS+ and CS- were both concentration-dependent and dependent on which odorant of a pair was reinforced. Subsequent comparison of differences in detection thresholds between odorant pairs accurately predicted this odor-dependent effect. To assess the effect of salience on discrimination learning, as well as cross validate the above odor-dependent effect, groups of moths were differentially conditioned at lower concentrations in the dilution series. These groups were tested at the conditioning and at neat concentrations 24 and 48 h after conditioning. ANOVA results confirmed the odor-dependent effect and indicated that conditioning at lower concentrations had a minor effect on discrimination performance. These results provide a behavioral basis for future matched neurophysiological studies.

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Poster: Central Taste and Chemosensory Behavior

More Time Means Better Detection and Discrimination of Odorants in the Honeybee (*Apis mellifera*)

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Physiological studies of the antennal lobe (AL) and olfactory bulb (OB) have revealed that sensory input to these neuropils is transformed over a short time period during and after odor stimulation. The temporal nature of this transformation is likely to arise from processing by local neural networks. Yet, the role of this transformation in olfactory coding remains controversial. If time is important for the production of the code for odor identity, we hypothesize that both stimulus duration and stimulus concentration will affect odor recognition. Here, we test whether these parameters of odor stimuli affect odor recognition by honeybees using Proboscis Extension Response conditioning. We first evaluated the effect of different stimulus durations (200, 500, 800, 1000 and 2000 ms) and three different stimulus concentrations (0.0002, 0.02 and 2.0 M) on detectability and discriminability of odorants. We then evaluated the effect of two training durations (200 and 1000 ms) on response at the five durations and three stimulus concentrations listed above of the same odor. In general, increasing stimulus duration increased both detectability and discriminability of odorants. However, increasing the concentration of the stimulus improved detection and discrimination at shorter stimulus durations. Response latencies were approximately 500 ms, but animals continued to sample odor beyond the initiation of a response.

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Anatomical and Behavioral Investigations of *In Ovo* Odor Sensitivity and Odor Imprinting in Chick Embryos

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The chick olfactory system begins development at embryonic day 8 (E8) and is known to be functional by E15. Studies have shown that chick embryos exposed to odorants *in ovo* prefer these odorants after hatching, suggesting that the olfactory system perceives and decodes odor stimuli during development. This *in ovo* experience may shape the animals' behavior post-hatching, presumably to make them more well-adapted to their immediate surroundings. We employed anatomical and behavioral approaches to study the timecourse of development of the olfactory system *in ovo*. Chick embryos were exposed to amyl acetate or phenylethyl alcohol for 3 day periods from E12 through E21. Upon hatching and for 2 days after birth, chicks were tested for their preference for "familiar" versus "unfamiliar" odorants using a T-maze. A subset of chick embryos were taken at E20 and their olfactory bulbs were fixed and tested for cFos immunoreactivity to determine olfactory bulb activation as a result of odorant exposure. Preliminary results show that chicks prefer familiar odorants that were given during, but not prior, to E15 through E18. Anatomical staining patterns suggest differences in odorant-elicited bulbar activation corresponding to this time window. These results collectively suggest that the critical period for odor imprinting occurs between E15 and E18, highlighting the importance of the sensory environment in shaping the developmental pathways of the olfactory system.

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Functional Anatomy of Synaptic Plasticity Mediating Olfactory Learning

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This study examined the expression of neural plasticity genes to further understand the functional organization of brain regions mediating olfactory fear learning. Specifically, we examined the expression of brain-derived neurotrophic factor (BDNF) and the potassium/chloride cotransporter KCC2. *In vitro*, BDNF downregulates KCC2, which may provide a mechanism for learning by altering the effects of GABAergic signaling via KCC2's effect on the cellular chloride gradient. For this experiment, adult male C57/B6 mice were divided into four groups. Mice received 10 pairings of amyl acetate with a 0.4 mA footshock ($n = 16$), the same number of odors and shocks in an unpaired fashion ($n = 8$), shock alone ($n = 8$), or remained in their home cage ($n = 8$). Two hours following training, half of the paired group and all of the other groups were anesthetized and brains were removed, frozen and sectioned. The remainder of the paired group was behaviorally tested the next day. We then performed *in situ* hybridization to examine relative expression levels of BDNF and KCC2 within the olfactory bulb (OB), the anterior piriform cortex (APC), the posterior piriform cortex

(PPC), and the basolateral amygdala (BLA). We found increased BDNF mRNA expression and a corresponding decrease in KCC2 expression within all areas in the paired group. In contrast, in the unpaired group, there were significant changes in these genes in the OB and APC only. No significant changes were found in the shock only control group. These results are consistent with a model in which OB and APC respond to olfactory stimuli regardless of the predictive qualities of shock. In contrast, the PPC and BLA respond differentially only when there is predictive information to be integrated.

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Olfactory Masking in Behaviorally-Trained Mice

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Under natural conditions odors are rarely, if ever, experienced in isolation. Yet, surprisingly, little is known about how the olfactory system accomplishes this critical task. Here we report on use of a behavioral technique to study olfactory masking in behaviorally-trained mice. Mice (C57BL/6) were trained to perform a two-odor discrimination task in an automated liquid-dilution olfactometer (Knosys, Bethesda, MD). Animals were trained to insert their head into a glass sniffing port to activate a trial sequence. During a trial, either the target odorant, Henkels100 (S+), diluted in de-ionized water, or de-ionized water alone (S-) were presented. Water reinforcement was contingent on the animal reporting the presence of the S+ in the air stream by licking a water spout and activating an electrical switch. Trials were in blocks of 20 (10 S+ and 10 S- in quasi-random order). The concentration of the S+ was decreased in 10-fold steps following two consecutive blocks of $\geq 85\%$. Threshold was estimated to be the lowest S+ concentration at which the animal was capable of discriminating the S+ from the S- stimulus with accuracy at $\geq 85\%$. Discrimination acquisition was compared for target stimulus presentations in a null background (no masking odorant) and in the presence of a continuous supra-threshold level masking odorant (ethyl acetate, $10^{-5}\%$ v/v). Discrimination-acquisition thresholds were, as predicted, shifted to higher S+ concentrations when measured in the presence of the masking odorant. The goal of this research program is to develop psychophysical paradigms to characterize the detection of complex odorants and simple odorants in the presence of background masking odors.

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The Role of the Cellular Prion Protein PrP^C in an Olfactory-Driven Behavior

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Despite over a decade of research, the normal physiological function of the cellular prion protein (PrP^C), encoded by the *Prnp* gene, remains unknown. We found PrP^C to be highly expressed in murine olfactory tissues and have used the olfactory system as a model in which to study PrP^C function. We observed the behavior of *Prnp* knockout mice and other *Prnp*-related transgenics in the hidden

cookie test. A mouse was placed in a novel cage in which a cookie had been buried under fresh bedding. The time the animal took to find the cookie was recorded. Each individual was given two trials, the first (T1) lasting 10 min and the second (T2) 5 min. Zürich I (ZI) *Prnp* knockout mice (medians: T1 = 233 s, T2 = 127.5 s, $n = 20$) scored lower than wild type control animals (medians: T1 = 73 s, T2 = 20 s, $n = 9$). In addition, a significant proportion of the ZI knockouts ($n = 6/20$) failed to find the cookie altogether, whereas none of the control mice failed the test. Further testing on numerous transgenic lines in which *Prnp* had been placed under control of various promoters showed cell-type specific requirements for PrP^C function. In particular, mice in the ZI knockout background expressing PrP^C only in neurons were rescued (medians: T1 = 68 s, T2 = 22 s, $n = 9$). Thus, the presence of PrP^C in neurons restored wild type behavior in this olfactory-driven test. In conclusion, we have demonstrated neuronal expression of *Prnp* is necessary for wild type performance in the hidden cookie test. These results are a first step towards the elucidation of a function for PrP^C.

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Effects of the MGLUR4 Antagonist CPPG on a Learned Taste Aversion in Rats

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Umami stimuli such as monosodium glutamate (MSG) and L-amino acid taste stimuli are believed to be detected by broadly-tuned, G protein-coupled T1R1 + T1R3 taste receptors. However, studies with T1R3 knockout mice have reported contradictory findings about the function of this receptor. Zhao *et al.* (2003) found that T1R3 knockout mice lack the ability to detect umami taste. Others with independently developed T1R3 knockout mice report only a reduction in umami taste preference and nerve recordings, no loss of taste thresholds and some ability to discriminate between MSG and sucrose, suggesting other receptors such as taste-mGluR4 may be involved. We used conditioned taste aversion methods in brief access testing to determine if: (1) an aversion to L-MSG generalizes to L-arginine and (2) if that generalization is effected by 1 mM CPPG, an m-GluR4 antagonist. Rats were presented with 100 mM MSG (with amiloride to reduce Na⁺ taste) and then injected with NaCl or LiCl. Three days later, the rats were presented with two bottles of (1) water, (2) 100 mM L-MSG and (3) 50 mM L-arginine. CPPG (1 mM) was added to one of the two bottle of each substance. Amiloride was in all solutions, including water rinse trials. Lick rates emitted in 10-s trials were counted. CPPG reduced the aversion to MSG but did not alter generalization of the aversion to arginine. These findings suggest that MSG may be detected by a combination of T1R and mGluR4 receptors while L-arginine is detected only by the T1R1 + T1R3 heterodimer.

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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 secretes within 2 min after oral sensory stimulation, peak at 4 min and return to baseline in the 8–10 min poststimulus 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 such as '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.

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Taste Functions After Gastric Bypass Surgery in Dietary and Genetic Obese Rats

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Weight loss after gastric bypass surgery (GBP) is caused by restriction of food intake and malabsorption, but many patients also note decreased appetite for palatable meals. To investigate involvement of central taste mechanisms, we performed GBP in diet-induced obese (DIO) and CCK-1 receptor deficient Otsuka Long-Evans Tokushima Fatty (OLETF) male rats. After GBP, both DIO and OLETF lost body weight similar to that seen in humans (25–30% weight loss) and exhibited improved glucose tolerance compared to both their preoperative baseline and pair-fed sham-operated controls. In addition, GBP rats of both strains showed a significantly reduced 24-h two-bottle preference for sucrose (1.0 M) compared to sham-operated controls (preference ratio, DIO: 0.48 ± 0.04 vs. 0.90 ± 0.04 , $P < 0.001$, $n = 3/4$; OLETF: 0.69 ± 0.02 vs. 0.89 ± 0.06 ; $P < 0.05$, $n = 6/4$). Lick rate (10 s) analysis revealed decreased responsiveness by DIO-GBP to sucrose concentrations above 0.1M and by OLETF-GBP to sucrose 0.3 M through 1.5 M and to fructose above 0.4 M ($P < 0.05$). No difference was noted in either strain for the non-caloric sweetener saccharin, alanine, aversive taste solutions or trigeminal stimulation with capsaicin. These findings suggest that (1) GBP may result in altered taste function with a reduced preference for palatable sugars in animal models of obesity, irrespective to the etiology of obesity, and (2) CCK-1 receptors do not contribute to the beneficial effects of GBP, such as weight reduction and improvement in insulin sensitivity.

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Temperature Modulates Behavioral Responses to Sucrose Taste in the Rat

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We recently showed that temperature modulates the responsiveness of gustatory neurons in the rat geniculate ganglion that respond primarily to sucrose (sucrose-specialists). Specifically, responses to 0.5 M sucrose at 10°C were less than those at 25°C or 40°C, which were not different from each other. The goal of this study was to investigate whether temperature modulates behavioral responses to sweet taste in rats. We employed very brief (10 s) trials in a Davis MS80 Rig lickometer, modified with individual Peltier heat exchange devices located near the tip of each drinking tube, that allows solutions to be maintained at constant temperatures. We recorded the number of licks/10 s to 0.2 M sucrose and to 0.05 M sucrose at 10, 25, and 40°C. Lick rates to 0.2 M sucrose were greater than those to 0.05 M sucrose at all temperatures. For a given concentration of sucrose, licking was lowest at 10°C, most robust at 25°C, and intermediate at 40°C. Licking to 0.2 M sucrose at 10°C was 33% less than that at 25°C, whereas licking to 0.05 M sucrose at 10°C was 58% less than that at 25°C. The finding that lick rates to sucrose at 10°C decreased is consistent with our previous observation of reduced responses from sucrose-specialist neurons in the geniculate ganglion to sucrose at 10°C, and suggests that cold temperature modulates sweet taste perception and thereby affects behavioral responses to sweet taste.

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Evaluating the Limits of Canine Olfaction

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To assess the limits of a canine's ability to identify a specific human scent, a golden-retriever was trained to pick a T-shirt impregnated with a target human's scent from T-shirts worn by the target's relatives and non-related persons. After sampling three test boxes (one of which contained the target), the dog was trained to exhibit a sit/stay response when the target was identified. Correct responses were rewarded 90% of the time. The first series of experiments introduced olfactory "noise" to the testing environment by placing beakers containing increasing concentrations of pure olfactory and olfactory/trigeminal odors between the T-shirts and the sampling ports of the test boxes. The dog was able to correctly identify the target even when the air concentrations of these added distracter odors were at their highest vapor concentrations. A second series of experiments reduced the concentration of the target scent by covering the three beakers containing the T-shirts with plates that decreased the exposed surface area, thus reducing the number of molecules present for detection. The dog was able to detect the target scent when the surface area of the beaker was reduced by 93%. Combined, these two series of experiments illustrate the specificity and sensitivity with which a canine is able to detect a target human

scent and discriminate between this scent and that of competing environmental odors. The first series of experiments provides some hints as to the chemical nature of this particular type of detection task and the second series of experiments allows for an estimation of the minimum number of molecules necessary for detection.

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Inhibition of Muscarinic Acetylcholine Receptors Alters Performance of Mice in an Odor Discrimination Task

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The olfactory bulb (OB) is heavily innervated by cholinergic fibers originating in the horizontal limb of the diagonal band of Broca, and acetylcholine (ACh) has been postulated to modulate OB processing thereby affecting the ability to distinguish structurally similar odorants. In this preliminary study we have altered the function of nicotinic and muscarinic ACh receptors either through drug infusion into the OB, or by using a mouse defective for the $\alpha 7$ ACh receptor. Mice were implanted with bilateral cannulae allowing direct drug injection into each OB. Immediately following drug injection, mice were tested on a go-no go odor discrimination task in which one odor was rewarded. Mice treated with scopolamine, an inhibitor of muscarinic receptors, displayed a delay in attaining criterion compared to controls. In contrast, mice treated with mecamylamine, a nicotinic receptor inhibitor, did not display differences from controls in the go-no go task. In order to determine whether mice defective for the $\alpha 7$ ACh receptor have a deficiency in discrimination threshold, we used a Maximum Likelihood Parameter Estimation by Sequential Testing (MLPEST) procedure. We did not find any differences between $\alpha 7$ knockouts and controls. Further studies are being performed to determine whether the threshold measured using MLPEST is different in $\alpha 7$ knockouts and controls when the mice are subjected to extensive training.

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Speed-Accuracy Tradeoff in Olfaction

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The basic psychophysical principle of speed accuracy tradeoff (SAT) has been used to understand key aspects of neuronal information processing in vision and audition, but has not yet been reported in olfaction. We present the first direct observation of SAT in olfaction and resolve a seeming controversy between results obtained in reaction-time experiments by Uchida and Mainen (2003) and Abraham *et al.* (2004). We developed a behavioral paradigm for mice in which both the time of odor exposure and the difficulty of the odor discrimination task were controlled by the experimenter. The mouse was trained to keep its nose in the odor sampling port during odor

delivery until an auditory signal indicated the availability of water reward in one of two water ports. The odor stimulus indicated whether reward was available in the left or right water port. The difficulty of the task was varied by presenting more or less similar pairs of mixtures. We found that longer enforced odor exposure (from 200 to 1200 ms) led to more accurate odor discrimination (from 60 to 95%), even beyond the level at which mice performed voluntarily, in the reaction-time paradigm developed for rats by Uchida and Mainen (2003). The presence of SAT in olfaction provides strong evidence for temporal integration in olfaction and constrains the applicability of different models of olfactory information processing.

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Role of Cholinergic Modulation in the Main Olfactory Bulb in Rats for Olfactory Acuity and Discrimination Learning

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The objective of this study was to investigate the specific role of cholinergic modulation in olfactory bulb processing. We tested the role of cholinergic modulation in the olfactory bulb of cannulated rats by bilateral injections of vehicle (6 μ L saline), the cholinergic antagonists scopolamine (20 and 100 μ g), the nicotinic antagonist MLA (20 and 100 μ g), a combination of both drugs, or the cholinesterase inhibitor neostigmine (dosage) 20 min before the behavioral tasks. We tested the role of cholinergic modulation on spontaneous odor discrimination in the absence of reward conditioning using a habituation task. A second test involved an olfactory discrimination task in which an odor-reward association has to be formed. We found that spontaneous discrimination between chemically related odorants was impaired when nicotinic, but not muscarinic receptors were blocked in the olfactory bulb. Additionally, the spontaneous discrimination between very similar odorants was enhanced when cholinergic modulation was increased. Interestingly, no effect of modulating the action of acetylcholine in the olfactory bulb was seen when rats were trained on a forced choice, two odor discrimination task.

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Broad Activation of the Olfactory Bulb Produces Long-Lasting Changes in Odor Perception in 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. As a consequence of these changes, across mitral cells in the olfactory bulb, individual odors should be better discriminated due to

previous exposure. We have previously shown that a daily 60 min exposures to odorants during 2 weeks enhance rats' ability to discriminate between chemically similar odorants in a relative odor-unspecific manner. Here we first show that the perception of test odorants is only modulated by enrichment with odorants that activate at least partially overlapping regions of the olfactory bulb. Second, we show that a broad activation of olfactory bulb neurons by daily local infusion of NMDA into both olfactory bulbs enhances the discrimination between chemically related odorants in a manner similar to the effect of daily exposure to odorants. The results strongly suggest that (1) changes in olfactory processing are responsible for the observed modulation of odor perception and (2) increased activity in the olfactory bulb network is sufficient to produce these changes.

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Sniffing Patterns of Rats During Learning and Performance of Odor Discrimination Tasks

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Odor sampling (sniffing) is a dynamic behavior. Most earlier studies have focused on sniffing strictly in the context of odor discrimination. In this study, we monitored sniffing behavior continuously in rats learning and performing odor discriminations under two different behavioral paradigms and asked how sniffing relates to odor discrimination as well as to other behavioral parameters. In both paradigms, rats were habituated to head restraint and trained to perform a simple lick-no-lick two odor discrimination. Sniffing, measured as intranasal pressure transients, was monitored during task acquisition and performance. In paradigm 1, the test odorant was presented after a random intertrial interval, with no other associated cues, while in paradigm 2 the rat was allowed to initiate each trial by pressing a bar after a tone was presented. In paradigm 1, rats sniffed at a fairly consistent slow frequency of 1–2 Hz. Surprisingly, sniff frequency did not increase around the time of odor presentation. Presenting a novel odorant as a CS- induced high frequency (6–10 Hz) sniffing which habituated within 1–2 trials despite continued successful performance of the task. In paradigm 2, rats consistently showed bouts of fast sniffing. Fast sniffing began immediately following the tone and just preceding bar press, but ceased as soon as odor presentation began. Odor discrimination difficulty did not influence the likelihood or duration of fast sniffing. Thus, fast sniffing is more strongly associated with the expectation of or search for an odor stimulus than with the discrimination of the odor itself.

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Olfactory Sensitivity for Enantiomers and Their Racemic Mixtures—A Comparative Study in Mice and Spider Monkeys

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Enantiomers appear to be particularly valuable tools to assess odor structure-activity relationships. Using an operant conditioning paradigm, we determined olfactory detection thresholds for the optical antipodes of carvone and limonene as well as for their racemic mixtures in CD-1 mice. We found that (a) with few exceptions the mice were able to detect all six stimuli at concentrations below 1 ppm, (b) all animals were more sensitive for (–)-limonene compared to (+)-limonene, whereas no systematic difference in sensitivity was observed for the optical antipodes of carvone, and (c) racemic mixtures of both limonene and carvone were not perceived at lower concentrations compared to the single compounds. Also using an operant conditioning paradigm, five spider monkeys were tested in parallel. We found that (a) the spider monkeys were at least as sensitive for all six stimuli as the mice, (b) all animals were more sensitive for (+)-limonene compared to (–)-limonene, whereas no systematic difference in sensitivity was observed for (+)- and (–)-carvone, and (c) racemic mixtures of both limonene and carvone were perceived at lower concentrations compared to the single compounds. Possible reasons underlying the different patterns of sensitivity found with mice and spider monkeys are discussed.

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Brief Stimulus Presentations Permit Gustatory Detection of Linoleic Acid but Not Oleic Acid in Rats

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We have shown that Sprague-Dawley rats can detect and avoid both linoleic and oleic acid during 15-min two-bottle preference testing following a conditioned taste aversion pairing. This study characterized the ability of rats to detect a variety of concentrations (44, 88, 176 μ M) of linoleic, oleic, and lauric acid following a conditioned taste aversion pairing with either 88 μ M linoleic or oleic acid as the conditioned stimulus. Furthermore, the role of the chorda tympani nerve was examined through bilateral transections in a subset of the subjects. All testing was conducted in the Davis Rig using 30-s stimulus durations. Rats with intact gustatory systems and a conditioned stimulus of 88 μ M linoleic acid showed significant ($F_{1,160} = 20.230$; $P < 0.01$) avoidance of 44, 88, and 176 μ M linoleic acid and a slight generalized avoidance of oleic acid at the 88 and 176 μ M concentrations with no avoidance of lauric acid. There was a significant effect of chorda tympani nerve transection ($F_{1,160} = 10.381$; $P < 0.01$) eliminating the avoidance of oleic acid and 44 μ M linoleic acid with a diminished avoidance of linoleic acid at 88 and 176 μ M concentrations. Rat with intact gustatory systems and a conditioned stimulus of 88 μ M oleic acid did not demonstrate any avoidance of linoleic, oleic, or lauric acid. Based on the current study and our previous findings, it appears that the chorda tympani nerve plays a role in the selective detection of linoleic acid but not oleic acid. Furthermore, there appears to be another mechanism of free fatty acid detection that may be based on olfactory or post-ingestive cues.

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Salt Discrimination in Rats with Cross-regenerated Lingual Gustatory Nerves

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Because chorda tympani nerve (CT) transection in rats severely disrupts their performance in salt discrimination tasks, but glossopharyngeal nerve (GL) transection does not, we tested whether rats with either the CT cross-regenerated to the posterior tongue or the GL cross-regenerated to the anterior tongue would be competent on a NaCl vs. KCl discrimination using a two-response operant procedure. In both groups of rats with cross-regenerated nerves, overall performance decreased significantly relative to before surgery. Performance was also significantly lower than rats with intact or normally regenerated CT nerves, and no better than rats with bilateral CT transection. With further postsurgical testing, some rats in all groups improved their performance, seemingly learning a new discrimination. Amiloride treatment significantly decreased performance in all groups both pre- and postsurgically. Functional connectivity in the cross-regenerated nerves was confirmed electrophysiologically in a subset of animals and regeneration in all animals was verified histologically. These results suggest that neither cross-regeneration condition emulates the normally regenerated CT with regard to this task. It is possible that the cross-regenerated nerves are capable of providing discriminable signals generated by these salt taste stimuli, however the contribution of the greater superficial petrosal nerve to the performance cannot be dismissed.

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Short-Term Licking by Potassium-Deprived Rats

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Potassium-deprived rats have elevated intakes of KCl relative to replete animals. It is not known, though, whether this appetite depends on learning about the post-ingestive consequences of tastant samples. To investigate the contribution of learned versus unlearned cues to potassium appetite, we tested eight potassium-deprived and eight control rats for on their short-term intake. Animals were trained to lick for water after fluid deprivation. They were then given brief-access tests (20 s per solution) to lick for a diverse array of taste stimuli. Measurement of plasma potassium levels and a 24-h two-bottle test with KCl and water at the end of the experiment confirmed that the potassium-deprivation treatment was effective at inducing potassium deficiency. Deprived rats drank less water and 10 mM citric acid in the brief-access tests than did replete rats, but intakes of 200 mM KCl, 200 mM NaCl, 100 mM CaCl₂, and 100 mM MgCl₂ were significantly greater ($P < 0.05$) in the deprived group. The results suggest that potassium deprivation inhib-

its thirst in rats, but that it also induces an appetite that generalizes across several mineral solutions. The brief periods of access precluded post-ingestive learning, and therefore the mineral appetite must have been guided solely by unlearned factors, such as taste.

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Discrimination and Post-ingestive Effects in TRPM5^{-/-} Mice

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Mice lacking functional TRPM5 channels (TRPM5^{-/-}) do not detect sweet (sucrose) and bitter (quinine) tastants. A comparison of the behavior and electrophysiological responses of male TRPM5^{-/-} mice permit the investigation of two issues relevant to gustatory physiologists. The first is whether they can detect bitter tastants (e.g., nicotine) using sensory inputs other than taste, and the second is whether they can distinguish the post-ingestive effects of sucrose and water independently of taste. Preliminary results with a two-bottle preference test suggest that TRPM5^{-/-} mice do discriminate between 1 mM nicotine and water as well as between 1 mM nicotine and 10 mM quinine (=water). We suggest that TRPM5^{-/-} mice use oral somatosensory input to detect nicotine. Regarding post-ingestive effects, food and water-deprived TRPM5^{-/-} mice were given daily 30 min free access to either water or 0.4M sucrose from one of two sipper tubes while access to the other tube is blocked. This procedure alternates access to either solution for 6 consecutive days followed by preference testing sessions where animals are given free access to both tubes simultaneously. We found that in comparison to pre-conditioning preference tests, TRPM5^{-/-} mice develop a preference to the tube previously associated with sucrose, suggesting that post-ingestive reinforcing properties of sucrose that are independent of taste mediate this learning process. In both experiments related recordings will be presented.

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Poster: Central Taste and Chemosensory Behavior

Behavioral Analysis of the Taste of L-Amino Acids in Mice

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Recent studies showed that the mouse T1R1 + T1R3 receptor is broadly tuned to respond to L-amino acids when it is heterologously expressed *in vitro*. Activation of the same taste receptor suggests that L-amino acids share the same taste quality, which contradicts mouse behavioral data showing that taste qualities of L-amino acids are not identical. In order to investigate this apparent contradiction, we examined taste quality perception of three L-amino acids at concentrations that activate T1R1 + T1R3 receptor *in vitro*: 50 mM

L-serine (Ser), 50 mM L-methionine (Met), and 50 mM L-glutamate (Glu). Separate groups of C57BL/6J mice were exposed to Ser, Met, Glu (presented as a mixture of 50 mM MSG, 2.5 mM IMP and 30 μ M amiloride added to block sodium taste) or water (control) and injected with LiCl to form a conditioned taste aversion (CTA). Mice were presented with each of the three L-amino acid stimuli, and four basic taste solutions. An aversion to Ser generalized to both Met and Glu, and also to Sucrose. However an aversion to both Met and Glu did not generalize to any of the stimuli tested. Generalization of CTA from Ser to Met and Glu suggests that consistent with *in vitro* predictions, these amino acids elicit identical taste sensation in mice. However, our results also suggest that each of these L-amino acid stimuli have additional unique sensory qualities. For example, Ser evokes a sucrose-like taste that Met and Glu lack. Why CTA does not reciprocally generalize from Met and Glu to Ser requires further investigation.

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Poster: Central Taste and Chemosensory Behavior

A High-Throughput Method to Measure NaCl Detection Threshold in Mice

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Currently known procedures to measure taste detection thresholds are not suitable for high-throughput genetic studies due to their harmful aspects or complexity of training. We have developed a simple procedure to measure NaCl detection threshold using conditioned taste aversion and two bottle preference tests. First, we compared three conditioning procedures, all followed by 48-h two-bottle tests: (a) presenting water-deprived mice with a single NaCl concentration followed by LiCl injection (multiple groups were tested with different concentrations); (b) presenting each water-deprived mouse with three NaCl concentrations near expected detection threshold, followed by LiCl injection; (c) presenting water replete mice with 150 mM LiCl for 24 h. Mice that consumed LiCl started avoiding NaCl at lower concentrations than did LiCl-injected mice. Next, we altered taste intensity and toxicity of consumed LiCl by changing LiCl concentration or adding NaCl to LiCl and concluded that 150 mM LiCl is the optimal stimulus for conditioning. Finally, we compared sensitivity of 48-h and 30-min tests for detecting thresholds in mice conditioned by LiCl consumption and found that the 48-h test is more sensitive. We have concluded that ingestion of 150 mM LiCl followed by the 48-h tests of ascending NaCl concentrations is the most efficient and sensitive method suitable for high-throughput genetic studies.

Poster: Central Taste and Chemosensory Behavior

A New Method of Assessing Taste Quality Generalization in Rats

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Currently, taste generalization behavior in rats is assessed by using the conditioned taste aversion paradigm, which has some practical limitations including concentration and extinction effects. We modified a procedure from Morrison (1967) in which we trained 4 groups of thirsty rats to lick one response spout after sampling (5 licks) a representative compound (standard stimulus) from one of 4 prototypical taste qualities (NaCl, sucrose, quinine HCl, citric acid) and to lick another response spout after sampling any of the other 3 prototypical taste stimuli (comparison stimuli). Concentration of all compounds was varied to render intensity an irrelevant cue. Correct responses were reinforced with water and incorrect responses were punished with a time-out. Rats readily learned to discriminate the solutions representing the putative 4 basic taste qualities from each other. We delivered unreinforced/unpunished test trials on approximately 15% of the session trials to identify how the trained animals would categorize the novel stimuli including new concentrations of the 4 standard compounds, mixtures of NaCl and sucrose, and water. Rats correctly classified the test solutions. Although, surprisingly, when water was used as a test stimulus, the rats categorized it as quinine-like suggesting that the weakest concentration of quinine may have been too low (i.e., "water-like"). We have now adapted the task to include water in the array of comparison stimuli. Soon, we will be poised to obtain profiles for several novel taste compounds.

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Poster: Central Taste and Chemosensory Behavior

Genetic Influences on Taste Preference for Ethanol

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Although many factors contribute to alcoholism, the palatability of its taste may facilitate initial ingestion. The taste of ethanol is a collection of qualities, including sweet and bitter. Since individual variation in the perception of taste qualities is in part genetically determined, it might be anticipated that PROP sensitive individuals would consume less ethanol due to its bitter quality. However, studies examining human PROP sensitivity and ethanol consumption have produced conflicting results, possibly due to the complex social factors surrounding ethanol ingestion. The present study utilized mice to investigate the relationship of bitter taste to ethanol ingestion. It was hypothesized that PROP sensitivity would predict ethanol preference. Four mouse strains (C57/BL6: Ethanol preferring, BALB/c: Ethanol avoiding, SWR/J: PROP sensitive, C3HeB/FeJ: PROP insensitive) were evaluated for taste preferences (relative to water) for PROP and for ethanol with a Brief Access Taste Test in order to minimize post-ingestive consequences of the stimuli. Three of the four mouse strains supported the hypothesis. That is, as predicted, if sensitivity to PROP was low, ethanol preference was high (C57, C3HeB). Conversely, if sensitivity to PROP was high, ethanol preference was low (SWR). Only the BALB/c strain showed a high lick ratio to PROP despite a low level of preference for ethanol. The basis for the latter result is unclear. Nonetheless, these findings suggest that bitter perception has a role in ethanol preference.

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Poster: Central Taste and Chemosensory Behavior**Behavioral Testing of Salt Taste Sensitivity in TRPV1 Knock-Out Mice**

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Current evidence suggests salt taste transduction involves at least two mechanisms, one that is amiloride-sensitive (AS) and appears to utilize apically located epithelial sodium channels relatively selective for Na⁺ and a second that is amiloride-insensitive (AI) and utilizes non-specific cation channels. Electrophysiological recordings show that *Trpv1* knock-out mice lacking the vanilloid receptor-1 (VR-1) demonstrate no AI chorda tympani (CT) responses to NaCl suggesting that AI salt taste transduction depends on the product of the *Trpv1* gene. To extend these findings into a functional context, we trained *Trpv1* knock-out (KO) and wild type (WT) C57BL/6J mice ($n = 9-10/\text{group}$) in a two response operant discrimination procedure to lick a response spout upon sampling from an array of NaCl concentrations and to lick another response spout upon sampling water. Mice were also tested in sessions in which all solutions contained 100 μM amiloride hydrochloride. Correct responses were reinforced with water and incorrect responses were punished with a time-out. The mice were then tested with an array of KCl concentrations. Both the KO and WT mice had similar detection thresholds for NaCl and KCl. Amiloride shifted the NaCl sensitivity curve to the same degree in both groups and had no effect on KCl thresholds. Thus, contrary to predictions based on CT recordings, these findings suggest the VR-1 variant is not necessary for normal taste detection of NaCl or KCl with or without 100 μM amiloride.

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Poster: Central Taste and Chemosensory Behavior**Cycloheximide: No Ordinary Taste Stimulus**

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Rats and mice avoid cycloheximide, a focus of work on bitter taste, at concentrations much lower than thresholds for most bitter stimuli. Hamsters, *Mesocricetus auratus*, also find this toxic antibiotic uniquely aversive; two-bottle aversion thresholds approximated 1 μM ($P < 0.001$). Preference vs. water fell from $36 \pm 5\%$ for 0.3 μM to $17 \pm 6\%$ for 3 μM cycloheximide. Like other non-ionic stimuli avoided by hamsters, 1 mM cycloheximide did not activate the hamster chorda tympani (CT) nerve. A small consistent CT response was elicited to 10 mM cycloheximide ($P = 0.004$), a concentration 10,000 times higher than behavioral threshold. Thus, hamsters' aversions to 1 μM to 1 mM cycloheximide are not tied to CT responses. Given low behavioral thresholds, naive hamsters, surprisingly, drank an average 1.5 mL of 0.5 mM cycloheximide, an amount near the rat LD₅₀, when presented in a one-bottle test. The cycloheximide did not kill them; however, they found it acceptable just once. When tested 2 days in a row or with as many as 21 days intervening, the second intake averaged 21% of the first intake ($P < 0.000001$), demonstrating a persistent "acquired" aversion. Sources

of the enhanced aversion are unknown but may involve induction of receptors or learned aversions. Rats, to which cycloheximide is multisensory, learn odor aversions induced by intake of 0.4 mM cycloheximide. Evidently, chemosensory properties of cycloheximide, nearly tasteless to humans, require further definition.

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Symposium: Neural Dynamics and Chemosensory Behavior**Noradrenaline Modulation of Main Olfactory Bulb Network Activity: Behavioral Consequences**

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The role of noradrenaline (NE) in the main olfactory bulb (MOB) has been characterized for early preference learning (EPL), where neonatal rats learn to prefer an odor associated with stroking. EPL is blocked by adrenergic antagonists. Adult rodents utilize a more complex neural system allowing for increased behavioral flexibility. Thus, NE modulation in the MOB would be expected to take on a subtler context-dependent role in the adult. Our goal is to link behavioral deficits caused by blockade of NE signaling with perturbations in mitral cell ensemble activity observed during the behavioral task. We have studied the consequences of localized blockade of NE signaling in the MOB of adult mice performing go-no go odor discrimination tasks. Mice received bilateral 2 μl injections of saline, phentolamine, alprenolol, or a combination of the two drugs immediately preceding the task. The odor pairs were of varying molecular similarity. Animal groups receiving saline, alprenolol, or phentolamine did not differ in the number of trials to discrimination. The injection of both drugs resulted in an odor pair-dependent effect, ranging from complete blockade for similar odors to no disturbance. We conclude that blockade of NE signaling in the MOB does not impair odor discrimination behavior per-se, but does impair the ability to discriminate similar odors. We have begun to characterize normal learning-related plasticity of mitral cell ensemble activity in mice performing the go-no go task. Once characterized, we will utilize NE signaling blockade to understand the network underpinnings of behavioral deficits caused by blockade of NE signaling in the MOB.

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Symposium: Neural Dynamics and Chemosensory Behavior**Towards Realistic Models of Concentration-Invariant, Background-Resistant Odor Recognition in the Mammalian Olfactory Bulb**

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Spike synchronization across neurons can be selective for the situation where neurons are driven at similar firing rates, a "many are equal" computation. This can be achieved in the absence of synaptic interactions between neurons, through phase locking to a common underlying oscillatory potential. Based on this principle, we

instantiate an algorithm for robust odor recognition into a model network of spiking neurons whose main features are taken from known properties of biological olfactory systems. Recognition of odors is signaled by spike synchronization of specific subsets of “mitral cells.” This synchronization is highly odor selective and invariant to a wide range of odor concentrations. It is also robust to the presence of strong distractor odors, thus allowing odor segmentation within complex olfactory scenes.

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Symposium: Neural Dynamics and Chemosensory Behavior

State-Dependent Changes in Taste Processing

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Sensory processing is a function of network states. In awake animals background activity and the overall state of cortical networks vary depending on the behavioral state of the subject. We will discuss results showing that rats engaged in a fluid self-administration task display a sudden shift between two very different behavioral states characterized by distinct patterns of oscillatory activity in the gustatory cortex. We will further show that gustatory processing differs in the two conditions and provide evidence that changes in such states specifically modify palatability-related information in neural taste responses, and that this modification is temporally specific. While recording multiple single units in the gustatory cortex, we delivered stimuli to rats before and after they went through the spontaneous state change (disengagement) that is associated with sudden reduction in interest in the experimental task and the simultaneous emergence of 7–12 Hz rhythms in cortex. The percentage of cortical neurons that responded to tastes remained stable with disengagement, but the particulars of these responses changed drastically. When analyzed at the population level the changes were palatability-related—the similarity among aversive tastes increased while the similarity between a highly aversive taste and the palatable tastes decreased. Furthermore, most of these changes were found near the time when palatability-specific information emerges in cortical responses. These data demonstrate that an animal’s state determines the meaning attached to sensory input, and that disengagement broadens palatability-related generalizations by modulating the time-course of responses.

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Symposium: Neural Dynamics and Chemosensory Behavior

Temporal Coding of Taste in the Brain Stem: Information and Function

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Most theories of taste coding in the central nervous system have focused on the spatial aspects of neural responses, utilizing the

sum of response-related spikes over time as the relevant response measure. However, recent data have shown that the temporal arrangement of spikes may also convey information about taste. In a series of related experiments, temporal coding in the mammalian gustatory system was investigated in two ways. First, electrophysiological responses to taste stimuli were recorded in the nucleus of the solitary tract (NTS) in anesthetized rats. Information-theoretic analyses revealed that about half of the taste-responsive cells in the NTS conveyed a significant amount of information about taste quality through spike timing, especially in the initial response interval. Second, the function of temporal coding in taste-guided behavior was studied in awake, behaving rats with electrodes implanted in the taste-responsive area of the NTS. Lick-contingent electrical pulse trains, designed to mimic the temporal arrangement of spikes in a sucrose or quinine response of single NTS cells, were delivered to the NTS in water-deprived rats drinking only water. Rats avoided licking when these pulse trains mimicked quinine responses but licked avidly when randomized control patterns were presented. In addition, rats that learned an aversion to the sucrose-simulation pattern of electrical pulses generalized that aversion to natural sucrose, but not to NaCl, HCl or quinine. Collectively, these data strongly suggest that temporal coding is one of the methods used for communication about taste in the NTS.

Symposium: Neural Dynamics and Chemosensory Behavior

Temporal and Spatial Codes Mediate the Discrimination of ‘Bitter’ Taste Stimuli by an Insect

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A primary function of sensory systems is to discriminate functionally distinct stimuli. In the taste system, most theories about discriminative processing have focused on two spatial coding frameworks (labeled-line vs. across-fiber pattern), and largely ignored the potential contribution of temporal codes. I will discuss recent work on an herbivorous insect (the caterpillar of *Manduca sexta*), which displays unusually fine discriminating abilities for ‘bitter’ taste stimuli. I will present evidence that the caterpillar uses a variety of coding mechanisms to accomplish this discrimination: a labeled-line mechanism to discriminate salicin and *Grindelia* extract, an across-fiber mechanism to discriminate salicin and *Canna* extract, and a temporal coding mechanism to discriminate salicin and aristolochic acid. The only ‘bitter’ taste stimuli that cannot be discriminated are those that elicit the same spatial and temporal code (e.g., salicin and caffeine). These findings show that this herbivorous insect has evolved a complex set of gustatory mechanisms for distinguishing among a diverse range of potentially toxic ‘bitter’ compounds, which abound in its solanaceous foodplants.

This project was supported by NIH DC02416.

Poster: Developmental, Neurogenesis, and Consumer Research

Embryonic Origin Dictates Mature Gustatory Neuron Fate

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The vertebrate tongue receives gustatory and somatosensory innervation from nerves whose cell bodies lie in cranial ganglia. Taste and somatosensory neurons project centrally to specific hindbrain nuclei, and peripherally to taste buds and epithelium, respectively. These neurons arise from two distinct embryonic populations: epibranchial placodes and neural crest. We tested the hypothesis that taste neurons arise from placodes, while somatosensory neurons derive from neural crest, via fate mapping in embryos of an aquatic salamander, the axolotl. Embryos were globally labeled via injection of GFP mRNA at the 2-cell stage. At mid-neurula stage, presumptive placodal ectoderm or premigratory neural crest/dorsal neural tube from GFP-labeled donors was grafted isotopically into unlabeled hosts. Importantly, this method allowed visualization of both peripheral and central projections of neurons. Placodal neurons sent out peripheral fibers which contacted taste buds almost exclusively, while their central processes projected to the nucleus of the solitary tract. Neural crest derived neurons, in contrast, did not innervate taste buds; rather their peripheral fibers terminated as free nerve endings within oral epithelium. Central projections of crest derived neurons were obscured by GFP label in the hindbrain, as the initial neural tube grafts comprised both premigratory neural crest and presumptive hindbrain. In sum, our data indicate that embryonic origin dictates a concise segregation of mature neuron function; placodal neurons are gustatory, while neural crest neurons are somatosensory.

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Poster: Developmental, Neurogenesis, and Consumer Research

Embryonic Development of Nasal Solitary Chemoreceptor Cells and Associated Nerve Fibers in Mice

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Nasal trigeminal chemosensitivity in mice and rats is mediated in part by solitary chemoreceptor cells (SCCs) in the nasal epithelium. Mature SCCs express the G-protein gustducin as well as other elements of the bitter taste signaling cascade such as PLC β 2 and T2R (bitter) taste receptors. Currently nothing is known concerning the development of nasal SCCs. The present experiments were designed to answer two basic questions: (1) When do gustducin expressing SCCs appear in the nasal epithelium during development? and (2) When do SCCs become innervated by the trigeminal nerve? Wild type C57/B6 embryos were taken at various stages from E14.5–E18.5, decapitated, and fixed in 4% PFA. Dual-label immunocytochemistry was used to identify SCCs (rabbit anti-gustducin) and nerve fibers (rabbit anti-PGP9.5). No gustducin immunoreactive (ir) SCCs were present in E14.5 or E15 embryos. Although PGP9.5-ir growth cones were present in the mucosa at these stages, no fibers penetrated into the nasal epithelium. Gustducin-ir SCCs first appeared in the nasal epithelium at E15.5. At this stage, PGP9.5-ir nerve fibers innervated the nasal epithelium and occa-

sional SCCs. By E17.5, gustducin-ir SCCs were abundant and more frequently contacted by PGP9.5-ir nerve fibers. Further experiments are underway to better delineate the timing and sequence of events leading up to development and innervation of nasal SCCs.

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Poster: Developmental, Neurogenesis, and Consumer Research

Taste Bud Development in Chicks After Treatment with β -Bungarotoxin, or Otocyst Removal

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Chick taste bud (gemmal) primordia normally appear on embryonic day (E)16 and incipient immature, spherical-shaped buds at E17. *In ovo* injection of β -bungarotoxin at E12 resulted in complete absence of taste buds in lower beak and palatal epithelium at developmental ages E17 and E21. However, putative gemmal primordia (solitary cells; small, cell groupings) remained, lying adjacent salivary gland duct openings as seen in normal chick gemmal development. Oral epithelium was immunonegative to neural cell adhesion molecule (NCAM) suggesting gemmal primordia are nerve-independent. Some NCAM immunoreactivity was evident in autonomic ganglion-like cells and nerve fibers in connective tissue. After unilateral geniculate ganglion/otocyst excision on E2.5, at developmental ages E18 and posthatching day 1, 10–15% of surviving ipsilateral geniculate ganglion cells sustained ~54% of the unoperated gemmal counts. After E18, proportional stages of differentiation in surviving developing buds probably reflect their degree of innervation, as well as rate of differentiation. Irrespective of degree of geniculate ganglion damage, the proportion of surviving buds can be sustained at the same differentiated bud stage as on the unoperated side, or differentiate to a later bud stage, consistent with the thesis that bud maintenance, survival and maturation are nerve-dependent.

Poster: Developmental, Neurogenesis, and Consumer Research

Developmental Effects of Lingual Nerve Transection on Taste Bud Volumes in Rat

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The present study examined the role of the lingual nerve in the maintenance of taste buds located in fungiform papillae at various developmental ages. Rats underwent unilateral lingual nerve transection on postnatal days 10, 25, or 65. Care was taken to avoid injury to the chorda tympani nerve. Following 2, 8, 16, or 50 days survival time, analysis of taste bud volume was conducted. Results identified a developmental effect of this procedure; transection of the lingual nerve on P10 resulted in a complete absence of taste buds following 8 and 16 days survival time. Transection on P25 resulted in a significant reduction in taste bud volume relative

to control sides of the tongue following 8 days survival time ($P < 0.05$), but no apparent reduction in the number of taste buds on the control versus intact tongue sides. Transection on P65 did not significantly alter taste bud volumes or the number of taste buds identified, regardless of survival time. Furthermore, morphological analyses identified the presence of filiform-like structures as early as 2 days posttransection in all groups (i.e., P10, P25, P65), and the persistence of these structures following 50 days survival time in P10 and P25 transected rats. These findings further demonstrate earlier results from our laboratory showing the importance of sensory innervation during early gustatory development in rats and for the first time identify the lingual nerve as an integral component in the early maintenance of taste buds in fungiform papillae.

Poster: Developmental, Neurogenesis, and Consumer Research

WNT/Catenin Signaling Modulates Development of Taste Primordia

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Wnt genes are key regulators of embryonic development. These secreted factors bind Frizzled receptors to activate the β -catenin pathway. To determine if Wnt signaling is involved in the development of taste papillae, we examined Wnt reporter gene activity in tongues of embryonic TOPGAL mice. Wnt signaling is first evident in anterior lingual epithelium on embryonic day (E)12.0, then focuses to papillary placodes as these taste primordia form (E12.5). Wnt activity persists in taste papillae, and at lower levels in lingual epithelium, as morphogenesis ensues. We have identified Wnt6 and Wnt10a via RT-PCR of lingual mRNA as likely activators of TOPGAL in embryonic tongues. Frizzled receptors 2-5, 7, and 8, secreted antagonists Dkk 1-3, and coreceptor LRP5/6 are also expressed in developing tongue coincident with Wnt6 and 10a. In sum, our data suggest that Wnts function in taste papilla formation, morphogenesis and/or differentiation. To test this hypothesis, embryonic tongue cultures were exposed at E11.5 to lithium (Li^+), which activates Wnt signaling by stabilizing cytoplasmic β -catenin. Explants treated with Li^+ had significantly more papillae than controls, implying that early Wnt signaling promotes taste primordia development. We are now exploring the expression patterns of Wnt6 and 10a with respect to embryonic papillae, and testing the specificity of Wnt action *in vitro*.

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Poster: Poster #7 - Developmental, Neurogenesis, and Consumer Research

Cell Signaling in EGF Regulation of Fungiform Papilla Patterning

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We demonstrated previously that exogenous epidermal growth factor (EGF) regulates patterning of fungiform papillae by reducing papilla number and increasing cell proliferation between papillae in embryonic rat tongue cultures. Using specific inhibitors, we also found that signaling through protein kinases, PI3K/Akt, MEK/ERK and p38 MAPK, mediates these responses to EGF. In the present study we investigate whether EGF-mediated effects on tongue papillae are induced via the EGF receptor (EGFR) and further explore intracellular signaling events. Compound 56, a specific inhibitor of EGFR, induced an increase in papilla number, completely blocking the EGF effect in embryonic day 14 tongue cultures. Using immunolocalization and immunoblot endpoint assays, EGF-mediated phosphorylation of Akt, ERK, and p38 MAPK in tongue cultures was observed. In the absence of EGF, inhibition of PI3K/Akt, MEK/ERK, and p38 MAPK with LY294002, U0126 or SB203580, respectively, showed no significant change in papilla number. However, MEK/ERK inhibition, in conjunction with inhibition of PI3K/Akt or p38 MAPK or both, increased papilla number, blocking any EGF-mediated action, consistent with a synergistic effect. In contrast, concurrent inhibition of PI3K/Akt and p38 MAPK had no effect. These results demonstrate that the EGF effect on fungiform papillae is mediated by EGFR, via PI3K/Akt, MEK/ERK, and p38 MAPK signaling and suggest a synergistic role of MEK/ERK pathway with PI3K/Akt or p38 MAPK in EGF-mediated papilla patterning.

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Poster: Developmental, Neurogenesis, and Consumer Research

Canonical WNT Signaling During Taste Papillae Formation

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Taste tissue development in mice is marked by the emergence of the tongue swelling around E11.5, followed by formation of the tongue placode (E12.5), and taste papillae within the epithelium of the tongue (E13.5). The taste buds emerge at later stages, around the time of birth. As is the case with development of other epithelial tissues, formation and patterning of taste tissues are thought to be induced through epithelial-mesenchymal interactions. Sonic hedgehog, bone morphogenetic proteins and epidermal growth factor receptor are associated with initiation and patterning of taste papillae. Other signaling pathways, such as those involving the Wnts, essential in the development of many epithelial tissues, have not been examined for a role in taste tissue development. We have determined that specific Wnt signaling elements are expressed in developing taste tissue and that canonical Wnt signaling is associated with taste papilla formation. Topgal mice carry a beta-galactosidase (LacZ) reporter gene regulated by the Tcf/Lef1- β -catenin complex such that they can be used to monitor canonical Wnt signaling pathways. Using Topgal mice we observed that canonical Wnt signaling was robustly activated during early stages of taste papilla formation, but less so during later stages, and was also active during taste bud development. Work is in progress to identify the specific Wnts

underlying canonical signaling at various stages of taste papillae/bud development.

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Poster: Developmental, Neurogenesis, and Consumer Research

BMP-4 and Noggin Alter Neuron Survival and Differentiation in Embryonic Geniculate and Trigeminal Ganglia *In Vitro*

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By rat embryonic day 16 (E16), geniculate and trigeminal ganglion cells innervate fungiform papillae and surrounding tongue epithelium, respectively, and thus are exposed to target-derived signaling factors, upon which they become dependent for survival and differentiation. Bone morphogenetic protein 4 (BMP-4), known to be involved in patterning and regionalization of the nervous system, and its antagonist, noggin are expressed in tongue by E13 and dramatically influence taste papilla development. To determine if these proteins not only regulate peripheral taste organs, but also influence development of neurons that innervate these targets, E16 geniculate and trigeminal ganglia were explanted and cultured with exogenous BMP-4, noggin, or brain derived neurotrophic factor (BDNF) for 3–6 days. Ganglia were assessed for neuron survival and neurite outgrowth. Compared to geniculate ganglia exposed to BDNF, with exogenous BMP-4 or noggin there was a substantial decrease in neuron number and reduced neurite extension. Neuron reduction was especially pronounced with noggin. Furthermore, BMP-4 in particular induced neuron aggregation and neurite fasciculation. Although not as profound, neuron survival and neurite extension also were decreased in trigeminal ganglia exposed to either BMP-4 or noggin. For both ganglia, addition of BDNF, BMP-4, and noggin together increased neuron survival relative to BDNF alone. We propose that these proteins, present in embryonic papillae, have varying and balanced effects on ganglion survival and differentiation.

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Poster: Developmental, Neurogenesis, and Consumer Research

BDNF and NT3 Attract Trigeminal Neurites

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Trigeminal and geniculate axons are both attracted to gustatory papillae, but are restricted to non-overlapping areas within the epithelium. We recently found that BDNF is an attractant for geniculate neurites. We therefore investigated which neurotrophins stimulate trigeminal neurite growth by bath application and tested their ability to attract these neurites using slow release beads in collagen gels. Explants were dissected from E15 and E18 rat embryos corresponding to *in vivo* intralingual pathfinding and target penetration stages, respectively. Bath applied NGF was the most potent and efficacious

at eliciting outgrowth at E15 and E18. NT3 was more effective than BDNF at E15, but this reversed at E18. NT3 stimulated finer fascicles than BDNF or NGF. Beads soaked in BDNF, NT3, and BDNF + NT3 did not promote appreciable neurite growth from E15 ganglia, but BDNF- and NT3-soaked beads did attract E18 trigeminal neurites, as reflected by convergence of proximal neurites toward the bead vs. radial divergence of distal neurites from the opposite side of the explant. BDNF + NT3 beads elicited the most robust attraction. In preliminary experiments, NGF soaked beads biased outgrowth less than either BDNF or NT3 at E18. Taken together, our observations support the following model: NGF exerts the predominant trophic influence throughout pathfinding and targeting. BDNF and NT3 attract trigeminal axons to the papillae epithelium, and NT3 promotes defasciculation within the epithelium.

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Poster: Developmental, Neurogenesis, and Consumer Research

BDNF Attracts Geniculate Neurites, NT4 Doesn't

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In vivo studies raise the possibility that BDNF attracts geniculate axons to gustatory papillae: Geniculate axons in mutant mice lacking BDNF or misexpressing BDNF exhibit aberrant intralingual trajectories and mistargeting, and BDNF mRNA is concentrated in the papillae epithelium. However, it has not been determined if the guidance influence of BDNF is direct, i.e., if BDNF is sufficient to attract geniculate axons. To test this, *in vitro* studies are necessary. We co-cultured control or BDNF-soaked beads and geniculate ganglia dissected from rat embryos at intralingual pathfinding stages (E14-16) and targeting stages (E17-18) in collagen gels. Several observations argue that BDNF is not only trophic but tropic: geniculate neurites grow exclusively (E15) or predominantly (E18) toward the beads, and neurites turn toward, stop at or encircle the beads. Furthermore, 25 ng/ml BDNF in the bath did not block attraction to the BDNF soaked bead, suggesting a wide range over which BDNF gradients can be sensed. Control beads soaked in media have no effect. Curiously, beads soaked in NT4, which also signals through trkB, do not attract geniculate neurites under these conditions. These data and those from *in vivo* studies suggest a role for BDNF as an attractant for lingual geniculate axons *in vivo*. We also recently demonstrated that tongue explants promote and attract geniculate neurites, but the attractant is unlikely to be either BDNF or NT4. Future studies will assess the precise role and stages at which BDNF acts as an attractant *in vivo*, and identify the explant-derived attractant.

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Poster: Developmental, Neurogenesis, and Consumer Research

Hyperinnervation with Preservation of Taste Bud-Neuron Specificity in Mice Over-Expressing Neurotrophin in the Tongue Epithelium

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A mouse fungiform taste bud is innervated by only 3–6 geniculate ganglion cells that do not branch to other buds. Brain-derived neurotrophic factor (BDNF) may influence the degree or specificity of innervation. We examined this by counting labeled ganglion cells after injecting single buds with different color markers in BDNF-lingual-overexpressing (OE) mice. Counts of taste buds and receptors were obtained from progeny of: BDNF-OE mice X Gg (gustducin) GFP mice. Finally, palatal buds were assessed to establish whether the lingual receptoneural phenotype generalizes throughout the seventh nerve taste system. Fungiform bud numbers in BDNF-OE mice are 65%, yet geniculate cell numbers are 195%, of normal. Neurons labeled by single fungiform bud injections in BDNF-OE animals were increased 5x vs. control mice (22 ± 3.6 vs. 4 ± 1.9). Injecting 3 buds rarely labeled cells multiply, identical with the “exclusive” pattern of wild type mice. Thus, hyper-innervation of BDNF-OE buds involves more neurons innervating single buds, not increased fiber branching. Proportions of GgGFP/BDNF-OE buds with 0, 1–5 fluorescent cells was identical to those of GgGFP mice; i.e., hyper-innervation was not accompanied by a change in receptor cell numbers. Numbers and patterns of palatal buds of GgGFP/BDNF-OE mice were normal. Numbers and lack of branching of ganglion cells innervating palatal buds were also normal. Thus, both wild type and BDNF-OE mice exhibit, in fungiform and palatal buds, the same, “exclusive” receptoneural pattern. Neurotrophin-related decreases in bud numbers and increases in innervation density apply to only fungiform buds.

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Poster: Developmental, Neurogenesis, and Consumer Research

Expression of Trophic Factors and Their Receptors in a Primary Taste Cell Culture System

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The regenerative ability of the taste system is remarkably robust in the face of ongoing exposure to a comparatively harsh environment. However, in patients where taste loss occurs—such as those undergoing oral radiation therapy—taste loss is a major concern due to malnutrition resulting from the lack of food intake. In spite of the importance of our sense of taste, the molecular mechanisms involved in the generation of taste cells throughout life are not well understood. To study these processes in a controlled environment, we developed a method that maintains taste cells obtained from rat tongue foliate and vallate papilla in long-term culture and supports the de novo generation of new cells exhibiting molecular and functional characteristics of mature taste cells. To begin to study the trophic pathways that may be involved in taste cell generation, we examined the expression of several molecules previously implicated in this process. BDNF, EGF receptor or Trk-B immunoreactivity was detected on subsets of cells *in vitro*. Labeling with bromodeoxyuridine (BrdU) demonstrated proliferation, and a subset of BrdU labeled cells also exhibited EGFR or Trk-B immuno-

reactivity. These findings indicate that further work with this model system to investigate the processes of proliferation and differentiation of taste cells is warranted.

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Poster: Developmental, Neurogenesis, and Consumer Research

Differential Postnatal Development of Gustatory Nerve Terminal Fields in Control Rats and E3-E12 Sodium-Restricted Rats

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Rats given a low-sodium diet (0.03% NaCl) from embryonic day 3 (E3) to E12 yielded enlarged chorda tympani (CT), greater superficial petrosal (GSP) and glossopharyngeal (IX) nerve terminal fields in the NTS when compared to rats fed a sodium-replete diet (controls). As there is significant rearrangement of these three terminal fields during normal development, we sought to identify the developmental time course of terminal field development in E3-E12 sodium-restricted rats to better understand how the dramatic diet-related differences at adulthood occur. An anterograde, triple fluorescent labeling technique was used to label the three nerves in rats aged postnatal day 15 (P15), P25, P35 and >P40 (adult). Nerve terminal fields were imaged using confocal microscopy, and then measured with computer software. A biphasic development accounted for adult differences. First, control rat terminal fields decreased while field size remained similar in E3-E12 restricted rats: control CT terminal field volumes decreased from P25-P35; control GSP and IX terminal field volumes decreased between P15-P25. Second, a later increase in field size occurred in E3-E12 restricted rats while volumes remained constant in controls: all three nerve terminal field volumes increased from P35 to adulthood in E3-E12 restricted rats. The deviation from normal terminal field development in E3-E12 sodium-restricted rats may lead to physiological and behavioral consequences during development and in adulthood.

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Poster: Developmental, Neurogenesis, and Consumer Research

MYCN Is Required for Proper Olfactory Epithelium Development

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The olfactory epithelium (OE) has the unique capacity for sustained neurogenesis throughout adulthood. This process is responsible for ensuring the continuing functionality of the system in the face of stress, damage and age. The production of new olfactory sensory neurons (OSNs) is dependent on a series of progenitor cells, which proliferate and differentiate to replace lost OSNs. Many factors influence the basal cell progenitor population. The bHLH transcription factor *Mycn* (v-myc myelocytomatosis viral related oncogene, neuroblastoma derived) is expressed in a subpopulation of globose basal cells and is known to increase

during repopulation of the damaged OE. We are using a conditional knockout strategy to test whether the absence of *Mycn* leads to deficits in OE development. Nestin-Cre dependent deletion of *Mycn* in the OE appears to have a profound effect at early developmental stages. At postnatal day 0 (P0), the tissue is disorganized and exhibits disruption of the sustentacular cell layer. Additionally, an increase in OE thickness and in OSN number at this age (as marked by *Gap43* and *Omp* expression) is observed. The tissue recovers over time, however, as adult (6 weeks) animals show qualitatively normal amounts of OSNs. We hypothesize that *Mycn* is functioning in the OE to maintain the proliferative state of OSN progenitors and that its loss leads to premature neuronal differentiation.

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Poster: Developmental, Neurogenesis, and Consumer Research

Nestin Is Expressed by a Subset of Embryonic and Postnatal OE Progenitors

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The olfactory neuroepithelium (OE) generates new olfactory receptor neurons (ORNs) into adulthood. We do not know if the most pluripotent progenitors in adult OE are identical to, or adult progeny, of, embryonic progenitors that first create the OE, or if progenitors are zonally committed. Nestin, which is found in CNS neural stem cells, is expressed by radial glia-like cells (RGLCs) in embryonic (E13.5) and postnatal day 5 (P5), but not adult, OE. These RGLCs are highly mitotic developmentally, but rare in P5 OE. Nestin+ RGLCs co-express the glutamate transporter NGLAST, but not a neurogenic radial glial protein, brain lipid binding protein (BLBP), which is exclusively segregated to the OEC lineage. Clonal neural progenitor assays *in vitro* generate not neurospheres, but semi-adherent, rapidly expanding colonies from E13.5 OE, whereas P5 and adult OE generated adherent colonies and neurospheres. Embryonic OE semi-adherent colonies contain a high proportion of nestin+, neuronal/glial lineage negative cells, passage extensively and produce neurons and glia. OE progenitors progressively decrease their ability to passage from E13.5 to P5 and adult. Decreased passaging of P5 OE is accompanied by an increased frequency of Nestin+ OECs within primary spheres. Using a genetic lineage tracing strategy, nestin-cre:Z/EG double-transgenic mice indicate that ORNs in restricted zones of the OE and vomeronasal organ (VNO) are derived from a subset of nestin expressing precursors. These results indicate that the OE progenitor identity and self-renewal capacity changes during aging, and that defined zones are generated in embryonic development through defined progenitor lineages.

Poster: Developmental, Neurogenesis, and Consumer Research

Horizontal Basal Cells Are Multipotent Neural Progenitors in Olfactory Epithelium

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The continual neurogenesis and remarkable regenerative capacity of the olfactory epithelium (OE) suggest that a stem cell population is likely to persist in this system. Developmental and regenerative studies have located the potential OE stem cells among a population of basal cells that includes the GBCs (globose basal cells) and HBCs (horizontal basal cells). Their respective roles remain controversial. In culture, the ability of HBCs to self-renew and give rise to neuronal and non-neuronal cell types supports HBCs as potential stem cell candidates. However, the *in vivo* roles of HBCs remain elusive, due to their quiescent nature that renders traditional methods to study cell lineage inefficient and non-specific. We examined the cellular contributions of HBCs to OE cell populations in normal and lesioned epithelium using a fate-mapping strategy based on an HBC-specific Cre-LoxP system. Our results confirmed that HBCs divide infrequently and retain their identities in intact OE. HBCs remain quiescent in OE even after bulbectomy-induced neuronal ablation. Intriguingly, after acute OE lesion, HBCs are highly proliferative and give rise to all OE cell types, including GBCs, olfactory receptor neurons, sustentacular cells and duct cells. Together, our data suggest that HBCs are multipotent neural progenitors and lead to a model in which the HBC and GBC populations play distinct roles in the cellular dynamics associated with OE development and maintenance.

Poster: Developmental, Neurogenesis, and Consumer Research

Macrophage Depletion in Murine Olfactory Epithelium Leads to Decreased Neurogenesis

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Induced apoptosis of olfactory sensory neurons (OSNs) by olfactory bulbectomy (OBX) leads to the activation of resident macrophages within the olfactory epithelium (OE). These macrophages phagocytose the degenerating OSNs and subsequently secrete chemokines, most notably MIP-1 α , that recruit additional macrophages into the OE. This infiltration of macrophages has been shown to parallel the levels of basal cell proliferation, which leads to the differentiation and maturation of OSNs. Here we examined the effects of reduced macrophage levels in the OE on olfactory neurogenesis 48 h post-OBX by inducing apoptosis in macrophages using liposome-encapsulated clodronate (dichloromethylene bisphosphonate). We found a 38% and 35% reduction in OE macrophages in sham and OBX mice, respectively, compared to liposome-treated controls following intranasal and intravenous administration. The reduction in macrophages was accompanied by a decrease in OE thickness and basal cell proliferation in sham and OBX mice compared to controls. In addition, there appeared to be fewer mature OSNs in clodronate-treated mice. These results indicate that macrophages regulate the integrity of normal and target-ablated murine OE by influencing basal cell proliferation and replacement of mature OSNs.

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Poster: Developmental, Neurogenesis, and Consumer Research

Mouse Model of a Human Gene Polymorphism in the Prodomain of BDNF (VAL66MET) Alters Neurogenesis in the Olfactory System

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Neurotrophins are well known mediators of cellular division and survival throughout development. In adults, BDNF is an important regulator of synaptic connectivity, synaptic remodeling, and neuronal survival. We have developed a mouse line that carries a naturally occurring variation in the BDNF protein found to occur in humans, a valine (val) to methionine (met) substitution at position 66 in the prodomain (val66met). Approximately 30% of Caucasians and 50% of Asians carry the met allele. Human carriers of the met variant have been shown to have subtle impairments in hippocampal dependent memory tasks, have smaller hippocampal volume, and potentially be predisposed to the development of a suite of psychiatric disorders compared to subjects that are homozygous for the val allele. Our lab is interested in investigating the effect of this gene variant on hippocampal and olfactory bulb morphology, cognition, and behavior. We describe here differences in rates of cell division and survival between animals of differing genotypes.

Poster: Developmental, Neurogenesis, and Consumer Research

Fresh Postmortem Human Olfactory Bulb Cultures to Study Neurogenesis

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While neurogenesis in the olfactory bulb of lower animals is well accepted, this process in humans is much more controversial. In order to address this question in humans, olfactory bulbs from fresh postmortem cadavers were placed in primary tissue culture. The dissociated cells produced differentiated cells of diverse morphology that over time formed cellular networks. Fluorescent labeling suggested some of these cells to be of neuronal phenotype. Whole-cell voltage clamp revealed inward currents that appear to be sodium currents based upon their kinetics and voltage dependency, as well as an array of outward currents. In isolated cases, an action potential-like event could be stimulated. To address the issue of neurogenesis, BrdU was added to the cultures to label post-mitotic cells. Preliminary results revealed a few BrdU positive cells, suggesting that the human olfactory bulb intrinsically may possess a neurogenic process. Ongoing experiments are altering the culture conditions to increase the number of post-

mitotic cells and addressing the phenotypic makeup of the cells in culture.

Poster: Developmental, Neurogenesis, and Consumer Research

Growth Factors and Receptors in the Olfactory Epithelium

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The olfactory epithelium (OE) is a dynamic cellular environment where cell death, proliferation, and neural differentiation are continuous. These dynamics are coordinated by signaling events, some of which are known. Our microarray data predicts expression of 47 growth factors and 30 growth factor receptors in the OE. The data also predict whether they are expressed in olfactory sensory neurons (OSNs) or in other cells in the OE. We have used *in situ* hybridization to test these predictions. OSNs express growth factor receptors Acvr1, Arvcf, Bmpr1a, Fzd3, Igf2r, Pdgfrb, Pdgfr1, and Tgfbr1, and growth factors Ecgf, Fgf9, Fgf12, and Pdgfa. Basal cells expressed growth factor receptors Fzd3 and Tgfbr1, and growth factors Hdgf and Pdgfa. These data agree with evidence of local signaling in the OE by PDGF and TGF- β family signals. They also predict roles for Wnt, Ecgf, and Hdgf signaling in the OE, or in the case of signals expressed by OSNs, the possibility of signaling to cells outside the OE such as olfactory ensheathing cells or cells in the olfactory bulb.

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Poster: Developmental, Neurogenesis, and Consumer Research

Purinergic Receptor Activation Evokes Neurotrophic Factor NPY Release from Mouse Olfactory Epithelial (OE) Slices

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The signals for injury-evoked neuroregeneration are unknown; however previous studies implied that injured cells secrete neurotrophic factors which trigger neurogenesis. Extracellular purine nucleotides exert multiple neurotrophic actions in the CNS mediated via activation of purinergic (P2) receptors. Our previous work demonstrated that ATP acts as a neuromodulator and a stress signal in the OE. To determine whether ATP and P2 receptor activation evokes neurotrophic factor secretion in the OE, we monitored release of NPY, a neuroproliferative factor known to be present and functional in the OE. Neonatal mouse OE slices were cultured on nitrocellulose paper to visualize NPY release. Immunoassaying the nitrocellulose resulted in NPY immunoreactivity in the region corresponding to the OE of the nasal septum. We found that exogenous ATP (10–500 μ M) significantly increased the percentage of OE slices that released NPY from 22% to 46% ($P < 0.05$). P2 receptor antagonists PPADS (25 μ M) and suramin (100 μ M) reduced the number of OE slices exhibiting NPY release evoked by ATP by 53%, suggesting that activation of P2 receptors mediates NPY

release. Moreover, P2 receptor antagonists reduced the number of OE slices that released NPY under control conditions by 40%, suggesting tonic release of endogenous ATP evokes NPY release. This study directly verifies that neurotrophic factor ATP evokes neurotrophic factor NPY release in the OE and provides pharmacological targets to promote regeneration of damaged OE.

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Poster: Developmental, Neurogenesis, and Consumer Research

SDF-1/CXCR4 Signaling Regulates Cell Migration in the Embryonic Olfactory System

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Two cell types that are derived from the olfactory placodes undergo important migratory phases during early stages of embryonic development in mice. (1) The Migratory Mass (MM) is a heterogeneous mixture of cells that is mainly composed of glial precursors that will populate the nerve layer of the olfactory bulbs. At embryonic day 10 (E10) in mice, the MM is visible as a single row of cells migrating through the nasal mesenchyme between the olfactory placodes and the rostral telencephalon. (2) Gonadotropin releasing hormone (GnRH) containing neurons migrate from the vomeronasal organ (VNO) in the nasal compartment to the basal forebrain in mice beginning at E11. These neurons use vomeronasal axons as guides to migrate through the nasal mesenchyme. *In situ* hybridization studies reveal that the cytokine stromal derived factor 1 (SDF-1) is expressed in the nasal mesenchyme at E10 and continues throughout embryonic development. SDF-1 is expressed in an increasing rostral to caudal gradient, which is most intense at the border of the nasal mesenchyme and the developing telencephalon. CXCR4, the receptor for SDF-1, is expressed by neurons in the olfactory epithelium and VNO, and by cells dispersed along migratory pathways in the nasal mesenchyme. These dispersed cells comprise two identifiable cell populations; migrating mass cells from E10 to E12, and migrating GnRH neurons from E11 to at least E18. Based on these studies, we suggest that CXCR4+ MM cells and GnRH neurons are attracted toward the telencephalon by SDF-1 from their origins in the olfactory placodes.

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Poster: Developmental, Neurogenesis, and Consumer Research

Protocadherin 20 Expression Is Restricted in the Newly Differentiated Olfactory Sensory Neurons

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Olfactory sensory neurons (OSNs) expressing the same odorant receptor project their axons to a predicted region of the olfactory bulb (OB) and converge into the same foci, the glomeruli. Guidance mechanisms for olfactory axons during development have been in-

vestigated intensely. In an effort to study this question, we have examined and detected the expression of *protocadherin 20* (*pcdh20*) transcripts in the nasal epithelium. Pcdh20 is a novel non-clustered protocadherin. 3T3 fibroblasts transfected with *pcdh20* expression constructs demonstrated Ca²⁺-dependent homophilic adhesion by cell aggregation assay. An antibody against the C-terminus region of Pcdh20 was developed. Pcdh20 immunostaining is restricted in the OSNs at embryonic and early postnatal stages. Pcdh20 was not detected in other regions of the brain. At P6, Pcdh20 is highly expressed in the olfactory nerve fascicles and continues in the olfactory nerve layer and in all glomeruli of the OB. In the adult, however, Pcdh20 is dramatically down regulated. Although most of the glomeruli are devoid of Pcdh20, a few glomeruli are found to have high Pcdh20 expression in adult OB. To examine whether Pcdh20 is expressed in newly generated OSNs in adult, we have performed diphtheria toxin mediated OSN specific ablation using OMP-DTR transgenic mice. At 17 days after OSN ablation, Pcdh20 is up-regulated in the olfactory axon fascicles and more Pcdh20 positive glomeruli are observed in the adult OB. These data indicate that *pcdh20* is an OSN specific adhesion molecule which is expressed exclusively in newly generated OSNs.

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Poster: Developmental, Neurogenesis, and Consumer Research

Localization of Nuclear Retinoic Acid Receptors, RAR and RXR in Postnatal Rodent Olfactory Epithelium

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Retinoic acid (RA) is a derivative of vitamin A (VA) that is known to affect developmental processes, including cell differentiation. RA is synthesized from VA, *all-trans-retinol*, via a biosynthetic pathway that includes a terminal oxidation step catalyzed by a retinaldehyde dehydrogenase (RALDH). We have recently published that RALDHs are present and relatively abundant in the postnatal rodent olfactory epithelium (OE) and underlying *lamina propria*, an indication that RA is produced by this tissue. RA is thought to facilitate gene transcription by activating nuclear RA receptors. Our work has demonstrated that VA deficiency (VAD) leads to a significant loss of mature olfactory neurons in postnatal rat OE, a continuously differentiating neural tissue. Our interpretation is that neuron development is impeded when RA is unavailable. An assumption of this hypothesis is that nuclear receptors are present. Using immunohistochemical methods, we show here that RAR β and RXR β are present in cell bodies in the central and supranuclear region of postnatal rodent OE. Positive staining of these cells colocalizes with markers for nuclei. The RAR β gene has an upstream RA response element that regulates transcription of the gene. Using quantitative RT-PCR, we find that RAR β transcript expression is significantly reduced in VAD olfactory tissue, an indication that RA levels are also reduced. These results and our previous findings suggest that RA is synthesized locally in postnatal rodent OE, where it interacts with nuclear RA receptors that appear to be expressed in sustentacular cells and cells of neuronal origin.

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Poster: Developmental, Neurogenesis, and Consumer Research

Methyl Binding Domain Proteins in the Stage-Specific Differentiation of Olfactory Receptor Neurons

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Methylation of cytosine residues is associated with epigenetic gene silencing and is critical for mammalian development. De novo DNA methyltransferases (DNMTs) catalyze the methylation, producing sites which may then be bound by methyl-CpG binding domain proteins (MBDs), forming repressor complexes that modify chromatin structure via recruitment of histone deacetylases. Disruptions in this process have been implicated in developmental disorders. The olfactory epithelium, where neurogenesis is ongoing, is an ideal system in which to study DNA methylation-dependent gene silencing during neuronal differentiation. The DNMTs are expressed in a stage-specific, sequential pattern during olfactory neurogenesis. DNMT3b is expressed in cycling progenitors, as they commit to the neuronal lineage, and is likely necessary to mediate this transition. DNMT3a is expressed in immature receptor neurons, and is down-regulated as they functionally mature, suggesting that it initiates gene silencing necessary to transition from an immature to a mature neuron. The MBD proteins MBD2 and MeCP2 are similarly expressed at distinct stages of olfactory development. Expression of MBD2 is initiated in the progenitors of the OE, and is maintained throughout differentiation. MeCP2 is expressed in immature receptor neurons as they functionally mature. Furthermore, MBD2 and MeCP2 knockout mice display stage-specific defects in olfactory neurogenesis corresponding to their observed expression patterns. Our findings indicate that the sequential recruitment of epigenetic modifiers may be essential for the successful stage-specific differentiation of olfactory receptor neurons.

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Poster: Developmental, Neurogenesis, and Consumer Research

Expression of Transcriptional Regulators in Olfactory Sensory Neurons

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Microarray comparison of a purified population of mature olfactory sensory neurons (OSNs) against all other cells in the olfactory epithelium predicts that OSNs express 238 mRNAs whose genes are annotated as regulators of transcription. Signal intensity distributions predict which of these mRNAs are expressed in mature OSNs versus other cell types in the epithelium. We attempted *in situ* hybridization on 63 of these mRNAs. Of the 36 that gave *in situ* hybridization signals, 28 were detected in the OSN layers of the olfactory epithelium, with some being restricted to either the mature or immature OSN layer. Those mRNAs encoding transcriptional regulators involved

in differentiation and maturation should show preferential expression in immature neurons. Six1, Sox6, Msx1, Tle1, Tle3 and Mef2b fit this pattern, and these genes are known to be associated with developmental events in other tissues. In contrast, mRNAs associated with mature neurons are likely to be associated with homeostasis or the final stages of maturation of OSNs. Nfat5, Creb1, Xbp1, Nfatc1, Nfe2l2, Ches1 and Usf2 fit this pattern. These genes share links to several types of stress responses. The type of transcriptional regulators expressed in OSNs gives an insight into the development and homeostasis of the two recognized OSN phenotypes.

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Poster: Developmental, Neurogenesis, and Consumer Research

WNT and FZ in the Developing Mouse Olfactory System

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The general principles of axon extension and regeneration can be effectively studied in the olfactory system. Olfactory sensory neurons (OSNs) are broadly distributed in the olfactory epithelium (OE) yet their axons target restricted areas of olfactory bulb (OB) neuropil, glomeruli, with exceptional precision. The odor receptors have been strongly implicated in the targeting of these axons. Nevertheless, how OSN axons navigate from the OE up to the OB is still not known. Formerly known as morphogens, there is increasing evidence that Wntless-Int (Wnt) molecules, signaling through Frizzled receptors (Fz) contribute in a variety of processes, including development of neuronal circuits. In this context, the aim of the present work was to study the expression of several Wnt, Fz and secreted Fz-related protein (sFrp) in the developing OB and OE. RT-PCR was performed from mouse embryonic (E13, E17) and early postnatal (P0, P4) tissues. Wnt-1 mRNA was the only one not found either in the OB or OE at any age. All Fz and sFrp studied were expressed at all analyzed ages. Immunohistochemical characterization showed that Wnt-5a is expressed by OMP positive cell bodies throughout the OE while, the expression Fz-7 is restricted to cells as yet unidentified in zones 2 to 4. In the OB Fz-7 was present in olfactory ensheathing cells, olfactory nerve layer and glomeruli, while Wnt-5a showed a relatively uniform staining throughout the OB. Taking into account that during the period of study OSN axons are growing towards the OB and starting to synapse in the glomeruli, it could be suggested that Wnt and Fz are involved in the development of the primary olfactory pathway and in synaptogenesis in the OB.

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Poster: Developmental, Neurogenesis, and Consumer Research

Expression of Axon Growth and Guidance Genes in Immature OSNs

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Newly differentiated olfactory sensory neurons (OSNs) grow axons into glomeruli in the olfactory bulb. The identity of the single odorant receptor expressed in each OSN determines which axons converge together. However, extrinsic guidance cues and neuronal activity must also be involved in guiding and targeting OSN axons. Differentially abundant mRNAs from two microarray experiments have identified mouse genes that may be involved in OSN axon growth and guidance. *In situ* hybridization revealed that several of these mRNAs are present only in immature OSNs, or in immature OSNs and basal cells. These mRNAs include *Cxcr4*, *Dpysl3*, *Mlp*, *Ppp2cb*, and *Dbn1*. The proteins encoded by these mRNAs are known to regulate axon growth in other parts of the nervous system. For example, *Cxcr4* and its ligand *Cxcl12* have been shown to direct the ventral trajectory of ventral motor neurons. We find that *Cxcl12* is expressed in the stroma of postnatal day 0 mice while *Cxcr4* is expressed in immature OSNs, suggesting that *Cxcl12* could direct the trajectory of nascent OSN axons. At postnatal day 21, expression of *Cxcl12* is absent from the stroma and restricted to underlying bone while *Cxcr4* is detectable only in a subset of the immature OSNs that express *Gap43*. These data suggest that if *Cxcr4/Cxcl12* signaling orients OSN axons, it is most important during development and the initial outgrowth of the axon.

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Poster: Developmental, Neurogenesis, and Consumer Research

Gene-Targeted Deletion of Kv1.3 Channel Alters Olfactory Receptor Gene Expression and Modifies Primary Olfactory Projections

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Kv1.3, a member of the Shaker family of potassium channels, plays a key role in the excitability of olfactory bulb neurons. Previously we have demonstrated that gene-targeted deletion of Kv1.3 created a "super-smeller" phenotype with an increased ability to discriminate and detect odors, an increased firing frequency of mitral cells, and an increased number of glomeruli. Since sensory activity plays a key role in axonal targeting, we asked whether deletion of Kv1.3 affects axonal targeting during development. We generated a mouse line that carries M72-IRES-*taulacZ* in a Kv1.3-null background (double-mutant) and compared its projection pattern with that of mice expressing M72-IRES-*taulacZ* (wildtype) at P20. The number of olfactory sensory neurons (OSNs) expressing M72 receptors was dramatically reduced in the double-mutant mice. The morphology of M72 OSNs was also altered in the double-mutant mice; OSNs were comparatively slender with an elongated dendrite. In the olfactory bulb, M72 positive axons were less numerous and their axons coalesced into two or three novel glomeruli at positions different from that observed in wildtype mice. We are currently analyzing M72 double-mutant mice over postnatal development and comparing with patterns of P2 expressing OSNs in the Kv1.3-null background. These data indicate that Kv1.3 influences the guidance of primary sensory axons and the targeting to specific glomeruli that may be receptor-type dependent.

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Poster: Developmental, Neurogenesis, and Consumer Research

Signaling Molecules Involved in Regulating Mouse Olfactory Axon Outgrowth

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Olfactory sensory neurons (OSNs) in the olfactory epithelium project their axons into defined glomeruli in the olfactory bulb. Although several cell surface molecules have been shown to play a role in olfactory axon growth and targeting, the signaling pathway in the OSNs involved in this process is largely unknown. To investigate the signaling mechanisms for OSN axon outgrowth and guidance, we established a dissociated OSN culture system, which allows the visualization of axon growth and manipulation of single OSN. Pharmacological studies were conducted to investigate the involvement of PKC, PKA, PKG, and Rho GTPase pathways in OSN axon elongation. We observed that OSN axons were dramatically shortened at 30 h after the treatment of Rottlerin (20 nM), a PKC δ specific inhibitor. However, increased elongation of OSN axons was observed after the treatment of Go6976 (100 nM), a specific inhibitor for PKC α and β I. These results suggest that isoforms of PKC play antagonistic roles in the OSN axon outgrowth and targeting. Small GTPases are also involved in the OSN axon outgrowth. Rac1 promotes the elongation of OSN axons indicated by Rac1 inhibitor (NSC23766, 30 μ M) result. However, downstream regulator of RhoA, Rho kinase, negatively regulates the outgrowth of OSN axons, suggested by Rho kinase inhibitor (Y27632, 10 μ M) results. Unexpectedly, PKA activator, Sp-cAMPS; PKA inhibitors, H89 and Rp-cAMPS; PKG activator, 8-bromo-cGMP; PKG inhibitors, KT5823 and Rp-8-pCPT-cGMPs, did not affect the axonal outgrowth of OSNs under our culture condition.

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Poster: Developmental, Neurogenesis, and Consumer Research

Mediation of Cell Signaling Events in Developing Olfactory System of *Manduca sexta* by Lipid Rafts

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During development of the adult olfactory system of the moth *Manduca sexta*, axons of olfactory receptor neurons (ORNs) extending into the brain induce centrally derived glia to migrate and populate a sorting zone; encountering these glia causes later-growing ORN axons to sort and fasciculate according to their target glomerulus. Past work showed that EGF receptor/neuroglial interactions in the sorting zone promote axonal outgrowth and sorting, that FGF receptors present on glia are activated at critical times, and that ORN axons sort according to expression of the IgCAM fasciclin II. Furthermore, ORN axons display glycosphingolipids (GSLs) in patterns that change during development. In other systems GSLs are concentrated in membrane domains called lipid rafts, which have been shown to be important in the function of EGF and FGF receptors and IgCAMs. Current experiments explore the role

played by lipid rafts in cell signaling events in the sorting zone. Sucrose gradient flotation reveals two low-density bands that may represent different types of lipid rafts; Western blots reveal that only one contains GPI-linked fasciilin II, which previous studies showed is associated with glial cells. General disruption of raft assembly with methyl-cyclodextrin causes aberrant glial migration and abnormal glomerular microarchitecture. Ongoing experiments will further characterize raft components and examine the functional organization of those rafts with respect to neuron-glia signaling in the sorting zone.

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Poster: Developmental, Neurogenesis, and Consumer Research

Disruption of Kallmann and *fgfr1* Gene Function in Zebrafish Differentially Affects GnRH and Olfactory Cell Development

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Human Kallmann syndrome is characterized by hypogonadic hypogonadism (deficits in GnRH) and anosmia (loss of sense of smell). Two of the genes known to underlie Kallmann syndrome are KAL1 (*anosmin1*) and KAL2 (*fibroblast growth factor receptor 1, fgfr1*). Though KAL1 has not been found in mouse, zebrafish have two KAL1 homologues, *kallmann1.1 (kall.1)* and *kallmann1.2 (kall.2)*, and also have one KAL2 homologue, *fibroblast growth factor receptor 1 (fgfr1)*. To identify mechanisms controlling GnRH and olfactory sensory cell development, we disrupted *kallman* and *fgfr1* gene function in the developing zebrafish. We used morpholinos (modified oligonucleotides; MO) to block protein translation of *kall.1*, *kall.2*, and *fgfr1*. “Knockdown” of gene function caused reduction of endocrine GnRH cells, but had no effect on neuromodulatory midbrain or nervus terminalis GnRH cells. The olfactory nerves of these animals were disrupted but not absent. Our data indicate that knockdown of *kall.1*, *kall.2*, and *fgfr1* results in a different GnRH cell and olfactory sensory system phenotype for each gene. Also, we studied the developmental origins of endocrine GnRH cells in relation to anterior pituitary and hypothalamic development. The anterior pituitary development was not greatly disrupted in *kall.1* MO injected fish, yet a hypothalamic marker was completely absent in many injected fish. Our data suggest *kall.1* is involved in both endocrine GnRH neuron and hypothalamic development.

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Poster: Developmental, Neurogenesis, and Consumer Research

Expression of Gonadotropin-Releasing Hormone (GnRH) and Gonadotropin Releasing Hormone Receptors (GnRH-R) in the Zebrafish

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We have shown that GnRH cells originate from precursors lying outside the olfactory placode: the region of the anterior pituitary

gives rise to hypothalamic GnRH cells and the cranial neural crest gives rise to the GnRH cells of the terminal nerve and midbrain. Our analysis of the molecular forms of GnRH expressed in these cells suggests that zebrafish have a third form of GnRH as has been observed in other fishes. Concurrently with the examination of the GnRH decapeptide expression, we are examining GnRH-R expression. We cloned GnRH-Rs from the zebrafish and our analysis of these putative receptors confirms that there are four receptors: two type I receptors (*GnRH-R2*, *GnRH-R4*) and two type II receptors (*GnRH-R1*, *GnRH-R3*). Using digoxigenin labeled mRNA probes generated against these sequences we were able to detect signal for putative receptor *GnRH-R3* (Type II) and *GnRH-R4* (Type I) at 56 h post fertilization. Using an antibody recognizing the Type II receptor (ISPR3) we were able to identify two cell types in the olfactory sensory system, large diameter cells in the respiratory epithelium and smaller, apparently neuronal cells in the sensory epithelium. This last observation suggests that GnRH may affect the olfactory sensory epithelium via a Type II GnRH receptor.

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Poster: Developmental, Neurogenesis, and Consumer Research

Odorant Modulation of 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. Our previous work has shown that one IEG, *c-fos* is expressed in the developing olfactory epithelia as early as 24 h post fertilization (hpf) and is retained in a small and variable number of cells up to 72hpf. To investigate whether odorant exposure modulates the expression of *c-fos* in the olfactory epithelia, we have used *in situ* hybridization to compare *c-fos* expression in the olfactory epithelia of odor-exposed and control embryos. Two odorants shown to be environmentally significant in adult goldfish, prostaglandin (PGF2 α) and Progesterone (17 α , 20 β dihydroxyprogesteronesodiumsulphate), have an effect on olfactory epithelial *c-fos* expression in the zebrafish. After 2 days of chronic exposure to these odorants (10–8 M), the frequency of embryos displaying high numbers of *c-fos* expressing cells increased relative to wild-type control siblings. The difference was found to be statistically significant by a Mann-Whitney rank-sum test ($P < 0.005$) in both cases. Our results suggest a possible role of odor environment in influencing gene expression during the development of the olfactory epithelium.

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Poster: Developmental, Neurogenesis, and Consumer Research

Formation of the Olfactory Placode in the Zebrafish, *Danio rerio*

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In zebrafish, the olfactory placodes are formed by a convergence of two fields of cells located on either side of the developing neural tube. In order to determine the extent of cell mixing during formation of the olfactory placodes and cranial neural crest derived structures of the face, we are visualizing cell movements in the developing embryo. Using a transgenic line expressing GFP we are able to visualize the neural crest cells *in vivo*. At approximately 13 h post fertilization, the neural crest cells migrate anteriorly as a group and separate at the anterior end of the neural tube. Some neural crest cells appear to migrate to the region of the forming olfactory placodes. We are generating a transgenic line that expresses RFP in the olfactory placode fields. By generating animals carrying both the neural crest GFP and olfactory placode RFP expression we will be able to visualize the movements of both of these cell types during development. Our investigations will provide insight into how the olfactory placode and the neural crest fields mix together during development to form the nose.

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Poster: Developmental, Neurogenesis, and Consumer Research

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 development the antennal disc in *M. sexta*. Disc growth occurs throughout most of the fifth larval instar. The antennal imaginal disc grows inward from an epithelial ring surrounding the base of the larval antenna. We have quantified DNA content during disc growth as an indicator of cell number, observing a sharp decline in DNA content just prior to disc eversion. We have subsequently identified apoptotic activity in a spatial pattern which is reflected in the spatial organization of the adult antenna. We have explored the role of ecdysteroids regulating disc growth. Prior to pupation the imaginal discs elongates and everts; we have demonstrated ecdysteroid sensitivity of disc eversion, and are currently exploring the role of ecdysteroids in regulating the post eversion apoptotic events. 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: Developmental, Neurogenesis, and Consumer Research

MMP-9 Elevation in the Early Response to Olfactory Nerve Injury

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Matrix metalloproteinases (MMPs) have been implicated in extracellular remodeling that occurs in developmental, reparative and homeostatic processes. MMP-9 (gelatinase B) has been reported in the central nervous system and may be associated with injury processes, including neuronal degeneration and gliosis. We used a well-documented model of olfactory nerve injury to study the role of MMP-9 during degeneration and regeneration processes in the olfactory bulb. By means of Western blot and immunohistochemistry, we studied MMP-9 and markers for olfactory neuron degeneration and regeneration (olfactory marker protein, OMP and X-gal staining) and gliosis (glial fibrillary acidic protein, GFAP) in the olfactory bulbs of P2-tau-lacZ mice following bilateral olfactory nerve transection. Data from control mice and sham surgeries showed almost no MMP-9 in the olfactory bulbs. However, we found that MMP-9 levels rose sharply and abruptly (within hours) following olfactory nerve injury and peaked at 5–7 days post-injury. Immunohistochemical analysis showed that MMP-9 was localized to the anterior portion of the olfactory bulb, the area that sustained the greatest injury in our model. After 7 days, MMP-9 levels decreased, returning to near control levels at later recovery time points. This is the first report demonstrating an elevation in MMP-9 levels in the olfactory bulb during the early response to injury, suggesting that MMP-9 may play a role in neuronal degeneration and gliosis.

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Poster: Developmental, Neurogenesis, and Consumer Research

Supporting Cells and Olfactory Neurons Express Different Inhibitory Apoptosis Proteins

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In the olfactory epithelium neurogenesis and neuronal apoptosis are thought to occur continuously throughout life. We believe that equilibrium between genesis and death of neurons is highly regulated. In this study we used RT-PCR and Northern blot methods to examine the expression of three members of the inhibitory apoptosis proteins (IAPs) gene family in rat olfactory epithelium. These IAPs are known to inhibit apoptosis by inhibiting caspase activity and are probably involved in apoptotic regulation. We established that mRNAs of Survivin, X-linked IAP (XIAP) and neuronal apoptosis inhibitory protein (NAIP) are expressed in olfactory mucosa. Cellular localization of two proteins for which antibodies were available was studied by immunohistochemistry whereas localization of mRNA for NAIP was analysed using *in situ* hybridization. All supporting cells are Survivin-positive, but only a subtype with a zonal distribution expresses both XIAP and Survivin. NAIP is restricted solely to olfactory neurons. Northern blots

suggested that the quantities of survivin and XIAP mRNAs did not change significantly after bulbectomy and could explain the low turnover of these supporting cells compared to turnover of neurons. We have also shown by Northern blots that, NAIP expression is down-regulated 1 day after bulbectomy and up-regulated 3 to 5 days post-lesion. Our data are consistent with the idea that apoptosis is regulated in rat olfactory epithelium. Further we suggest that apoptosis in olfactory sensory neurons and supporting cells are regulated by different molecular mechanisms.

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Poster: Developmental, Neurogenesis, and Consumer Research

Microglia in the Zebrafish Immune Response to Injury

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The zebrafish olfactory system is a good model for studies of neuronal plasticity and recovery from brain injury or disease. This study attempts to determine the immune response of the zebrafish brain to injury, with particular interest in the role of microglia. Microglia are phagocytic cells that respond to neuronal death by removing cellular debris and they can be identified using a variety of histochemical labels including certain plant lectins. They play an important role in the defense of the central nervous system and may increase at the site of injury as part of an inflammatory response. Here we establish the normal microglial composition of the adult zebrafish brain and examine the microglial response to damage. Normal and injured fish were analyzed at various time points for the presence of microglia using the lectin marker IsoB4. In normal animals, very few IsoB4 lectin-positive profiles were seen in the olfactory bulb and optic tract, although profiles were more prevalent around the ventricles. Injured animals underwent either olfactory deafferentation or optic nerve crush. In deafferented olfactory bulbs there were very few lectin-positive cells and no evidence of microglial proliferation. Following optic nerve crush, however, a large number of lectin-positive microglia were visualized in the optic tract and diencephalon. Thus, we conclude that olfactory deafferentation elicits a different type of wound response from other peripheral injury; the olfactory damage response does not involve a proliferation of IsoB4 lectin-positive microglia. We will continue to investigate the mechanisms by which the olfactory bulb responds to damage by examining microglia following direct bulb injury.

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Poster: Developmental, Neurogenesis, and Consumer Research

Coumarin Produces Selective Deafferentation of the Olfactory Bulb

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Coumarin (1,2-benzopyrone), a compound found in the essential oils of many plants, had been employed as a fixative and flavoring

agent but is now banned in food products because of its potential hepatotoxicity. In rats, doses subthreshold for liver toxicity produce cytotoxicity in the olfactory epithelium, due, in part, to OE-specific P450 bioactivation of the toxicant. We assessed the effects of ip injections of coumarin on projections from the OE to the olfactory bulb. Anterograde transport of HRP*WGA from OE to the OB was evaluated 7 or 21 days after treatment with 50 or 100 mg/kg coumarin. Rats appeared normally active 24 h after treatment. 50 mg/kg had little effect on anterograde transport. Seven day 100 mg/kg survival cases ($n = 5$) had dense HRP*WGA reaction product in glomeruli of the AOB but no reaction product in the MOB or very light reaction product in some glomeruli in the mid lateral and posterior ventral medial bulb. There was considerable recovery of input to all but glomeruli on the dorsal and medial wall of the MOB in 21 day survival cases ($n = 5$). Surprisingly, patterns of deafferentation were not bilaterally symmetrical and, in most cases, one bulb had considerably less input than the other. Higher doses (200–500 mg/kg) were not necessarily more effective than the 100 mg/kg dose. A study of the effects of coumarin on odor detection and discrimination is in progress.

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Poster: Developmental, Neurogenesis, and Consumer Research

Morphological and Functional Regeneration of the Olfactory Epithelium Depends upon the Extent of the Ablation

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Regeneration of olfactory lamellae occurs following partial ablation of the olfactory organ of black bullhead catfish (*Ameiurus melas*). Depending upon the size of the remaining tissue, the olfactory epithelium regenerated into either small roseta, single lamellae or epithelial tissue. Four months post the ablation, large medial sections of the olfactory lamellae (1–3.6 mm × 2 mm × 0.3 mm) regenerated into either small rosetae with 14–22 lamellae or fan-like rosetae with 5–11 lamellae. The regenerated rosetae contained ciliated and microvillous olfactory receptor neurons (ORNs). Axons of the ciliated ORNs connected to the anterior area and axons of microvillus ORNs connected to the lateral area of the ventral olfactory bulb which indicated full functional regeneration. In a second experiment, small medial sections of the olfactory lamellae (0.6–1.4 mm × 1 mm × 0.3 mm) regenerated into either flat or fingerlike lamellae or into small deformed epithelial tissues. Four months after the ablation there was no behavioral evidence of olfactory discrimination. The small regenerated lamellae did not contain ORNs and did not respond to amino acids electrophysiologically. A year after the ablation, four of the catfish with the small regenerated lamellae discriminated the conditioned L-norvaline from other amino acids, whereas three catfish with few regenerated lamellae and four catfish with deformed epithelial tissues did not discriminate amino acids. Catfish with functionally regenerated olfactory organs responded to olfactory stimulation, whereas anosmic catfish responded to taste stimulation only.

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Poster: Developmental, Neurogenesis, and Consumer Research

Hemocyte Infiltration of Olfactory Receptor Neuron Clusters After Aesthetasc Damage in the Spiny Lobster

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In the spiny lobster, *Panulirus argus*, olfactory sensilla (aesthetascs) are comprised of large clusters of olfactory receptor neurons (ORNs) and ensheathing cells. Aesthetascs are continuously generated in adults, and after severe damage they degenerate and subsequently regenerate. To study cellular events underlying the local de- and regeneration of aesthetascs, we monitored the tissue composition in the olfactory organ with confocal microscopy after focally damaging aesthetascs in two ways: shaving off their entire setae, or clipping them at about 50% of their length. Shortly after the damage (6 h to 3 days), infiltration of damaged ORN clusters by granulocytes—a prominent type of circulating hemocytes containing large granules and f-actin—was observed in both treatments. However, this infiltration was much more substantial after clipping the aesthetascs than after shaving them. Later time points revealed radically different fates of the ORN clusters in both treatments: after clipping, ORN clusters remained massively infiltrated by granulocytes for several more days and appeared normal after 3 weeks; after shaving, ORN clusters completely degenerated within ca. 2 weeks and then started to regenerate by mitotic activity, without granulocytes being present. These findings indicate that granulocytes are the main agents of an immune response induced by physiological relevant damage to aesthetascs and that they contribute to repair mechanisms allowing the damaged ORNs to survive.

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Poster: Developmental, Neurogenesis, and Consumer Research

Ethanol *In Vivo* Causes Degeneration of Olfactory Sensory Neurons

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Alcoholism is a major medical problem resulting in damage to many organ systems. Neuronal degeneration in the CNS is reported from human and animal studies particularly in limbic system areas. Deficits in olfactory function in alcoholics are reported to be reversed on abstinence. We hypothesized that these deficits result from the degeneration and death of mature olfactory sensory neurons (OSNs) after EtOH administration and that upon abstinence OSNs are reconstituted from mitotically active progenitors in OE. Nevertheless, the effect of ethanol on this neuronal population is uncharacterized. Therefore, we are studying the effect of EtOH on mouse olfactory neuroepithelium. Administration of EtOH by i.p. injection results in reduced OMP expression in olfactory neuroepithelium (OE) and olfactory bulb (OB), and significant loss of mature neurons in the OE as measured by molecular and immunohistochemical techniques. After several weeks of abstinence the OMP levels and tissue morphology return to control values. We also monitored gene ex-

pression in non-neuronal cells in OE and have shown the elevation of EtOH-inducible P450 (CYP2E1) and carnosinase after EtOH administration. These data demonstrate that multiple cell types, in addition to OSNs, in OE are influenced by *in vivo* EtOH treatment.

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Poster: Developmental, Neurogenesis, and Consumer Research

NG2-Expressing Cells: A 4th Class of Macroglia in the Mouse Olfactory Bulb

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The NG2 chondroitin sulfate proteoglycan (CSPG) is a large integral membrane proteoglycan comprising a ~300 kDa core protein and at least one chondroitin sulfate glycosaminoglycan (GAG) side chain. In the CNS, two distinct populations of NG2-positive cells have been described: (1) a population of oligodendroglial precursors and (2) a population of mature neuroglial cells termed synantocytes that are distinct from astrocytes, oligodendrocytes and microglia. We examined NG2 expression in both developing and mature murine olfactory bulb (OB). NG2 was expressed by a population of stellate cells in the glomerular, external plexiform and granule cell layers of the OB. These stellate cells did not express the astrocytic marker GFAP or the oligodendrocyte marker RIP. Ultrastructurally, they displayed all the morphological characteristic of synantocytes: an irregularly shaped pale nucleus; with a thin rim of heterochromatin beneath the nuclear envelope; and few organelles in the cytoplasm. They also received synapses, but did not express the neuronal markers NeuN, Dcx or MAP-2, thus appear to be glia rather than neurons. Moreover we demonstrate that these glia are proliferative. We describe for the first time that NG2+ glia comprise a significant population of glia within the adult mouse OB, and are the predominant glial population within the EPL.

Poster: Developmental, Neurogenesis, and Consumer Research

Heterogeneous Generation of Periglomerular Cells in the Adult Mouse

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The olfactory system of adult mammals has a continual influx of new neurons. Stem cells in the subventricular zone (SVZ) lining the lateral ventricles give rise to neuroblasts that migrate into the olfactory bulb (OB), via the Rostral Migratory Stream (RMS). In the OB, they differentiate into the two main populations of interneurons, granule cells and periglomerular (PG) cells. The PG cells, because they represent a small proportion of the new cells, have received relatively little attention. PG cells can be divided into several subtypes, based on expression of neurotransmitters and calcium-binding proteins. It is not known if all the subtypes continue to be generated in adulthood, or if the adult-generated neurons comprise only one or a few subtypes

of PG cell. We have examined this question in mouse by using BrdU incorporation as a marker of new cells. Animals are given BrdU, followed by a 30 day survival period to allow for migration and differentiation. Tissue is then double labeled for BrdU and calcium binding proteins, such as calbindin, calretinin, and parvalbumin, or markers of neurotransmitter phenotype, such as tyrosine hydroxylase (TH) and glutamic acid decarboxylase (GAD). For each subtype of PG cell, we have found some labeled with BrdU, but when the proportion of total cells expressing each marker is compared to the proportion of BrdU labeled cells expressing each marker, there are marked differences among the subtypes. Our data indicate that the generation and perhaps replacement of PG cells is not uniform and may reflect different functional roles for PG cells or their integration into glomerular circuits.

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Poster: Developmental, Neurogenesis, and Consumer Research

Tarsh Gene Expression in the Developing Mitral Cell

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Mitral cell is the first relay in the olfactory system. During development, mitral cells first extend elaborated dendritic processes at embryonic stages and then undergo dendritic pruning at early postnatal stages to acquire the single primary dendrite morphology. Genome-wide screen was performed using oligonucleotide microarray to compare the transcriptional differences between P6 and E16 mouse olfactory bulbs (OB). TARSH was identified as one of the upregulated genes in P6 OB. TARSH mRNA is first detected in E18 mouse brain and exclusively expressed in the mitral/tufted cells by *in situ* hybridization. At early postnatal stages, TARSH is expressed in the mitral/tufted cells in the main OB and also the anterior olfactory nucleus (AON). At P35, TARSH expression can not be detected in the OBs but the AON expression remains. Quantitative RT-PCR indicates TARSH transcription level reaches the peak at P6 and is down-regulated after P6. The changes of TARSH transcription level in the OB are correlated with the mitral cell pruning event. A previous study found 5 alternative splicing forms of TARSH mRNA. We have identified 6 alternative splicing forms in the P6 OB and 4 of them are new alternative splicing variants in the SH3-binding motif region. This suggests that TARSH may have different binding affinities with its interacting molecules. Full length cDNA of TARSH was cloned from the mouse OB. The over-expression and knockdown effects of TARSH in mitral cell morphogenesis are currently under investigation.

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Poster: Developmental, Neurogenesis, and Consumer Research

Time Lapse Confocal Microscopy on Migrating Neuroblasts in the Mouse Rostral Migratory Stream

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Neural progenitors cells born in the subventricular zone (SVZ) migrate along the rostral migratory stream (RMS) to the olfactory bulb, and differentiate into several classes of interneurons. Tangential migration in the RMS takes place in 'chains' of cells as compared to individual cells in cortical radial migration. To examine the biophysics of migration in this pathway we labeled SVZ progenitors with Cell Tracker Green (CTG) in P2 and P17 mice. At 3 days post injection acute sagittal slices were time-lapse imaged on a confocal microscope. The centroid of the cell soma was tracked for at least 60 min. Individual cells in the RMS migrate in a salutatory manner with bursts of high speed followed by periods of slower speed (mean 26–31 $\mu\text{m}/\text{h}$). Neurotransmitters, particularly GABA, have been implicated as modulators of neuroblasts migration in the RMS. To test the role of GABA and glutamate in this model slices were incubated with specific agonists/antagonists. Incubation of the slices with the GABA_A receptor antagonist gabazine increases the migratory speed by 45% while the agonist muscimol decreases speed of 34%. NMDA/AMPA and mGluR group I receptors are also expressed along the RMS; however, inhibition of these receptors does not significantly affect migration. Migratory cells can interact and modify the extracellular matrix through expression of a family of proteins, the matrix metalloproteinases (MMPs), which we found expressed in the RMS. In the presence of inhibitor neuroblasts migration in the RMS was reduced by $\approx 35\%$, suggesting a role for these proteases in CNS neuroblasts migration.

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Poster: Developmental, Neurogenesis, and Consumer Research

Odorant Deprivation Reversibly Modulates NR2B-Mediated CREB Phosphorylation In Mouse Piriform Cortex

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The olfactory system is an outstanding model to characterize activity-dependent plasticity in mammals. The goal of this study is to elucidate molecular mechanisms underlying neuronal plasticity in mouse piriform cortex (PC). Although the functional organization of the olfactory bulb (OB) to PC network has been studied electrophysiologically the molecular mechanisms remain elusive. To understand the influence of the periphery on trans-synaptic gene regulation in the PC, we used intranasal zinc sulfate irrigation as well as permanent and reversible naris occlusion. We characterized reductions in NMDA receptor NR2B subunit expression in OB and PC layer IIb by measuring its immunoreactivity and mRNA level 7 days after zinc sulfate lesion. No evidence for neuronal death was observed after deafferentation. We also found the same reduction 5 days after naris occlusion, implying that the reduction in NR2B expression in PC is activity-dependent. We further demonstrated an activity-dependent reduction in phosphorylation of transcription factor CREB, which is in the NR2B-mediated signal transduction pathway, and subsequently characterized the subset of pyramidal

cells that shows high sensitivity to odor deprivation, using retrograde tracers. We confirmed that the activity-dependent reduction of CREB phosphorylation can be reversed by 10 days of odor exposure. Taken together, the present results demonstrate the molecular mechanisms underlying functional organization and odor-evoked activity-dependent neuronal plasticity of PC.

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Poster: Developmental, Neurogenesis, and Consumer Research

Genesis and Migration of Mitral Cells in the Developing Mouse Olfactory Bulb

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Olfactory sensory neurons expressing the same odorant receptor converge their axons to the same glomeruli where they synapse with dendrites from a small group of mitral cells. It is not clear, in vertebrate, whether mitral cells are genetically programmed to target the defined glomerulus in the olfactory bulb. Mitral cells are born during early embryonic stages and migrate to form a single cell layer in the adult olfactory bulb. To investigate the genesis and the migration of mitral cells, we have employed a double labeling technique to follow two cell populations simultaneously. Two different thymidine analogs, CldUrd and IdUrd, were injected into timed pregnant mice 2 days apart. Cells in S phase at time of injection will be labeled by either CldUrd or IdUrd and their numbers and distribution are analyzed. We have obtained evidence that, in contrast to the development of cortical tissue, older cells, born at E11, are pushed outward while the younger cells, born at E13, remain more central in the olfactory bulb at E15. The older cells were measured to be significantly farther away from the ventricular zone than the younger cells ($45.8 \pm 0.74 \mu\text{m}$ versus $28.0 \pm 0.76 \mu\text{m}$, $P < 0.001$). Consistent with Hinds' studies, the majority of the mitral cells are born between E10 and E12. Mitral cell genesis peaks at E12 and is dramatically decreased at E13. The distribution of mitral cells born between E10 and E15 were analyzed in the adult olfactory bulb. No clustered distribution was found between different labeled populations.

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Poster: Developmental, Neurogenesis, and Consumer Research

Molecular Cloning, Characterization and Expression Patterns of a Novel RapGAP Gene in the Developing Olfactory Bulb

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Regulation of dendritic patterning is critical for the formation of a precise neuronal circuit. In the olfactory bulb, mitral cells undergo

dendritic remodeling during development to achieve specific connectivity with afferent olfactory sensory neurons. Using this model to study dendritic morphogenesis, our lab has established profiles of gene expression in the developing olfactory bulb. With this approach we have been able to identify candidate genes for their potential roles in dendritic remodeling. From our profile, the expression of a putative Rap1GAP is upregulated, when comparing levels before and during dendritic remodeling. Rap1GAPs are an exclusive negative regulator of Rap1 activity. Rap1 is a small GTPase recently shown to promote dendritic outgrowth and elaboration in cortical neurons. We cloned the putative Rap1GAP's full-length cDNA using 5' RACE method. This putative Rap1GAP has a 93% overall protein sequence homology to human Rap1-GAP2b. *In situ* hybridization shows that the putative Rap1GAP is expressed in many cell types of the olfactory bulb as well as the neocortex. Transcript signal is particularly strong in the mitral cell layer of the olfactory bulb. Quantitative RT-PCR confirms the putative Rap1GAP is upregulated in the olfactory bulb during the developmental period when pruning occurs. We have prepared a non-radioactive assay, which utilizes active Rap1-GTP effector RalGDS (RBD), to test the putative Rap1GAP for Rap1-specific GAP activity.

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Poster: Developmental, Neurogenesis, and Consumer Research

Oxidative Stress-Mediated Degenerative Changes in the Aging Murine Olfactory Bulb

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Our recent proteomic analysis of the aging olfactory system demonstrated changes in several oxidative stress proteins. Here, we have investigated oxidative stress markers in olfactory bulbs (OB) of old (20 months) vs. young (1.5 months) mice. Total protein carbonylation was significantly higher in OBs of old mice. 2D gel electrophoresis and Western blotting detected over 300 oxidized proteins. Specific carbonylation levels of 4 proteins identified by mass spectrometry-based redox proteomics significantly differed in old vs. young mice, demonstrating the selectivity of age-associated changes in protein oxidation. Of these, aldolase 1, localized in astroglia in the granule cell layer, showed a 13-fold increase in carbonyl levels with aging. The oxidative stress management protein ferritin heavy chain 1 was also carbonylated; it was up-regulated and localized in mitral/tufted cells. Endothelial cells and microglia in the glomerular layer were immunoreactive for 3-nitrotyrosine, an oxidative protein modification, in OBs of old mice. We initiated GeneChip analyses to study global changes in the oxidative stress response. Genes regulated in the aging OB include key antioxidant proteins, chaperones and apoptosis pathway members. Our results provide fundamental insight into the role of oxidative stress in the aging OB.

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Poster: Developmental, Neurogenesis, and Consumer Research

Odor Increases Infants' Attention to Dynamic Emotion Display

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Infants show specific preferences when it comes to looking at emotional expressions. Infants look longer at happy than sad expressions. Infants look longer to dynamic displays of emotion and longer when displays have sound. How might another sensory input (odor) affect infants' looking time? Thirty infants were shown both happy and sad dynamic expressions (counterbalanced) in one of three conditions: no odor, pine or baby powder. Infants looked longer when they were in the odor conditions, regardless of the emotion displayed ($P < 0.049$). This suggests that the addition of the odor sensory channel increases alertness and enables an infant to maintain visual attention, even to stimuli usually avoided (e.g., sad emotion expression).

Poster: Developmental, Neurogenesis, and Consumer Research

Effects of Aroma on Amateur Ten-Pin Bowling Performance

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Introduction: While odor has been noted to have an impact in some sports, effects on bowling have never been addressed. Methods: Twenty subjects in a single-blind fashion bowled two frames. One frame was while wearing a blank surgical mask and the other was while wearing a mask impregnated with the aroma of jasmine. These were presented in a counterbalanced order. On an analog scale, subjects rated the hedonics of the jasmine aroma. Results: With the blank mask, the average score was 6.00, whereas while bowling with the jasmine impregnated mask the average score was 8.35 ($P = 0.0053$). No effect on bowling score was seen in relation to absolute hedonics ($P = 0.29$), or relative hedonics—like/dislike ($P = 0.64$), of the jasmine aroma. Conclusions: Ten-pin bowling in the presence of the odor of jasmine improved scores. Possible mechanisms of action include mood regulation, enhanced alertness or concentration, anxiolysis, increased self-confidence, and improved hand-eye coordination. A similar effect of jasmine may be observed in other sports requiring precise hand-eye coordination or precision in execution.

Poster: Developmental, Neurogenesis, and Consumer Research

Floral Odor Prompts Positive Emotional Searches

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Flowers preferred by humans increase Duchenne smiling and social behavior and decrease negative mood reports. Would a floral odor also affect emotional behavior? 123 participants (66 females) rated their own emotional state, rated the emotional response to short fear/anger videos and reported a recent memory in either a gardenia, peppermint or Etoh condition. There is no effect of odor on ratings of pleasantness or intensity (both odors are different from Etoh only). Condition did not effect self-rating of mood. There is an effect of emotional response to the fear/anger videos for positive emotion ($P < 0.002$) and negative emotion ($P < 0.02$); both gardenia and peppermint prompt higher positive emotion than Etoh but peppermint also prompts more negative emotion than Etoh. There is an effect of odor on social reference words in the memory narrative ($P < 0.02$); gardenia prompts more than Etoh. These results support the hypothesis that floral odor specializes in searches for positive emotion and social reference.

Poster: Developmental, Neurogenesis, and Consumer Research

Beauty Is in the "Nose" of the Beholder—Unconscious Smells Influence Perceived Likability

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The direct projections between the olfactory bulb and the limbic system suggest that olfactory input may induce emotion without neocortical involvement, and perhaps independently of conscious awareness of smell. We used sub-threshold concentrations of pleasant (citric acid), unpleasant (valeric acid) and neutral (anisole) odorants to test whether the emotional content of smells could be accessed subliminally so as to influence affective judgments. In each subject, detection thresholds were determined for each odorant by requiring five consecutive hits in a two-alternative forced-choice ascending staircase procedure (using 1/3 serial dilutions). We then presented odorants 3 dilution steps below threshold for presence/absence judgments. In each of 80 trials, the subject sniffed from a bottle containing one of the odorants or mineral oil alone. After sniffing the subject saw a photo of a neutral face (80 different faces) and rated how much they liked that person. Performance on the odor-detection task was at chance level in 22 out of 39 subjects. These subjects thus demonstrated lack of conscious awareness of odors. Also, they showed a shift in likability judgments towards the valence of the preceding odor; faces preceded by unpleasant odor were rated less likable than faces following pleasant or neutral odors. These data provide clear evidence that emotional properties of a subliminal odor can be processed so as to influence one's emotional reaction to another person. Furthermore, these findings imply that unconscious smells can potentially influence everyday behavior.

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Poster: Developmental, Neurogenesis, and Consumer Research

Effects of Pleasant and Unpleasant Odors on Exercise Performance

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This study evaluated the effect of the smell of lavender (pleasant), butyric acid (unpleasant), and octane (unpleasant) on the exercise performance of female college students. During a test session, subjects were instructed to peddle as fast as they could for 20 min on a stationary bike. During a session, subjects were exposed to the smell of lavender, octane, butyric acid or to an odorless control. The subject's heart rate, speed, and distance traveled were recorded every 2 min. Each experimental condition (lavender, octane, butyric acid and odorless control) was repeated 3 times for a total of 12 trials per subject. As expected, exposure to lavender improved exercise performance compared to the performance seen with the water control. Although exposure to unpleasant smell of octane also increased performance, the smell of butyric acid did not alter performance. The increase in performance seen with lavender was accompanied by a decreased heart rate for at least part of the exercise period whereas the increased performance seen with octane was accompanied by an increase in heart rate. Perhaps pleasant odors like lavender facilitate entering the "zone"—a relaxed state in which actions are more reflexive and attention is focused in a good way on the task at hand. On the other hand the increased performance seen with unpleasant odors like octane may be related more to their irritating psychological properties.

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Cognitions Influence Color-Odor Correspondences

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It has been demonstrated previously that certain colors are selected as corresponding to certain odors. We further investigate this effect using complex scents (the unisex fragrances CKOne and CKbe). In addition, we examine the influence of telling subjects that the scents are male or female fragrances. Subjects ($n = 68$) smelled both CKOne and CKbe from identical sniff-bottles. Half of the subjects were told that the scents were female fragrances and the other half were told that they were male. They were asked to assign five "appropriateness" points to the following 11 colors: red, orange, yellow, green, blue, purple, pink, brown, white, grey, and black. For the two scents, both when subjects were told that they were male and when they were told that they were female fragrances, the points were not uniformly distributed among the 11 colors. Some colors were chosen more frequently than were others [Friedman chi-square (10) = 21.25 to 31.07, $P < 0.02$ in all cases]. In addition, the pattern of color selection depended on whether subjects were told that it was a male or female scent [chi-square (10) = 52.31, $P < 0.01$ for CKOne and chi-square (10) = 44.75, $P < 0.01$ for CKbe]. Subjects who were told that the CKOne was a female scent predominately chose pink whereas those who were told that it was

a male scent chose blue. Subjects who were told that CKbe was a female scent predominately chose yellow whereas subjects who were told that it was a male scent chose blue. While the actual odor of a scent plays some role in odor-color correspondences, how a subject thinks of the odor may play an even more important role.

Poster: Developmental, Neurogenesis, and Consumer Research

Measuring Odor Attitudes in an Implicit Way

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Introduction: Beliefs and attitudes about odors and exposure effects can influence odor perception in an implicit way. So far, researchers have tried to map these odor attitudes by using self-report measurements, and thus by asking people to think explicitly about their attitudes. Objective: To measure odor attitudes in an implicit way, we developed an odor version of the Implicit Association Test (IAT). Validation of this odor-IAT will be discussed. Methods: Three experiments were conducted to test the odor-IAT, which is a computerized reaction time task, during which participants have to associate words from the concept "odor" with positive and negative words, by pressing the corresponding keys on a computer keyboard. Participants not selected on specific odor attitudes (Experiment 1 and Replication Experiment 2) and participants who frequently use aromatherapy products (Experiment 3) completed the test. They were all psychology students. Implicit odor attitudes were inferred from examining response latencies and error rates. Results: In general, lower response latencies and error rates were observed during phases of the test where "odor" had to be associated with positive words, reflecting an overall positive attitude towards odors in Experiment 1 and 2. This odor attitude was even more positive within an aromatherapy group in Experiment 3 compared to participants who reported they never use aromatherapy products. The odor-IAT was capable of making distinctions between various implicit odor attitudes. Participants with a negative attitude towards odors are currently being tested.

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Poster: Developmental, Neurogenesis, and Consumer Research

Pleasantness Information Facilitates Detection in Taste

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Affective context has been shown to influence performance on a number of tasks in several sensory modalities. It is not known whether affective context is able to influence perceptual processing in taste. In two experiments we investigated a special cross-modal top-down example of affective priming, namely detection of taste targets preceded by visually presented words containing pleasantness information. In the first experiment with long prime presentation times (1000 ms) and long prime-target intervals

(750–1000 ms) we found that detection times of gustatory target stimuli are shortened if the pleasantness information in the prime is congruent with hedonic tone of the taste stimulus (e.g., “good”-sucrose; “bad”-caffeine; “neutral”-demineralised water) as compared to incongruent prime-target combinations ($F_{(1,14)} = 5.253$, $P = 0.038$). In the second experiment using shorter prime presentation times (200 ms), this facilitation of detection by an affectively congruent context was replicated ($F_{(2,24)} = 8.787$, $P = 0.001$). In the first experiment congruency facilitation was most prominent for sucrose, in the second experiment for caffeine. This finding supports the idea of preferential facilitation of the processing of negative stimuli under time-constraints. Without time-constraints positive stimuli might benefit most from congruency facilitation. This is in line with findings in the perceptual-defense literature, claiming that the defense against negative stimuli demand extra attentional resources leading to a delay of response.

Poster: Developmental, Neurogenesis, and Consumer Research

Correlation between Brain Activity and Online Psychophysical Measurement: How the Evaluative Task Affects Brain Activation

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We used event related fMRI (3T GE) to investigate cortical activations related to taste. Subjects performed two separate runs. In one, they evaluated the pleasantness of the stimuli, in the other, their intensity, while stimuli were presented to the mouth as 0.3 ml of solution in 1 s boluses alternating with water. An important question related to this type of paradigm is whether the cognitive task, i.e., evaluating intensity or pleasantness, affects the perception of the stimuli. To investigate this question, we compared the level of correlation between the psychophysical measures of intensity of pleasantness and the brain activity in both cognitive tasks, in 4 regions of interest, amygdala, orbitofrontal cortex BA13, BA47 and insula. Image analysis was conducted using AFNI. Results showed that for sucrose, saccharin, citric acid and caffeine, pleasantness ratings were significantly more strongly correlated with brain activity when

subjects evaluated pleasantness than when subjects evaluated intensity ($P = 0.039$); there was a similar effect for intensity ratings that approached significance ($P = 0.057$) with intensity ratings more strongly correlated with brain activity when subjects evaluated intensity. Interestingly, 2 of the 6 stimuli, GMP and NaCl showed opposite effects. Although the interpretation of this observation is still unclear, it reinforces the importance of sampling a wide range of stimuli in order to understand the complex mechanisms at play in gustatory function.

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Poster: Developmental, Neurogenesis, and Consumer Research

Identification of Latent Variables in a Semantic Odor Profile Database Using Principal Component Analysis

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Many classifications of odors have been proposed, but none of them has yet gained wide acceptance. Odor sensation is usually described by means of odor character descriptors. If these semantic profiles are obtained for a large diversity of compounds, the resulting database can be considered representative of odor perception space. Few of these comprehensive databases are publicly available, being a valuable source of information for fragrance research. Their statistical analysis has revealed that the underlying structure of odor space is highly dimensional, not governed by a few primary odors. In a new effort to study the underlying sensory dimensions of the multivariate olfactory perception space, we have applied Principal Component Analysis to a database of 881 perfume materials with semantic profiles comprising 82 odor descriptors. The relationships identified between the descriptors are consistent with those reported in similar studies, and have allowed their classification into 15 groups.

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