

ABSTRACTS

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

Givaudan-Roure Lecture

Why new neurons in the adult olfactory bulb

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New neurons continue to be formed in adult brains. These cells incorporate into mature circuitry raising questions on how these cells are formed and what is their functional contribution. Adult neurogenesis is most prominent in the subventricular zone (SVZ) on the lateral walls of the lateral ventricles. This process has now been described in many species including primates. Young neurons born in the SVZ engage in an extraordinary journey, along the walls of the lateral ventricle and the rostral migratory stream that leads into the olfactory bulb, where these cells differentiate into granule and periglomerular interneurons. These cells must be continually replaced in adult life, as their total number remains unchanged in adults. I will review recent advances on the origin, mechanism of migration and integration of these new neurons in adult brain. I will describe the different stages in the maturation and present electrophysiological evidence demonstrating that these neurons become functionally incorporated in adult olfactory bulb circuits. Surprisingly, soon after full maturation and incorporation many of the new neurons die in a process that appears to be dependent on olfactory activity. A computer model of a neural network that approximates the circuitry of the olfactory bulb predicts that replacement of neurons through an activity-dependent mechanism greatly improves odor discrimination. A similar principle could apply to other circuits that receive new neuron in adults.

Slide: Olfactory Bulb Physiology

Understanding the role of gap junctions in olfactory function

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Previously, we reported that we had generated OlfDNCX mice that express a dominant negative connexin 43 protein (Cx43/ β -gal) in mature olfactory receptor neurons (ORNs) to inactivate Cx43 gap junctions (*Chem. Senses* 27: 664). The use of olfactory marker protein promoter to drive the expression of Cx43/ β -gal ensures the dysfunction of gap junctions occurs only in mature ORNs, without affecting the function of gap junctions in other epithelial cells and the maturation of olfactory epithelial cells. Using electro-

olfactogram (EOG) recordings, we found that 500 μ M IBMX, a phosphodiesterase inhibitor that elicits responses by increasing intracellular cAMP, consistently induced large olfactory responses in various location in the olfactory epithelium and that the magnitude did not differ between OlfDNCX and controls. This suggests that Cx43/ β -gal expression in the olfactory epithelium in OlfDNCX did not result in a gross interference of signal transduction machinery in ORNs. We decided to conduct a systematic study to understand how dysfunction of gap junctions in mature ORNs will affect responses to odors at the peripheral level, odor maps in the olfactory bulb and olfactory sensation. We found that OlfDNCX had reduced EOG responses to select odors, including octaldehyde, which led to 'simpler' odor maps in the olfactory bulb as compared with controls. Gap junctions in the olfactory epithelium are likely to play a role in odor detection and discrimination.

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

Slide: Olfactory Bulb Physiology

Real-time imaging of odorant-stimulated nitric oxide production in the antennal lobe of *Manduca sexta*

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In the antennal lobe (AL) of the moth, *Manduca sexta*, nitric oxide synthase is expressed exclusively in the axons of most or all olfactory receptor neurons (ORNs), and soluble guanylyl cyclase is expressed in subsets of AL neurons, which suggests that the gaseous signaling molecule, nitric oxide (NO), has a role in processing olfactory information. To determine if NO is produced by odorant-stimulated ORNs, we used real-time imaging with a fluorescent NO marker, DAF-FM diacetate, to record odorant-stimulated NO production in AL glomeruli. Odorants caused a reproducible, spatially distinct and concentration-dependent production of NO. Different plant odorants evoked spatially distinct patterns of NO production in sexually isomorphic glomeruli in males and females, and pheromone activated the sexually dimorphic macroglomerular complex in males. The NO response was concentration-dependent both for pheromone and for linalool, a plant volatile. The NO-induced fluorescence was reduced by bath-applying NO signaling antagonists. These results show clearly that NO is produced in the AL in response to odorant stimulation of the antennae. The resulting NO may then stimulate soluble guanylyl cyclase in a subset of AL neurons to affect the processing of olfactory information.

Supported by NIH-NIDCD DC04292.

Slide: Olfactory Bulb Physiology**Responses in the mouse main and accessory olfactory bulbs to general odorants and pheromones revealed by fMRI**F. Xu¹, N. Liu², I. Kida³, M. Schafer⁴, D.L. Rothman², F. Hyder³, D. Restrepo⁴ and G.M. Shepherd¹¹Neurobiology, Yale University, New Haven, CT, USA, ²Yale University, New Haven, CT, USA, ³Diagnostic Radiology, Yale University, New Haven, CT, USA and ⁴University of Colorado Health Sciences Center, Denver, CO, USA

High resolution fMRI has been used to study the responses in the mouse main and accessory olfactory bulb (MOB and AOB) to general odors and pheromones. General odorants elicited strong signals across the MOB; a few odors were also able to activate the AOB. Urine, a rich source of pheromones, stimulated both the MOB and the AOB. The patterns in the MOB and AOB were distinctly different for urine and amyl acetate. The patterns of urines from different strains shared significant domains, and correlated well with other mapping methods. For both general odors and pheromones, the pattern intensity in the MOB increased with concentration, with the pattern topography remaining similar. In the AOB, most odors did not significantly activate any region at low concentration, but did at high concentration. Both pattern intensity and topography were concentration dependent in the AOB. However, pheromones stimulated the AOB at lower concentrations. Interestingly, the most activated regions were not significantly affected by concentration, making the topography of the pattern at different concentrations similar, as in the MOB. The response time course was slower in the AOB than in the MOB. The results give insights into the similarities and differences between responses of the MOB and AOB to general odors and pheromones.

This research was supported by MURI and NIH grants DC-00086 (G.M.S.) and DC-03710 (F.H.).

Slide: Olfactory Bulb Physiology**Main olfactory bulb detection of social recognition cues in mouse urine**

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In rodents, chemicals present in urine play a critical role in reproductive behaviors and mediating aggressive interactions. Both behavioral tests and immediate early gene expression indicate that the main olfactory bulb (MOB) participates in individual identification. Little is known about how urine responsive neurons are organized in the MOB, or which semiochemicals in urine carry important information. To address these issues, we examined extracellular activity of mitral cells in the mouse MOB in response to volatile components of urine from different sexes and strains of mice. Recordings from >1000 cells revealed that <5% of neurons responded selectively to different sources of urine. The urine responsive cells were confined to a small region in the ventral and lateral parts of the MOB. To ascribe a role to specific chemicals present in urine, we combined gas chromatography with extracellular recording in the MOB, so that the activity of individual mitral cells could be monitored while delivering

the separated urinary components. The majority of neurons which showed an excitatory response to complete urine responded identically to just a single peak out of the hundreds present in the gas chromatogram of mouse urine. Surprisingly, over 20% of the urine responsive cells responded to one particular component, which has been implicated as a mouse pheromone. Preliminary behavioral data indicate the concentration of this pheromone in male mouse urine significantly correlates with the duration of female mice investigation of the urine. Thus, mitral cells selectively respond to individual components in mouse urine, and a disproportionate number of these neurons are involved in detecting a volatile pheromone.

Slide: Olfactory Bulb Physiology**Spatio-temporal firing rate interactions amongst an ensemble of mitral/tufted cells may subserve odorant discriminations**

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Populations of output neurons in the mammalian olfactory bulb (OB) exhibit distinct spatial and temporal activation patterns when stimulated with enantiomers of the same odorant. If, as has recently been suggested, a two-stage network of inhibitory and excitatory center-surround connections link spatially distributed glomerular activity with underlying mitral and tufted neurons within olfactory bulb, then it is likely that these interactions affect the ensemble response to odorant stimulation. Here we investigate in the anesthetized rat the rate response temporal kinetics in neuronal ensembles by comparing pair-wise response similarity of a population of single- and multi-unit mitral/tufted cells aligned to breathing in the presence and absence of enantiomers of limonene. Such comparisons can be used to quantify differences in the kinetics of the response of one neuron from other neurons in the recorded population. It is shown that aligning unit responses to the inhalation phase of the animals' breathing tends to synchronize unit responses. Instances of high-similarity and dissimilarity within the population are enantiomer-specific with these instances occurring for pairs of units recorded over electrode separations of 400 μm –1.7 mm. These results support the center-surround hypothesis and indicate that spatio-temporal firing rate interactions amongst an ensemble of OB neurons produce odorant representations beyond simple firing rates that may subserve odorant discrimination.

Slide: Olfactory Bulb Physiology**Presynaptic center-surround inhibition shapes odorant-elicited input to the mouse olfactory bulb**

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Synaptic transmission from olfactory primary afferents onto neurons of the olfactory bulb is modulated by presynaptic GABA_B receptors (e.g. Wachowiak and Cohen, 1999; Ennis *et al.*, 2001; Wachowiak *et al.*, 2002). To study the role of this modulatory circuit *in vivo* we recorded the fluorescent signal from Calcium Green-1 dextran, loaded into the axon terminals of olfactory receptor neurons, in

response to odorant presentations to the nose. We bath-applied agonists and antagonists of the GABA_B receptor onto the olfactory bulb and looked for an effect of these agents on the amplitude, spatial map and time course of odorant-elicited signals. The GABA_B antagonist CGP46381 caused a significant and long-lasting increase in the average amplitude of glomerular activation in most preparations. Large changes in signal amplitude were accompanied by a change in the input map such that many weakly activated glomeruli underwent relatively larger increases in signal amplitude than the strongly activated ones. We find that the magnitude of this effect correlates with how strongly activated the surround of a glomerulus is. This finding indicates that a form of GABA-mediated center-surround inhibition modifies the sensory input map even before the first synaptic transmission, increasing its contrast.

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Slide: Olfactory Bulb Physiology

Long-term odor exposure increases survival and functional integration of interneurons in the olfactory bulb

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While our previous work indicates that activity is critical for the survival of bulbar interneurons, the rules by which new neurons become integrated into existing neural circuits remain unclear. If olfactory activity guides the functional integration and survival of newly born cells, then repeated exposure to an odorant should increase the number of cells that participate in the coding of that odor. Adult subjects received daily injections of BrdU for 5 days to label a cohort of newly born neurons. They were then exposed daily to menthyl isovalerate, β -pinene, butyric acid, or mineral oil (control) for 3 weeks. For half of the animals in each group, the odorant was paired with Froot Loops to increase salience. On the last day, animals were exposed to ultra-pure odorants intermittently for 1 h, and tissue stained for BrdU and Fos protein. Long-term odor exposure doubled the survival of interneurons compared with controls. Double-label immunohistochemistry showed a statistically significant increase (44%; $P < 0.05$) in BrdU/Fos positive cells, demonstrating integration of newly born cells during the training period. Additional analyses confirmed that new neurons integrated specifically into the areas of the bulb activated by the odorant presented. This study demonstrates that survival and integration of newly born bulbar neurons depends on the odor environment present during their development. We conclude that repeated activation of synaptic ensembles might change neural requirements, thereby influencing the cellular milieu.

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Slide: Olfactory Bulb Physiology

A LimK disease model reveals defects in glomerular development and olfactory neurodegeneration

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The molecular mechanism by which the precise connectivity of the olfactory map is formed is poorly understood. We have investigated the role of the *Lim Kinase (Limk)* gene in the *Drosophila* olfactory map development. *Limk* has also been at the center of much attention lately because its loss is implicated in the human mental retardation disease, Williams Syndrome. We have created a *Limk* disease model by knocking out and overexpressing the fly *Limk* gene. At the neuromuscular junction, loss of *Limk* leads to enlarged synapses while expression of a hyperactive *Limk* mutant (*LimkK^d*) leads to stunted synapse. Expression of *LimkK^d* in the olfactory receptor neurons (ORNs) results in the formation of numerous ectopic glomeruli. The ability of the *LimkK^d* to induce ectopic glomeruli is abolished by either mutations in the *Pak* gene or overexpression of *cofilin* gene. This result indicates that *Pak*, *Limk* and *cofilin* function in a signaling pathway to regulate synaptogenesis in the fly antennal lobes. Interestingly, loss of *Limk* also leads to an adult-onset, progressive loss of ORN axons from the antennal lobes. This adult-onset neurodegeneration phenotype is mimicked by increased *cofilin* expression. In summary, *Limk* performs two functions *in vivo*, a developmental function in which it regulates synapse development and an adult function in which it protects neurons from premature demise by keeping *cofilin* activity in check.

Symposium: Developmental Regulatory Genes in the Taste and Olfactory Systems

Making a neuron: proneural bHLH factors during retinal and olfactory development

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Basic helix-loop-helix (bHLH) transcription factors play a central role in regulating the acquisition of neuronal cell fate in sensory tissues such as the olfactory epithelium and neural retina. For example, we identified the bHLH factor Xath5 in *Xenopus laevis* and demonstrated that it can regulate neuronal differentiation during retina and olfactory placode development. In general, proneural bHLH factors are both necessary and sufficient to promote the neural fate and do so by activating a core program of neuronal differentiation in progenitors. We have performed a differential screen to define the genes that are directly regulated by proneural bHLH factors in *Xenopus* and are examining how these genes contribute to the process of neuronal differentiation. In addition, specific factors act to regulate the onset of proneural gene expression during development. Frizzled 5, which is a transmembrane receptor that binds to wnt ligands, is exclusively expressed in the developing neural retina during early eye development in *Xenopus laevis*. We used antisense morpholino oligonucleotides to disrupt Frizzled 5 expression and found that this receptor regulates the onset of neurogenesis during retinal development. In the absence of normal Frizzled 5 expression, retinal progenitors fail to activate the expression of genes required for neural competence and subsequently fail to initiate proneural gene expression on schedule. Thus we can place proneural bHLH factors in a genetic cascade that leads to the final acquisition of a neural fate.

Symposium: Developmental Regulatory Genes in the Taste and Olfactory Systems

Common molecular signals regulating progression through the neuronal lineage in olfactory and other sensory epithelia

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To understand how signaling molecules regulate the generation of neurons from proliferating stem cells and neuronal progenitors in the mammalian nervous system, we have focused on studies of neurogenesis in the olfactory epithelium (OE) of the mouse. By analyzing neurogenesis in the OE of normal animals and mouse developmental mutants, we have identified distinct stages of stem and transit amplifying progenitor cells in the differentiation pathway of olfactory receptor neurons (ORNs); each of these cell stages can be identified by distinct molecular marker(s). Studies of progenitor cells in developing and regenerating OE have led to the understanding that (i) each progenitor cell type is regulated by signals produced both within the OE itself and by its underlying stroma; (ii) the same regulatory gene(s) play critical roles in neurogenesis in different regions of the primary olfactory pathway; and (iii) the same signaling molecules that regulate neurogenesis in OE likely play similar roles in other sensory epithelia.

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Symposium: Developmental Regulatory Genes in the Taste and Olfactory Systems

Shh signaling and taste bud maintenance in the adult mouse

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Continuous cell renewal in adult mammalian taste buds implies the requirement of inductive signals for growth and differentiation of taste bud precursor cells. And they should be dependent on the taste nerve. We have previously reported the expression of Sonic hedgehog (Shh) in the basal cells of taste buds. Various inductive events are mediated by the Shh signal in animal development. In adult taste epithelium, the expression of Patched1 (Ptc), Shh receptor, was observed in the surrounding region of the Shh expression, where mitotic cells contributing to taste buds may be distributed. Moreover, Nkx2.2 expression was found in taste buds: Nkx2.2 is transiently expressed in the neuronal precursor cells in the ventral region of neural tube, being activated by Shh signal. The Nkx2.2-expressing cells in the taste buds were found to express Mash1, a marker for the neuronal precursor cells. These observations suggest that Shh signaling may be involved in the taste bud maintenance in the adult mouse. In addition, the denervation revealed the strong nerve dependency of the basal cell-specific Shh expression in the taste bud. A quick loss of Shh after denervation led

the decrease of Ptc expression. The decrease might be involved in the arrest of precursor cell proliferation after denervation, which causes the taste bud disappearance. Shh signaling and the regulatory gene expressions in taste buds will be discussed in relation to the taste cell-specific genes.

This work was supported by Bio-Oriented Technology Research Advancement Institution of Japan.

Poster: Taste Hedonics & Psychophysics

Understanding vegetable acceptance: role of early experience

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Although infants differ substantially in their acceptance of foods during weaning, the source of such differences remains a mystery. Recently, we have identified a particularly apt system to explore these issues: the inherent flavor variations characteristic of infant formulas. Within each of these categories of formulas are a number of varieties that differ among themselves in formulation and flavor but the differences between the categories, and, in particular, between the hydrolysate and milk-based varieties in sensory quality (flavor) are striking and profound. The present study tested the hypothesis that the type of formula fed to infants would influence their acceptance of vegetables that shared a similar flavor note (e.g. sulfur volatiles) with the formula (e.g. hydrolysate formula). In counterbalanced order, we evaluated 87 infants' acceptance of pureed carrot on one testing day and pureed broccoli on the other. Infants who were feeding a hydrolysate formula consumed significantly less broccoli relative to carrots when compared with those who were currently fed milk based formulas. Such findings are consistent with previous research that demonstrated a sensory specific satiety following repeated exposure to a particular flavor in either formula or mothers' milk in the short term.

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Poster: Taste Hedonics & Psychophysics

Analgesic effects of intraoral sucrose: the more they like sweet taste, the better it works?

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During infancy and childhood, preference for sweet tastes is heightened and sweet-tasting substances can be analgesics. The goal of the study was to evaluate individual differences in sweet preferences and to determine whether such differences are related to sucrose's analgesic effects in 5- to 10-year-old children and their mothers. To this aim, the preferred level of sucrose was determined by using a forced-choice, paired comparison, tracking procedure, and the analgesic effect of sweet taste was determined by the Cold Pressor Test. As a group, children preferred significantly higher concentrations of sucrose than mothers. Ethnic differences in sweet preferences were observed in both children and adults such that Blacks preferred significantly higher concentrations when compared with Whites. Regardless of race, children who preferred high sweet concentrations kept their hand in the cold water significantly longer

when sucrose was held in their mouths when compared with water ($P = 0.002$). These findings suggest that the analgesic effects of sweet tastes may be more pronounced in those pre-pubertal children who have heightened sweet preference.

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Poster: Taste Hedonics & Psychophysics

Influence of concentration on taste–taste interactions in foods by elderly and young

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An increase in concentration of one of the tastants in a 'real food' might not only effect the perception of the taste quality of the manipulated tastant, but also the other perceivable taste qualities. The influence of concentration increase of sodium chloride, potassium chloride, sucrose, aspartame, acetic acid, citric acid, caffeine, quinine-HCl, monosodium glutamate (MSG), and inosine 5'-monophosphate (IMP) on the other perceivable taste qualities was studied in different foods. Twenty-one young (19–33 years) and 21 older subjects (60–75 years) rated the saltiness, sweetness, sourness, bitterness and umami taste of the food stimuli on 9-point scales. Repeated measures and multivariate analysis showed that an increasing concentration of sodium and potassium chloride diminished the sweetness more for the young than for the elderly, but enlarged the bitterness, sourness and umami taste in tomato soup more for the elderly than for the young. The saltiness of ice tea was decreased with an increase in sucrose, while a larger decrease in bitterness was found for the young than for the elderly. An increase in sucrose or aspartame concentration in ice tea also decreased the sourness. No influence was shown by an increment of acetic acid or citric acid in mayonnaise. The increasing concentration of caffeine and quinine induced a decrease in sweetness of the chocolate drink. The increase in MSG showed an increase in the saltiness of broth, whereas an increase in IMP led to a decrease in saltiness and an increase in sweetness. Young subjects took advantage of their sense of smell in the no noseclip condition.

Slide: Taste Hedonics & Psychophysics

Accounting for between-subject variance in discrimination and preference tasks

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The binomial statistic is typically used to determine the number of correct discriminations needed to indicate a significant difference between items. This statistic does not account for differences between subjects, making it inappropriate to combine responses across subjects and repetitions, which is often something researchers would like to do. The beta-binomial model is able to account for between-subject variance (measured by gamma), making such analyses possible. This study examined panel overdispersion (gamma)

for one group of subjects (53–58 subjects per group, depending on stimuli set) performing two replications of both paired comparisons (2AFC) and paired preferences on the same stimuli set. Stimuli tested included fruit-flavored beverages (with different sucrose levels), and snack foods (with different fat contents). Results showed that significant overdispersion with one task were not predictive of overdispersion in the other. Further, the stability of gamma across discrimination methods (2AFC, 3AFC, triangle and duo–trio) for a group of 103 subjects was examined. Stimuli were cherry-flavored fruit beverages at two different sucrose levels, and order of the discrimination tasks was counterbalanced across subjects. Results indicated that gamma was largely consistent across the 2AFC, 3AFC and triangle tasks, but it was higher in the duo–trio task. In all cases, the use of the beta-binomial model allowed for the combining of discriminations across subjects and replications, increasing the discrimination power achieved for a given panel size.

This project was self-funded.

Poster: Taste Hedonics & Psychophysics

Responses of PROP taster groups to variations in tastes and oral irritation within a beverage

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Despite considerable evidence that variations in sensitivity to the bitterness of 6-n-propylthiouracil (PROP) are also reflected in responses to both other tastes and also chemesthetic qualities in solution, there has been little research examining the impact of PROP sensitivity on response to these sensory modalities in foods or beverages. The present study examined responses of PROP taster groups to systematic variations in sourness and oral irritation in a carbonated beverage. Taster groups were defined according to ratings of a 0.0032 M PROP solution using the labelled magnitude scale (LMS). Using the LMS, subjects rated the sweetness, sourness and oral irritation of carbonated fruit drinks that systematically varied in citric acid (0.32, 0.64, 1.28, 2.56% w/v) and CO₂ (25, 50, 75 psi) concentrations. Ratings of sourness as a function of citric acid, and irritation as a function of both citric acid and CO₂ levels, were significantly different between groups, with highest ratings for STs and lowest for NTs. There were no group differences for sweetness ratings. These data are some of the first to show PROP taster group differences in tastes and irritation within a real product, and provide a basis for reported differences of PROP groups in their hedonic responses to foods.

Poster: Taste Hedonics & Psychophysics

Genetic sensitivity to 6-n-propylthiouracil, and perception of high-intensity sweeteners in model soft drinks

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Intense sweeteners (e.g. saccharin, aspartame, and acesulfame-K) impart sweetness, bitterness and other aftertastes to foods.

Individual variation in sensitivity to sweeteners is well known. Those who are genetically sensitive to the bitterness of 6-n-propylthiouracil (PROP) perceive more sweetness and bitterness from saccharin. Findings for other sweeteners are less clear. This study examined the role of PROP taster status in perception and acceptance of intense sweeteners in citrus-flavored model soft drinks. Young adults were classified as non-tasters (NT; $n = 29$) or supertasters (ST; $n = 30$) of PROP using a filter paper screening method (Zhao *et al.*, 2003). The following sweeteners were used: 10 and 8% high fructose corn syrup (HFCS) (controls), sucralose (SUC), aspartame (ASP), acesulfame-K (ACE), ASP/ACE and SUC/ACE. Subjects rated the intensity of nine attributes with a 15-cm line scale and liking with the Labeled Affective Scale. ST perceived more bitterness from SUC/ACE and 8% HFCS ($P = 0.05$); ACE was marginally more bitter to ST ($P = 0.06$). ST also perceived more persistence of sweetness across all sweeteners ($P = 0.05$). Three-dimensional models best described the perceptions of the sweeteners. For ST the dimensions were bitterness (43% variance), persistence of sweetness (22%) and carbonation (11%). For NT the dimensions were: sweetness and citrus flavor (37%), bitterness (24%) and carbonation/thickness (11%). Liking was uniquely related to low bitterness for NT but was related to multiple attributes for ST. These data suggest that ST experience intense sweeteners differently than NT but these differences play a modest role in soft drink acceptance.

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Poster: Taste Hedonics & Psychophysics

6-n-Propylthiouracil bitterness and tastes from alcoholic and non-alcoholic beverages in of-age undergraduates

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Previous research has shown associations between taste genetics and alcohol behaviors. We tested the taste genetic and alcohol intake relationship and linked this relationship to oral sensations from alcoholic beverages. Taste genetics was measured via perceived 6-n-propylthiouracil (PROP) bitterness; nontasters taste PROP as mildly bitter while supertasters as intensely bitter. Sixty-two adults (mean age = 22 years) used the general Labeled Magnitude Scale to rate PROP bitterness and oral sensations from and preference for sampled Pilsner beer, scotch, strong black coffee and unsweetened grapefruit juice. Subjects also completed validated questionnaires on behaviors toward alcohol. Data were analyzed with regression analyses. Those who tasted greater PROP bitterness reported all beverages as more bitter and less preferred except beer. Neither PROP bitterness nor beer bitterness were strong predictors of beer preference. PROP nontasters tasted scotch as less bitter but more sweet than did supertasters. Those who tasted scotch as more bitter consumed less alcohol; PROP bitterness only tended to associate with alcohol intake. Beer sensations did not associate significantly with alcohol intake. Summary: Nontasters get less negative (bitter) and more positive (sweet) tastes from alcohol than supertasters. Sensory differences translated into greater liking for bitter beverages by nontasters, except for beer. Positive social influences

to drink beer on college campuses may override negative oral sensations to hinder intake.

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Poster: Taste Hedonics & Psychophysics

Personality traits and PROP sensitivity

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Although theories of personality vary widely, most trait theories include the dimension of introversion–extroversion. Physiological differences are thought to exist between people who are represented by the extremes on this dimension, with introverts experiencing a high level of baseline arousal. This higher arousal level suggests that introverts experience elevated sensitivity to perceptual stimuli, including sour tastes (Eysenck, 1976), as compared with extroverts. As the ability to taste PROP can also affect taste sensitivity, the current study investigated the relationship between PROP sensitivity and personality traits, particularly introversion. The NEO-PI, a personality measure that evaluates five personality traits, was administered to 50 college students (39 women, 11 men). After completing the personality test, participants were given a PROP sample and asked to rate its intensity on a labeled magnitude scale (LMS; Green, 1993). No correlation was found between introversion, conscientiousness, or openness to experience and PROP tasting ability. However, a relationship was seen between the domain trait of neuroticism and intensity ratings of PROP on the LMS ($r = 0.346$, $P = 0.014$), in particular those facets concerned with anxiety and depression. Although agreeableness was not overall associated, the facet concerned with trust was inversely ($r = -0.336$, $P = 0.017$) associated with LMS scores. These associations suggest that personality traits may interact with genetic abilities as partial explanation for individual differences food preferences.

Poster: Taste Hedonics & Psychophysics

Orosensory and genetic taste markers predict alcohol intake across age cohorts

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Supertasters, identified by heightened 6-n-propylthiouracil (PROP) bitterness and fungiform papilla (FP) number, report greater negative and less positive sensations from alcohol, possibly hindering intake. Work by others (e.g. Kranzler *et al.*) show positive associations between oral sensations and intake, findings that contradict genetic taste (GT)-mediated effects. To characterize age, sensory, and GT effects on intake, 99 healthy women (aged 20–84 years) used the general Labeled Magnitude Scale to rate PROP bitterness, regional and whole mouth intensity of prototypical tastants and ethanol on the tongue tip. Intake was assessed via a frequency interview and FP by videomicroscopy. In young subjects, lower PROP bitterness and greater whole mouth taste were significant

predictors of greater alcohol intake, yet a spatial taste measure diminished the predictive ability of whole mouth taste. Across the entire sample, age (young > aged), FP number (higher number, less intake) and whole-mouth taste responses (greater intensity, more intake) were significant predictors of intake. Cross quality whole-mouth and chorda tympani, but not circumvallate, intensity showed age-related loss. In summary, GT markers and additional taste markers contribute to predicting alcohol intake across age cohorts. Reasons for higher taste response associating with greater alcohol intake remain unclear. Spatial interactions among sensory nerves could heighten whole mouth orosensation (e.g., sweetness) and change the sensory appeal of alcohol.

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Poster: Taste Hedonics & Psychophysics

Influence of fatty acid sensitivity on taste perception

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Following previous work (Gilbertson *et al.*, Kamphuis *et al.*) demonstrating chemosensitivity to fatty acids in the taste system, we investigated whether sensitivity to fatty acids in humans: (i) is influenced by olfaction; and (ii) is associated with differences in taste perception in general. Methods: a group of (linoleic acid) LA-tasters (≥ 9 out of 10 correct detections of 10 μ M LA) was compared with a non-taster group ($n = 20$ total). To investigate (i), triangle tests were conducted using 0% fat mayonnaise with and without LA (10 μ M), with nose clips on and off. To investigate (ii), taste profiles were generated for various products/solutions containing e.g. LA and basic tastants, with and without noseclips. Results: (i) LA-tasters showed above-chance performance without noseclips ($P = 0.04$), but not with noseclips. Non-tasters did not perform above chance. (ii) There was a main effect of taster status ($P < 0.001$) on the evaluations of the sensory attributes of stimulus solutions. Tasters gave higher scores than non-tasters to taste intensity, bitterness, prickling and astringency, but lower scores to fattiness and softness. Wearing noseclips did not affect results. Conclusions: The differences found between the sensory experiences of LA-tasters and non-tasters may have an important impact on dietary preference. Olfaction seems to contribute to the ability to detect LA in products by LA-tasters, but this does not account for the differences found in sensory attribute scores.

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Poster: Taste Hedonics & Psychophysics

Effects of the sweet taste inhibitor lactisole on the umami taste of MSG

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Both sweeteners and monosodium glutamate (MSG) are transduced by TAS1R receptors. Specifically, the TAS1R2–TAS1R3

heterodimer is responsive to sweeteners and the TAS1R1–TAS1R3 heterodimer is responsive to MSG. The compound lactisole Na⁺[2-(4-methoxyphenoxy) propionic acid] is a very effective sweet taste inhibitor and known to inhibit the T1R2–T1R3 dimer. The present experiment determined whether lactisole can block the umami taste of MSG and mixtures of MSG and 5'-ribonucleotides. Because lactisole was employed at very high concentrations, we also determined its impact on exemplars of other taste qualities (sucrose—sweet, NaCl—salty, citric acid—sour, quinine—bitter) as a general control for mixture effects. The experimental test stimuli (100 mM MSG; 20 mM MSG + 3 mM IMP; 20 mM MSG + 3 mM GMP) were mixed with six levels of lactisole (0, 200, 800, 1600, 3200, 6400 p.p.m.) in three randomized replications. In addition, the four exemplar solutions intensity matched to 100 mM MSG were tested with 0 and 3200 p.p.m. lactisole with two replicates. Subjects rated intensities on a computer LMS screen with five quality scales available. The umami taste of MSG was inhibited by lactisole, but only at relatively high concentrations. The presence of 5'-ribonucleotides prevented lactisole from inhibiting umami taste. As expected, lactisole at high concentrations inhibited the sweetness of sucrose, but had no effect on sour, salty or bitter taste. To the degree that human umami and sweet taste are initiated by activation of T1R1–T1R3 and T1R2–T1R3 respectively, lactisole appears able to inhibit the T1R1–T1R3 receptor almost completely but with a much lower affinity than it inhibits the T1R2–T1R3 receptor.

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Poster: Taste Hedonics & Psychophysics

Do compounds that elicit sweet water-taste also inhibit sweetness? A paradoxical case study with saccharin

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Saccharin is a well-known sweetener and has been used commercially in the soft-drinks industry. Unlike other sweeteners, saccharin is not sweet tasting at high concentrations. A potential explanation for this phenomenon is the strong bitter taste that accompanies high concentrations of saccharin. However, the degree of bitter taste does not predict the low level of sweetness observed at high concentrations. An alternative explanation derives from the curious observation that after tasting strong concentrations of saccharin, water rinses taste very sweet; a phenomenon known as sweet water-taste. We hypothesized that compounds with associated sweet water-taste are sweet taste inhibitors, and we further proposed that saccharin inhibits its own sweetness at high concentrations. We demonstrated in fourteen subjects that 200 mM Na saccharin, 200 mM acesulfame-K, 1 mM lactisole and, to a lesser degree, 500 mM MgSO₄ stimulate sweet water-taste. All four compounds inhibited to varying degrees the moderate sweetness intensity of 300 mM sucrose, 4 mM Na saccharin, 3 mM aspartame and 17.5 mM Na cyclamate. These results demonstrate that sweet water-taste can be used to identify compounds with sweetness inhibiting activity. These data further illustrate that the sweeteners Na saccharin and acesulfame-K are paradoxically sweet taste inhibitors at high concentrations. At these concentrations, they no longer taste sweet because they are likely autoinhibiting their own sweet taste. Some potential mechanisms for how a single compound can be both a sweet taste agonist

at low concentrations and sweet taste inhibitor at high concentrations will be discussed.

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Poster: Taste Hedonics & Psychophysics

Inhibition of bitter taste by zinc and Na-cyclamate

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Previous research has shown that zinc is a potent inhibitor of the bitterness of quinine-HCl (R.S.J. Keast, 2003, *J. Food Sci.* 68: 1871–1877) and the sweet taste of most sweeteners (except Na-cyclamate) (Keast *et al.*, 2004). We investigated the influence of zinc on perceived bitterness of six structurally divergent bitter compounds. We also determined the impact of a combination of zinc + Na-cyclamate or zinc + sucrose on the bitterness of specific compounds. In experiment one, six diverse bitter tasting compounds [quinine-HCl (QHCl), tetralone (TET), sucrose octaacetate, dextromethorphan, denatonium benzoate (DB) and pseudoephedrine (PSE)] were matched for 'moderate' bitterness intensity. To these compounds one of five salts was added: 25 or 300 mM Na acetate, 25 mM Mg sulfate, 25 mM Mg acetate, and 25 mM Zn sulfate. All possible binary combinations of bitter compounds and salts were tested. In experiment two, the influence of Zn sulfate on bitter-sweet mixtures was investigated. The bitter compounds used were DB and PSE, and the sweet compounds were sucrose and Na-cyclamate. Zn sulfate, 300 mM Na acetate, and Mg acetate significantly inhibited bitterness ($P < 0.05$). Adding a combination of zinc and a sweetener (Na-cyclamate or sucrose) to DB and PSE allowed the cognitive effects of sweetness and the oral peripheral effect of zinc on bitterness to combine.

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Poster: Taste Hedonics & Psychophysics

Changes in taste perception and emotional face recognition in chronic back pain

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Chronic pain patients are thought to have a heightened sensitivity to pain. Neuroimaging studies have shown overlapping representation of taste, smell and pain in the insula. We evaluated taste detection, recognition and suprathreshold intensity estimation in patients with chronic back pain ($n = 11$) and healthy normal subjects ($n = 11$). Given the proposed role for the insula in discriminating emotional facial expression we also tested our subject's ability to discriminate faces with displayed emotions (anger, disgust, fear, happiness, sadness, surprise). All subjects also completed a control task in which they rated the intensity of gray slides. No group differences were observed on the control task. Compared with the age- and gender-matched control group, patients with chronic back pain

had lower taste detection thresholds (i.e. increased sensitivity). No group differences were observed with recognition thresholds. Chronic back pain patients also gave higher intensity ratings for suprathreshold salty, sour and sweet tastes ($P < 0.05$), with a trend for higher intensity ratings for the bitter taste ($P = 0.016$). Patients were also less accurate at discriminating disgusted ($P < 0.01$), fearful ($P < 0.05$) and angry ($P < 0.05$) faces. These results support the notion that patients with chronic pain are more sensitive to some classes of sensory experience (i.e. those with limbic representation), while simultaneously exhibiting impaired discrimination of some classes of emotional stimuli (i.e. faces).

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Poster: Taste: Fats

Behavioral taste responses to linoleic acid by female rats

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Previous studies in male rats showed that, in short term tests, licking responses to solutions of linoleic acid (LA) mixed in sucrose decreased with increasing LA concentration. This study evaluated licking responses to LA by ovariectomized (OVX) female rats ($n = 11$) that were trained to consume fluids in brief 10 s trials. OVX rats were given estradiol benzoate (EB: 10 μg) or the oil vehicle (OIL) and then tested using water, 0.0375 M sucrose, 0.2 M sucrose and LA (11, 22, 44, 88 μM) mixed in 0.2 M sucrose and in 0.0375 M sucrose. Licking was maximal to 0.2 M sucrose (EB: 68.3 ± 1.1 licks/10 s; OIL: 69.0 ± 1.0 licks/10 s) and lower to 0.0375 M sucrose (EB: 39.2 ± 5.0 licks/10 s; OIL: 40.9 ± 4.9 licks/10 s). EB and OIL treated OVX rats sustained maximal licking rates to all LA concentrations mixed in 0.2 M sucrose. Interestingly, when LA was mixed in 0.0375 M sucrose, OVX rats increased licking with increasing LA concentration reaching maximal levels to 44 μM (EB: 60.6 ± 3.8 licks/10 s; OIL: 61 ± 9 licks/10 s) and then decreased slightly to 88 μM (EB: 53.4 ± 6.3 licks/10 s; OIL: 54.4 ± 5.1 licks/10 s). These results indicate that, unlike male rats in previous studies, female rats do not decrease licking to increasing LA concentrations. In fact, the use of LA mixed in two sucrose concentrations revealed that female rats, regardless of hormone treatment, actually increase licking relative to 0.0375 M sucrose with increasing LA concentration. Thus, there may be sex differences in the behavioral taste responses to LA; however, this difference does not depend on EB.

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Poster: Taste: Fats

Gustatory detection of oleic acid and stimulus generalization to linoleic acid in rats

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Previous research has shown that taste receptor cells are capable of transducing free fatty acid chemical components of dietary fat. Our laboratory has provided evidence that rats are capable of detecting and avoiding the principle free fatty acid in corn oil, linoleic acid,

at micromolar concentrations. This study characterizes the ability of Sprague–Dawley rats to detect and avoid a secondary free fatty acid in corn oil, oleic acid, using a conditioned taste aversion paradigm. Following the single pairing of a LiCl injection with consumption of oleic acid at a concentration $\geq 66 \mu\text{M}$, rats avoided future consumption of oleic acid as compared with controls; however, the pairing of a LiCl injection with consumption of $44 \mu\text{M}$ oleic acid did not produce a conditioned taste aversion. Given that rats form conditioned taste aversions to both linoleic and oleic acid, the similarity between the orosensory properties of the two free fatty acids was examined using a stimulus generalization conditioned taste aversion experiment. Following the single pairing of a LiCl injection with either $88 \mu\text{M}$ oleic or linoleic acid, rats formed a taste aversion and subsequently avoided consumption of the other respective free fatty acid. These data suggest that rats can detect the presence of oleic acid ($\geq 66 \mu\text{M}$) and that oleic and linoleic acid share common orosensory properties such that rats generalize a conditioned taste aversion to either free fatty acid.

Poster: Taste: Fats

The gustatory sensation from free fatty acid

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Fat in food often increases the palatability of food, even though it does not give us obvious taste by itself. Some researches have reported that taste cells recognize free fatty acid, a hydrolysate from common dietary fat, though the free fatty acid has not been defined as one of tastants. Its perceptual mechanism is still unclear. We demonstrated immunohistochemically that CD36, one of fatty acid transport proteins, was localized on the apical part of the taste cells isolated from rat vallate papillae. This localization supports the hypothesis that CD36 plays a role in the oral recognition of dietary fat. In this study, we used CD36-null mice for behavioral studies and nerve recordings and compared with the wild type mice that had the same background. The wild type mice preferred oleic acid solution to mineral oil solution in short-time two-bottle preference tests, but the CD36-null mice did not show the preference for either solution. Neural recording revealed that small but significant response to oleic acid was observed on the lingual branch of glossopharyngeal nerve in the wild type mice, but not in the CD36-null mice. These results suggest that gustatory signal of fat was conveyed via the lingual branch of glossopharyngeal nerve and that the CD36 molecule, which exists on the tongue innervated by the lingual branch of glossopharyngeal nerve, plays an important role in enjoying a taste of fatty acid.

Poster: Taste: Fats

PROP taster status and perception of fats and free fatty acids

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PROP tasters, especially supertasters, are reportedly better able to perceive the level of fat in foods than PROP non-tasters. This study tested perception of fat in both foods and free fatty acids in water. PROP taster status was determined by the five-solution method. To date, seven non-tasters, 12 tasters and seven supertasters have been tested. Subjects were randomly presented 5 ml samples of milk (0.00, 3.25, 10.00, 18.00, 36.00% fat/wt), 5 ml of vanilla pudding (4.1, 8.2, 16.3, 24.4, 29.7% fat/wt) and 5 g of brownies (8.0, 16.0, 24.0, 31.8, 35.6% fat/wt) and rated the level of fat in duplicate using the gLMS. Milk and vanilla pudding were served at 7°C and brownies at 22°C. To mask texture, oleic and linoleic acids (0.40, 0.71, 1.267, 2.25, 4.00% w/w) were suspended in a solidified unflavored gelatin starch-thickened water mixture and 5 g samples were served at 22°C. Stearic acid (0.5, 1.0, 2.0, 4.0, 8.0% w/w) was suspended in starch-thickened water and 5 ml samples were served at 71°C. Samples were randomly presented in duplicate and the level of fat rated using the gLMS. Subjects correctly ranked the fat levels ($P < 0.0001$) in all foods and free fatty acids solutions, but no differences were observed between PROP taster groups. These data demonstrate a taste component for free fatty acids, but no differences in fat perception based on PROP taster status.

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Poster: Taste: Fats

Fat taste—are free fatty acids or conjugated dienes the effective stimulus?

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‘Fattiness’ is hypothesized to be a basic taste quality, most efficiently elicited by long-chain poly- and monounsaturated fatty acids. Free fatty acids (FFA) have been prepared in solutions containing an emulsifier or thickening agent to mask textural cues or in foods. Whether the effective stimulus was the FFA or oxidation product has not been established. This work examined the effects of addition of EDTA and sonification (to reduce oxidation) on oxidation product concentration. Linoleic acid was prepared at a concentration of 10 mg/dl and mixed in a solution of 5% acacia and demineralized water. The concentration of EDTA was 0.01%. Samples were sonified for 40 min. This preparation increased linoleic acid recovery 100 times over a simple oil–water mixture. These results suggest extensive degradation of FFAs in previously used fat taste samples resulting in conjugated dienes that may be effective taste stimuli. This is being explored through psychophysical studies.

Poster: Vomeronasal Organ

Regulator of G-protein signaling proteins in the vomeronasal organ of garter snakes

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The response to prey chemoattractants in garter snakes is mediated by the vomeronasal (VN) system. The chemosignal transduction pathway in the VN epithelium involves the binding of agonist to its G-protein coupled receptors leading to activation of Gi/o-proteins which in turn activate PLC, converting PIP₂ to IP₃ and DAG, and resulting in a transient cytosolic Ca²⁺ increase and changes in membrane potential. However, the desensitization mechanism so far remains unknown. We found that the chemoattractant-induced signal is modulated by two membrane-bound proteins, p42/44, which modulate the exchange of GTP for GDP on the Ga subunit. During recent years, regulators of G-protein signaling (RGS proteins) have been identified. These proteins enhance the activity of GTPase intrinsic to G alpha subunits and have been shown, in a wide number of biological systems, to play an important role in modulating agonist-induced signals. Using commercially available RGS antibodies, we detected the expression of several isoforms of RGS proteins in the VN sensory epithelium of garter snakes. In addition, by screening a snake VN cDNA library, we obtained several clones which show high homology to RGS2, RGS3 and RGS4. These RGSs may play a role in desensitization of chemoattractant-induced signal transduction.

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Poster: Vomeronasal Organ

Chemosignal transduction in the vomeronasal organ of garter snakes: chemoattractant-induced membrane potential changes

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We have demonstrated previously that in the snake vomeronasal (VN) organ chemoattractant (ESS) produce transient calcium cytosolic accumulation in the dendritic regions of VN neurons via two pathways: calcium release from IP₃-sensitive stores and a plasma membrane calcium influx. Using voltage-sensitive dyes, here we characterize the functional role of these events during VN chemosensory transduction. ESS evokes optical depolarizations which exhibit a time course similar to the EVG, but shorter than VN calcium transients. Peak depolarization in the apical VN region are reduced but not suppressed in the absence of external calcium, indicating that they probably depend on non-selective cation channels. Depletion of all internal calcium stores evokes a reduction of these depolarizations, which is not observed when responses are evoked by potassium depolarization. Interestingly, the depletion of ryanodine-sensitive calcium stores fails to evoke this reduction, and instead increases the duration of depolarizations in the cell body region. This effect is suppressed when potassium currents are blocked. These results support the notion of a functional compartmentalization between different calcium stores and indicates a dual and opposite action of calcium release in snake VN neurons during chemosensory transduction. Calcium release by IP₃-sensitive stores appears to enhance the initial dendritic depolarization, while calcium release by ryanodine stores seems to activate re-

polarizing potassium currents controlling the duration of these responses.

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Poster: Vomeronasal Organ

Vomeronasal organ signal transduction in the Chilean lizard, *Liolaemus bellii*

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Specimens of *Liolaemus bellii* (Chilean lizard) were live-captured in the mountains of central Chile and transported to the USA to determine their suitability for cellular studies of the vomeronasal organ (VNO). Rhodamine-conjugated dextran plus 2% Triton X-100 was introduced into the vomeronasal (VN) orifice to confirm the location of the VN epithelium and its degree of compartmentalization from the MOE. After migration of the vital dye for two weeks, individual vomeronasal sensory neurons (VSNs) were distinctly labeled and showed a bipolar morphology as reported in the VNO of all other vertebrates. Ten-micrometer cryosections of the VN epithelium were intensely labeled with antibodies against Giα1–3 and Gβ G-proteins. Extracts were prepared (1:300 dilution) from three body source secretions (skin, feces, cloacal) known to contain pheromones mediating chemical communication in these animals. Three-micromolar agmatine (AGB) was mixed with one of the body source secretions or control saline and lizards were stimulated with the pheromone plus AGB mixture to histologically map and determine secretion rank-order effectiveness. Using L-cysteine-activated papain for VSN isolation, we were able to preserve both voltage and chemosignal-activated conductances ($n = 69$ total recordings) using recording and pipette solutions optimized for turtle preparations. The tuning and percentage of chemosignal-activated responses (25 of 46 VSNs, 54% response rate), when presented a panel of five chemical extracts, will be favorable for future single cell electrophysiology studies combined with a distinct cadre of quantifiable behavioral displays such as the tongue flick, head bob, and scent marking.

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Poster: Vomeronasal Organ

Protein interactions with the TRPC2 ion channel in the vomeronasal organ

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The role of the inositol 1,4,5-trisphosphate (IP₃) second messenger system in vomeronasal sensory neurons (VSNs) of the vomeronasal organ (VNO) is unclear. Furthermore, how the functional connections between the type 3 IP₃ receptor (IP₃R3) and transient receptor potential channel type 2 (TRPC2) may influence the cationic receptor potential is also unknown. We have previously demonstrated that IP₃R3 and TRPC2 have an overlapping expression pattern in rodent VNO and participate in a protein-protein interaction.

Here we sought to demonstrate a functional role for the IP₃R3/TRPC2 protein complex. Pheromone-evoked whole-cell currents were blocked in four of five VSNs when a peptide, directed against the interaction domain between IP₃R3/TRPC2, was included in the recording pipette held (Vh) at -60 mV. Under our recording conditions, pipette dialysis of 240 mM IP₃ failed to evoke whole-cell current in 20 of 20 VSNs tested. Typical antagonists of the IP₃R (ruthenium red, 2-APB) also failed to block pheromone-evoked currents. SDS-PAGE and Western analysis of male and female VNO tissue reveals expression of one or more of the long isoforms of the adaptor protein Homer (1b, 1c, 2a, 2b, and 3; 45 kDa) as well as neuronal Shc (66, 53, 46 kDa), an adaptor protein known to communicate G-protein-coupled receptor (GPCR) and receptor tyrosine kinase (RTK) activation. The IP₃R3/TRPC2 protein complex may be associated within a larger protein scaffold whereby IP₃ and/or adaptor proteins could subserve regulatory functions of the primary transduction current.

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Poster: Vomeronasal Organ

Species specificity in rodent pheromone receptor repertoires

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The mouse V1R putative pheromone receptor gene family consists of at least 137 intact genes clustered at multiple chromosomal locations in the genome. Species-specific pheromone receptor repertoires may partly explain species-specific social behavior. We conducted a genomic analysis of an orthologous pair of mouse and rat V1R gene clusters to test for species-specificity in rodent pheromone systems. Mouse and rat have lineage-specific V1R repertoires in each of three major subfamilies at these loci as a result of post-speciation duplications, gene loss and gene conversions. The onset of this diversification roughly coincides with a wave of Line1 (L1) retrotranspositions into the two loci. We propose that L1 activity has facilitated post-speciation V1R duplications and gene conversions. In addition, we find extensive homology among putative V1R promoter regions in both species. We propose a regulatory model in which promoter homogenization could ensure that V1R genes are equally competitive for a limiting transcriptional structure to account for mutually exclusive V1R expression in vomeronasal neurons.

Poster: Vomeronasal Organ

Expression pattern of genes for notch signaling pathway in mouse vomeronasal organ during ontogeny and regeneration after removal of accessory olfactory bulb

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Vomeronasal receptor neurons (VRNs) proliferate and differentiate continuously throughout life. Proliferation of VRNs mainly occurs in the marginal region of the sensory epithelium of adult vomeronasal organ (VNO). The Notch signaling pathway is involved in cell fate decisions and differentiation during developmental CNS. We have studied whether Notch signaling pathway involves in differentiation and proliferation of VRNs during ontogeny and regeneration. In this study, we examined the expression patterns of Notch and their ligands, Delta and Jagged, using *in situ* hybridization during ontogeny and regeneration after removal of accessory olfactory bulb (AOBX) in mice. In adult VNO, a few Notch1(+), Delta1(+) and BrdU(+) cells appeared only in the marginal region, whereas Jagged2 was expressed in all VRNs. Notch1(+), Delta1(+) and BrdU(+) cells located close to the basal lamia at embryonic days 14.5 and 16.5, whereas expression level of Jagged2 was very low in comparison with adult. At AOBX day2, number and location of Notch1(+), Delta1(+) and BrdU(+) cells did not change. Expression pattern of Jagged2 did not change. At AOBX day7, large amount of Notch1(+), Delta1(+) and BrdU(+) cells appeared in the marginal region, whereas expression pattern of Jagged2 did not change. These results suggested that the interaction of Notch1(+) and Delta1(+) cells play important roles in vomeronasal neurogenesis of VNO during ontogeny as well as regeneration after AOBX mice.

Poster: Vomeronasal Organ

The role of the vomeronasal system in food preferences of the gray short-tailed opossum, *Monodelphis domestica*

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The vomeronasal system (VNS) is usually considered primarily a pheromone-detecting system. In snakes and lizards, this system is also important for feeding behavior. To date, no studies have reported feeding deficits in mammals deprived of a functional VNS. *Monodelphis domestica* is considered a primitive mammalian species that was recently introduced into laboratories. Since these opossums respond to a variety of foods, they are a good model to investigate the role of the VNS in food preferences in mammals. The six male and seven female gray short-tailed opossums used in this study were simultaneously presented with four foods, one from each of the following food groups: fruits (apples, oranges, peaches, cantaloupes), meats (mealworms, chicken, pork, crickets), processed vegetables (raisin bran, cheerios, whole wheat bread, bagel) and unprocessed vegetables (corn, peppers, carrots, broccoli). Before blocking access to the vomeronasal organ (VNO) with gel foam and Crazy Glue, the opossums selected meats most frequently and fruits more frequently than processed and unprocessed vegetables. Following VNO blockage, the opossums demonstrated no preference between the different food groups. This study suggests that without a functional vomeronasal organ, the food preferences of gray short-tailed opossums are significantly impaired.

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Slide: Vomeronasal Organ**Chemo-investigatory behaviour of male mice in detecting estrus: role of olfactory-vomeronasal system**

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The aim of the present study is to evaluate whether the male mouse is capable of discriminating the female urinary odour of different reproductive phases, with a view to detect oestrus using Y-maze apparatus and to establish the relationship of the olfactory–vomeronasal system and chemo-investigatory behaviour in oestrus detection. Hence, normal, vomeronasal organ (VNO)-ablated and zinc sulphate-irrigated mice were used as test animals. Various behaviours such as frequency of visit, duration of visit, sniffing, licking, body rubbing and grooming were recorded. The normal mice frequently visited and devoted more time in delivering various behaviours towards the oestrus urine sample in comparison to that of non-oestrus urine. The VNO-ablated mice showed significant reduction in response to estrus urine (duration of visit and self-grooming) than that of ZnSO₄-irrigated mice. However, the ZnSO₄-irrigated mice showed significant reduction in frequency of visits to the urine samples. These results clearly reveal that the VNO play a significant role in the detection of oestrus in mice. The present results also suggest that male mice preferentially communicate sexual interest via self-grooming towards the opposite sex. By self-grooming at higher rates, male mice may be broadcasting scents to attract potential mates or to inform their willingness to mate.

Poster: Vomeronasal Organ**Neurogenesis, migration and apoptosis in the vomeronasal epithelium of adult mice**A. Martinez-Marcos¹, W. Quan², C. Jia² and M. Halpern²*¹Departamento de Ciencias Medicas, Universidad de Castilla-La Mancha, Albacete, Albacete, Spain and ²Anatomy and Cell Biology, Downstate Medical Center, Brooklyn, NY, USA*

Neurogenesis in the adult mouse vomeronasal organ appears to occur in the central regions, but is more prevalent at the edges of sensory epithelium. Basal cells at the center of the epithelium participate in cell replacement. It is unknown whether dividing cells at the edges constitute a reservoir for growth, become apoptotic or participate in neural turnover. This latter possibility implies a process of horizontal migration. The present work addresses this controversy by injecting bromodeoxyuridine in adult mice and allowing them to survive for various intervals. The vertical and horizontal position of labeled cells was analyzed as a function of time. Both, vertical and horizontal migration of labeled cells were detected. Cells at the center of the epithelium migrate vertically to become neurons as demonstrated by co-expression of olfactory marker protein. Cells at the edges migrate horizontally toward the center. After 42 days, however, they have migrated <10% of the distance from the edge (0%) to the center of the epithelium (100%), thus making it likely that if these cells participate in neural turnover it is only in marginal regions. The pattern of distribution of apoptotic cells has been studied and, interestingly, it is similar to that of dividing cells. These results support the idea that sensory cell renewal in the

mouse vomeronasal organ occurs through a process of vertical migration.

Supported by grant DC02745.

Poster: Vomeronasal Organ**New method of vomeronasal nerve transection leaves the olfactory system intact**M. Matsuoka¹, M. Norita² and R.M. Costanzo³*¹Niigata University Graduate School of Medical and Dental Sciences, Niigata, Niigata, Japan, ²Division of Neurobiology & Anatomy, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Niigata, Japan and ³Physiology, Virginia Commonwealth University, Richmond, VA, USA*

Methods used to study degeneration and regeneration in the vomeronasal system typically result in collateral damage to olfactory system pathways. We set out to develop a new approach to selectively lesion the vomeronasal nerves and to leave the olfactory system and its nerve fibers intact. For this study we used OMP-tauLacZ mice and X-gal staining, which allowed us to label both vomeronasal and olfactory pathways containing the olfactory marker protein (OMP). Vannis microdissecting scissors were used to cut the vomeronasal nerves at a point just anterior to the accessory olfactory bulb (AOB). We then observed the vomeronasal epithelium, the olfactory epithelium, the main olfactory bulb (MOB) and the AOB at 1, 6, 20, 60 and 120 days after nerve lesion. At days 20 and 60 we found that there were no OMP-positive cells in the vomeronasal epithelium and no OMP-positive nerve terminals in the AOB. In contrast, OMP-positive cells in the olfactory epithelium and their OMP-positive nerve fiber projections to the MOB remained intact. In a few animals examined after 120 days of recovery we noticed OMP staining in both the vomeronasal epithelium and nerve projections within the AOB. These findings suggest that recovery in the vomeronasal epithelium may be associated with the retargeting of nerve fibers in the AOB. This new approach to vomeronasal nerve transection may prove important for behavioral studies of vomeronasal function, since it has the distinct advantage of leaving the olfactory system intact.

Supported by NIH grant DC00165.

Slide: Vomeronasal Organ**Proteomics analysis of olfactory systems: a generic approach using differential dye analysis and *de novo* peptide sequencing for identifying proteins potentially involved in pheromone transport and reception**D. Greenwood¹, M. Jordan¹, J. Cooney², D. Jensen², K. Plummer³, R. Newcomb¹ and L. Rasmussen⁴*¹Gene Technologies, HortResearch, Auckland, New Zealand, ²HortResearch, Hamilton, New Zealand, ³School of Biological Sciences, University of Auckland, Auckland, New Zealand and ⁴Oregon Graduate Institute of Science & Technology, Beaverton, OR, USA*

Mucosal proteins from the trunk and VNO duct of the Asian elephant and proteins, both soluble and membrane-bound, extracted

from the antennae of tortricid moths have provided us with diverse sources of olfactory proteins. We have developed a proteomics platform which concentrates on targeting proteins that are differentially expressed, such as exhibiting sex-specific expression. This approach enables us to significantly reduce the total number of proteins that feed into our proteomics pipeline. Proteins conjugated from reaction with multiplexed fluorescent dyes are separated on 1-D and 2-D polyacrylamide gels and scanned at segregating wavelengths. The resultant images are processed using gel analysis software. Those proteins displaying significantly altered levels or in a new position are excised and subjected to trypsin hydrolysis and released peptides are analyzed by nano-electrospray ion-trap mass spectrometry. Primary data crunching is performed using peptide search algorithms interrogating both public domain and proprietary databases. Emphasis is placed on data generated from *de novo* sequencing of peptides using the DeNovoX® package followed by BLAST analysis in an attempt to overcome the shortfall of having incompletely sequenced genomes.

Poster: Olfactory Transduction

G-proteins in *Anopheles gambiae* olfaction

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Chemoreception in general and olfaction in particular represent critical sensory inputs into many behaviors of hematophagous (blood-feeding) insects. Recently the first odorant receptors (ORs) of the malaria vector mosquito *Anopheles gambiae* (AgORs) have been identified and functionally characterized. In addition to these studies, it will be important to develop methodology to facilitate high throughput screening of substances, which could potentially lead to the development of a new generation of efficient insect repellents. To build an experimental setup that could allow such an approach, expansion of our current knowledge about signal transduction in insect olfaction is crucial. The current study identifies six genes in the *A. gambiae* genome that encode G-protein (α -subunit) homologues. We have investigated tissue-specific expression of these G α -genes and describe the expression pattern of a total of 11 transcripts in the adult mosquito. Furthermore we localized G α -proteins within the female mosquito-antenna and identify candidates for G α -proteins involved in olfactory signal transduction.

Supported by grants from NIAID and NIDCD.

Poster: Olfactory Transduction

The novel guanylyl cyclase MsGC-I may mediate pheromone-induced cgmp in the antennae of *Manduca sexta*

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The signal transduction cascade induced by pheromone detection results in a transient increase in calcium followed by a slow increase in cGMP, which is thought to play a role in adaptation in the olfactory receptor neurons (ORNs) of *Manduca sexta*. However, the

specific mechanism mediating this increase in cGMP is unknown. We wanted to determine if the novel guanylyl cyclase, MsGC-I, mediates this increase in cGMP. Using immunocytochemistry and *in situ* hybridization, we found that MsGC-I is likely to be expressed in at least a subset of pheromone-sensitive ORNs. Some neuronal calcium sensor (NCS) proteins are known to regulate calcium-dependent guanylyl cyclase activity in the olfactory system. To learn how the activity of MsGC-I might be regulated we are testing if MsFrequenin and/or MsNeurocalcin, two NCSs cloned previously and known to be expressed in ORNs, may mediate calcium-dependent regulation of MsGC-I activity.

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Slide: Olfactory Transduction

Evidence for alternate transduction pathways in the mouse: olfaction in the CNGA2 knockout

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In mammals the adenosine 3',5'-monophosphate (cAMP) pathway is widely believed to be the only transduction mechanism in the main olfactory epithelium largely due to the lack of odor responsiveness of mice defective for cAMP signaling. Here we report on odor responsiveness in mice with a disrupted cyclic nucleotide gated (CNG) channel subunit A2. Several odorants, including putative pheromones, can be detected and discriminated by these mice behaviorally. These odors elicit electro-olfactogram (EOG) responses in the olfactory epithelium and activate a subset of glomeruli in the main olfactory bulb and stimulate neurons in of CNGA2 knockout mice. In addition, EOG responses to odors detected by CNGA2 knockout mice are relatively insensitive to inhibitors of the cAMP pathway. These results provide strong evidence that cAMP-independent pathways in the main olfactory system of mammals participate in detecting a subset of odors.

This work was supported by NIH grants DC00566, DC04657, DC006070 (D.R.) DC0043 (W.L.) and MH6118 (B.S.).

Slide: Olfactory Transduction

Identification of the second messenger that mediates signal transduction in the newt olfactory receptor cell

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It has long been believed that vertebrate olfactory signal transduction is mediated by independent multiple pathways (utilizing cAMP and IP₃ as second messengers). However, the dual presence of parallel pathways in the olfactory cilia is still controversial, mainly because of the lack of information regarding the single cell response induced by odorants that have been shown to produce IP₃ exclusively.

In the present study, we examined two series of experiments. First, we compared responses induced by both cAMP- and

IP₃-odorants. Fundamental properties of responses were surprisingly homologous in spatial distribution of the sensitivity, waveforms, *I-V* relation and reversal potential, dose-dependence, time integration of stimulus, adaptation and recovery. By applying both types of odorants alternatively to the same cell, we observed cells to exhibit perfect symmetrical cross-adaptation. Second series of experiments was that the cytoplasmic cNMP concentration was manipulated through the photolysis of caged compounds to examine their real-time interactions with IP₃-odorant-induced events. The Properties of responses induced by both IP₃-odorants and cytoplasmic cNMP resembled each other in their unique characteristics. They showed symmetrical adaptation that is dependent on the Ca²⁺-influx. Furthermore, both responses were additive in a manner as predicted quantitatively by the theory that signal transduction is mediated by the increase in cytoplasmic cAMP. The data will provide evidence showing that olfactory responses are generated by a uniform mechanism for a wide variety of odorants.

Poster: Olfactory Transduction

Pharmacological properties of a possible TRP-related ion channel in lobster olfactory receptor neurons

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Lobster olfactory receptor neurons express a sodium-gated non-selective cation channel (Zhainazarov *et al.*, 1998, *J. Neurophysiol.*, 79:1349) that is a potential member of the growing family of trp channels. Here, we extend our pharmacological characterization of the channel by recording the effect of potential agonists and antagonists on the channel in cell-free patches. In addition to Na⁺, the channel is activated by Ca²⁺, PIP₂, PIP₃ and maitotoxin, and antagonized by H⁺, calmodulin inhibitors and the trp channel blockers 2APB, SKF96365, ruthenium red, Al³⁺, Gd³⁺, and La³⁺. Interestingly, decreasing extracellular pH from 8.0 to 7.5, a range in which pH is not known to block other ion channels, effectively blocked the lobster channel and could serve as a selective probe for the channel. We are in the process of using this enhanced pharmacological profile to implicate the channel as a target of odor-activated phosphoinositide signaling in the cells.

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Poster: Olfactory Transduction

Expression of TRP channels and odorant receptor signaling in Odora cells

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To examine signaling pathways directly activated by odorant receptor, we utilize the olfactory epithelial cell line, Odora. Odora cells functionally express transfected odorant receptor U131 without requiring concurrent transfection of additional signaling proteins

(Murrell and Hunter, 1999). Previously we showed that Odora cells express adenylyl cyclase III, phospholipase C isoforms, and G-proteins found in the olfactory epithelium (OE). Cai signals in Odora cells are not activated by the canonical cAMP cascade; transfected U131 receptors in Odora cells activate PLC, IP₃ and IP₃ receptor to elevate Cai without the release of intracellular Ca²⁺. Biotinylation studies reveal that there is cell surface expression of IP₃ receptors I–III in Odora cells, implicating one or more IP₃ receptors as a possible route for Ca²⁺ entry mediated by odorant. In this study, we explore the expression of transient receptor potential (TRP) channels to assess the possibility that they might participate in odor-activated Ca²⁺ entry through the plasma membrane. RT-PCR analysis of all seven mammalian *TRPC* channels reveals transcripts for *TRPC1* and *TRPC5* in both Odora cells and nasal epithelium. Western blotting further documents the expression of protein for TRPC5, but not for TRPC1. Current studies using antibodies to TRPC5 and TRPC1 examine the localization of TRPCs in the rat olfactory system. These observations continue the characterization and evaluation of a unique pathway activated by odorant receptor in Odora cells that may reveal an important role for alternative signaling through the odorant receptor in the olfactory system *in vivo*.

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Poster: Olfactory Transduction

Odor-induced calcium responses from squid olfactory receptor neurons

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Olfactory receptor neurons (ORNs) from the squid *Lolliguncula brevis* fall into five morphological subtypes. Previously we characterized odor responses in two morphological subtypes from isolated squid ORNs (Lucero *et al.*, 1992, *J. Exp. Biol.* 162: 231–249). Because the isolation process destroys the other three subtypes, we have developed a slice preparation to make physiological recordings from a relatively intact olfactory epithelium. Thus far, electrophysiological recordings from slices have been challenging because of the thick mucous that covers the olfactory organ. Initial Ca²⁺ imaging studies using the fluorescent Ca²⁺ indicator dye, Fluo-4, and laser scanning confocal microscopy were unsuccessful because the tissue underlying the olfactory epithelium spontaneously contracts after dissection from the animal. We tested numerous receptor antagonists, ion channel inhibitors and conotoxins that were unsuccessful in blocking spontaneous contractions. Both isotonic MgCl₂ and 62.5 μg/ml ketamine blocked the tissue contractions but also inhibited odor-induced Ca²⁺ responses. Fortunately, we found that 5 μM nicotine completely blocks spontaneous contractions without compromising odor responses. We are currently using the squid olfactory organ slice preparation to test the odor specificity and transduction pathway of the five morphological subtypes of ORNs. Application of this methodology will allow us to correlate morphology, odor specificity and signal transduction in real time across a relatively intact olfactory epithelium.

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Slide: Olfactory Transduction**Novel action of calmodulin on native rat olfactory CNG channels**J. Bradley¹, W. Bönigk², T. Gensch², K. Yau¹, B. Kaupp² and S. Frings³¹Department of Neuroscience/IHMI, Johns Hopkins University, Baltimore, MD, USA, ²IBI-1, Forschungszentrum, Jülich, Germany and ³Molecular Physiology, University of Heidelberg, Heidelberg, Germany

Adaptation to stimuli is an important process in sensory neurons. In vertebrate olfactory receptor neurons, rapid negative-feedback inhibition of Ca²⁺-permeable, cAMP-gated (CNG) transduction channels underlies adaptation. Previous studies with CNGA2, a CNG channel subunit that forms a homomeric channel when expressed heterologously, have implicated binding of Ca²⁺/calmodulin (Ca²⁺/CaM) to CNGA2 in adaptation. Native rat olfactory CNG channels, however, are a heteromeric complex of three homologous subunits, CNGA2, CNGA4 and CNGB1b. We now report that the CaM-binding site on CNGA2 does not mediate at all the modulation by Ca²⁺/CaM of native channels. We find that, with native channels in resting neurons, Ca²⁺-free calmodulin (apocalmodulin) is largely pre-associated. Accordingly, apocalmodulin-binding sites of the IQ-type are together necessary and sufficient for Ca²⁺/CaM feedback inhibition of heteromeric channels. Thus, calmodulin is permanently associated with native channels in ORNs, poised at the site of Ca²⁺ influx as a Ca²⁺ sensor to rapidly effect inhibition. Apart from mechanistically explaining the fast kinetics of adaptation to odorants, our findings caution against the practice of extrapolating findings from heterologously expressed homomeric channels to native heteromeric channels.

Slide: Olfactory Transduction**Amplification by a single GPCR molecule in the olfactory receptor neuron**V. Bhandawat¹, J. Reiser² and K. Yau³¹Neuroscience, Johns Hopkins University, Baltimore, MD, USA, ²Howard Hughes Medical Institute, Baltimore, MD, USA and ³Neuroscience (HHMI), Johns Hopkins University, Baltimore, MD, USA

Odorants bind to specific G-protein coupled receptors (GPCRs) on olfactory neurons, which, via a G-protein cascade, convert chemical signals into an electrical response. We have measured membrane current from single frog (*Rana pipiens*) olfactory neurons and analyzed the odorant-receptor interaction. We find that an odorant molecule rapidly unbinds from the receptor, often before the activation of a G-protein. This leads to low amplification between receptor activation and G-protein activation, which is confirmed by the small size of the unitary event as derived by quantal analysis. Although the individual events are very small, there is a non-linear summation of these events that amplifies the response greatly.

Funded by Howard Hughes Medical Institute.

Slide: Olfaction: Animal Behavior**A comparison of sensory hair distribution on the chelae and olfactory organs of crayfish (*Orconectes rusticus*)**

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Sensory hairs are found in great abundance on the appendages of crayfish and have been shown to be important for detecting both mechano- and chemosensory stimuli. Detailed analysis of the spatial distribution of these sensory hairs that is correlated with sex or reproductive forms allows us to determine if a particular sensory hair type or abundance could be important for mating. With this in mind, we examined the sensory hair distribution of form I (reproductive) and form II (non-reproductive) males, as well as females using scanning electron microscopy. We quantified and qualified all sensory hair types. Our research indicates that reproductive male crayfish have a greater number of feathered hairs on their chelae and antennules when compared with non-reproductive males and females. This increase in sensory hair distribution suggests a link between sensory hairs and mating chemical cues in the crayfish. Male and female crayfish have different distributions and abundance of sensory hairs on the sensory appendages. These differences are likely a function of the different reproductive life histories where males actively pursue and locate females. In all, the sensory hairs on all of the appendages appear to be important in mate recognition.

Poster: Olfaction: Animal Behavior**Aesthetascs, the olfactory sensilla, are mediators of chemosensory activation of antennular flicking in the spiny lobster, *Panulirus argus***

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Lobster antennules bear a number of different sensilla sensitive to odorants. We have found that two antennular behaviors, grooming (Wroblewska *et al.*, 2002, *Chem. Senses* 27: 769–778) and flicking (Fox *et al.*, 2003, *Chem. Senses* 28: 555), are mediated by aesthetascs and/or asymmetric setae located in the ‘tuft’ region of the lateral flagellum. Flicking also requires ‘non-tuft’ setae scattered across the lateral and medial flagella. Schmidt *et al.* (this meeting) have found through ablation experiments that grooming is solely activated by asymmetric setae. The present study used similar techniques to determine the relative importance of aesthetascs and asymmetric setae to activation of flicking behavior. Two groups of lobsters were sham-ablated by removing a row of guard setae and then tested for antennular responses to L-glutamate (the major chemical stimulus eliciting grooming) and squid extract (concentration). This was followed by either ablation of asymmetric setae ($n = 8$) or ablation of aesthetascs ($n = 6$), after which the behavioral assay was repeated. Lobsters with asymmetric setae removed no longer groomed in response to glutamate but showed no reduction in flick rate to squid extract compared with their responses to the same odorants following sham ablation. In

contrast, lobsters with aesthetascs removed showed no reduction in grooming to glutamate but no longer increased flick rates to squid extract. These results, along with those from the earlier study, suggest that olfactory and nonolfactory pathways are utilized in eliciting flicking behavior in which processing occurs via the olfactory lobes and the lateral antennular neuropils.

Poster: Olfaction: Animal Behavior

Behavioral discrimination of amino acids in zebrafish (*Danio rerio*)

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Based on the dissimilar patterns of glomerular activation during amino acid stimulation in recent calcium-imaging studies in zebrafish (Friedrich and Korsching, 1997, *Neuron* 18: 737–752), it is possible, but previously untested, that this species is capable of discriminating behaviorally different amino acids. As performed in catfish, olfactory discrimination was studied by conditioning zebrafish with a food reward that was presented 90 s after the conditioning amino acid solution was injected into the aquarium. Tests for discrimination began after ~50 conditioning sessions. Conditioned zebrafish, which associated a specific amino acid odor with a food reward, searched for food longer and more intensely (measurements of the swimming path by video-tracking or counting the turns $>90^\circ$ during 90 s) than after stimulation with a non-conditioned amino acid. We used 3×10^{-5} M L-Ala, L-Val and L-Arg, respectively, as conditioning stimuli and the following as test stimuli: Gly, L-Ala, L-Ser, L-Phe, L-Tyr, L-Trp, L-His, L-Asn, L-Val, L-Ile, L-Leu, L-Met, L-Arg, L-Lys, L-Glu, L-Asp, L-Pro and D-Ala. With the exception of the L-Val conditioning stimulus and the L-Ile test stimulus, zebrafish discriminated all the conditioning stimuli from the test stimuli. Our results clearly indicated that the amino acids that previously showed similar glomerular activity patterns (e.g. L-Val and L-Ile), were not discriminated behaviorally by zebrafish, but those in which the glomerular activation patterns were distinct were readily discriminated.

Poster: Olfaction: Animal Behavior

Olfactory communication: evolving new blends and novel preferences

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Male moths are often extremely sensitive and narrowly tuned to the blend of components emitted by conspecific females. The sexual communication channel is therefore under strong stabilizing selective pressure. How, then, do new female blends and male preferences evolve? Two closely related moth species, *Heliothis virescens* and *Heliothis subflexa*, can be hybridized under laboratory conditions in order to study the genetic basis of behavior and olfactory characteristics that accompany species divergence. Males of these two species prefer qualitatively distinct blends that include either Z9–14:Ald (*H. virescens*) or Z9–16:Ald (*H. subflexa*). In addition, *H. subflexa* males require Z11–16:OH. These behavioral preferences are corre-

lated with the specificity of olfactory receptor neurons and central interneurons arborizing within the glomeruli of the male-specific macroglomerular complex. Wind tunnel studies have shown that Z9–14:Ald/Z9–16:Ald preference segregates in a 1:1 ratio in back-cross males. Preliminary genetic (QTL) analyses have revealed that this trait is associated with a single autosomal chromosome suggesting that a major gene may control this phenotype, perhaps coupled to the expression of odorant receptors. Other divergent characters in this system, such as Z11–16:OH-agonism and Z11–16:OAc-antagonism, may involve odorant receptors but also likely involve shifts in the glomerular targets of receptor axons and interpretation of olfactory information by higher brain centers.

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Poster: Olfaction: Animal Behavior

Olfactory recognition in canines

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The overall objective of this work was to assess the specificity of the olfactory cues that canines use when identifying humans. That is, once a dog is trained to identify the smell of a human (the target), will the dog be confused by the smell of the target's siblings or by the smell of other people who use the target's soap or deodorant? A 1-year-old golden retriever was trained to pick her owner's scent out of three possible choices. After a trial had been initiated by 'go' the dog would smell each of three boxes containing T-shirts impregnated with human scent. The target was randomly assigned to one of the boxes. The boxes were constructed such that the dog could not use visual cues. The dog signaled recognition of the target's smell by a sit/stay response. The dog was trained to correctly identify the target over 90% of the time. When there was no target present the dog would repeatedly sample the three boxes and 80% of the time give no recognition signal. The dog was rewarded only for correct target responses. Probe trials consisting of the scent of the target's siblings or of the scent of other humans who had used the soap and/or deodorant of the target were inserted into the testing sessions. There was no reward given during a probe trial. The dog gave the recognition response more often to the smell of the target's siblings as compared with the smell of non-related humans. Likewise the dog gave the recognition response more often when non-related humans used either the soap or deodorant of the target. These results suggest that both genetic factors (such as HLA typing) and added smells (such as soap) may have some bearing on canine olfactory recognition of humans.

Poster: Olfaction: Animal Behavior

MLPEST as a measure of olfactory sensitivity threshold in mice

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Threshold is defined as the stimulus intensity necessary for a subject to reach a specified percent correct on a detection test. Unfortunately,

many measures of threshold in sensory systems are heavily influenced by decision making processes and are subject to extinction. MLPEST (Maximum-Likelihood Parameter Estimation by Sequential Testing) is a method that minimizes both decision-based bias and extinction. Originally developed for human auditory and visual tasks, it has been also utilized for human olfactory and gustatory tests. Indeed, a recent comparison of MLPEST with other methods to test olfactory and gustatory thresholds demonstrated reliable and precise threshold measurements (Linschoten *et al.*, 2001, *Percept. Psychophys.* 63: 1330). However, this comparison was limited to its application for human subjects. In order to modify this technique for olfactory testing in mice, we have adapted MLPEST methodology to the computerized olfactometer of Bodyak and Slotnick (1999, *Chem. Senses* 24: 637). Here we present data that demonstrate the utility of this technique in mice, and we discuss the ramifications of altering MLPEST test parameters on performance.

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Poster: Olfaction: Animal Behavior

Virus-induced body odors in mice

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Mouse mammary tumor virus (MMTV) provides an attractive animal model to study the effects of infection on body odor. MMTV is a retrovirus that either is passed from mother to offspring through her milk or is transmitted genetically as an endogenous provirus. Depending on the mouse strain, multiparous infected females often develop mammary tumors. The provirus genome consists of several genes, one of which, *Sag*, codes for a superantigen that is presented by MHC class II on B cells and acts to delete specific subsets of T cells in the host. We previously demonstrated that mice infected by MMTV by suckling on infected dams expressed a distinctive odor as shown by the ability of trained mice to discriminate odors of infected versus non-infected controls in a Y maze. Endogenously expressed MMTV can be used to investigate mechanisms of body odor change. In the first study with endogenous virus, we found that mice transgenic for an MMTV provirus (C3H/HeN) express a different odor than control littermates. In the second study, we demonstrated that mice differing from control littermates only by expressing the *Sag* gene (MMTV-ORF 16) also had a distinctive body odor. This result suggests that changes in immune function, likely alterations in the T-cell repertoire, underlie body odor changes following MMTV infection. Variations in immune function following infection may thus result in specific volatile signals useful in disease diagnosis.

Supported by NSF grant 0112528.

Poster: Olfaction: Animal Behavior

Olfactory fear conditioning and discrimination in mice

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A method for producing long-lasting olfactory learning and discrimination within a single session is required for future studies of the molecular bases of olfactory memory. In previous studies, we have shown that mice can learn olfactory-cued fear as measured by both freezing and fear potentiated startle, the two most common fear measures in rodents. We first show that mice have enhanced fear potentiated startle and freezing after pairing odors with footshocks in a paradigm that supports learning, but not when the same number of odors and shock presentations are presented in an unpaired fashion. Here we also show that mice can discriminate between an odor paired with a shock versus an unpaired odor. Using 10% amyl acetate or 5% acetophenone, we examined the unconditioned startle and freezing responses of mice prior to training. During training mice received five presentations of one odor alone interspersed with five pairings of the other odor with a footshock. The following day, we tested fear potentiated startle and freezing to these odors. Acoustic startle to the untrained odor and the trained odor were non-significant before training. After conditioning, the fear potentiated startle response to the untrained (8%) and trained (29%) odor were significantly different ($P = 0.002$). Additionally, freezing prior to training was non-significant, but after training, mice froze significantly more to the conditioned odor ($P = 0.03$). Ongoing studies investigating the role of the amygdala in these behaviors will pave the way for examining the molecular mechanisms of amygdala-dependent modulation of olfactory learning.

Poster: Olfaction: Animal Behavior

Opposing effects of D1 and D2 receptor activation on odor discrimination learning

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Dopaminergic modulation of cortical activity has been implicated in the planning, initiation, and control of movements, as well as in emotional responses, motivation, and the formation of reward associations. Additionally, there is abundant evidence for dopaminergic effects on olfactory processing, and in particular for the effects of dopaminergic modulation on odor detection thresholds. Using a simultaneous olfactory discrimination task, we here show that both D1 and D2 dopamine receptors can regulate rats' olfactory discrimination capacities, and furthermore that the effects of D1 and D2 receptor activation functionally oppose one another in the performance of this task. Specifically, injection of either the D1 agonist SKF 38393 (10 mg/kg body wt) or the D2 antagonist spiperone (0.62 mg/kg) facilitated the difficult discrimination of similar odorants but had no effect on the easier discrimination of dissimilar odorants, whereas both the D1 antagonist SCH 23390 (0.025 mg/kg) and the D2 agonist quinpirole (0.2 mg/kg) significantly impaired rats' ability to discriminate both similar and dissimilar odorants.

Poster: Olfaction: Animal Behavior**Not only—but also-adrenoceptors are involved in early odor preference learning in the rat**J.H. Mclean¹, J. McCann², A. Darby-King¹ and C.W. Harley²¹Basic Medical Sciences, Memorial University of Newfoundland, St. John's, Newfoundland, Canada and ²Psychology, Memorial University of Newfoundland, St. John's, Newfoundland, Canada

Early preference olfactory learning is important for the survival of infant rats because they need to associate the odor of the mother with reward (food). In the neonate, tactile stimulation activates the locus coeruleus (Nakamura *et al.*, 1987) in the brain stem. Noradrenergic (NE) neurons in the locus coeruleus (LC) have a strong axonal projection to the olfactory bulb (Shiple *et al.*, 1985; McLean and Shiple, 1989; McLean *et al.*, 1991). When NE activation is paired with odor in the neonate, preference for the odor (learning) takes place (Sullivan *et al.*, 1989, 1991, 2000; Langdon *et al.*, 1997; Price *et al.*, 1998). The LC β -adrenergic input has been shown to be necessary and sufficient for preference acquisition to occur in rats up to around postnatal day 10. Surprisingly, despite observations that α -adrenoceptors are present (Day *et al.*, 1997; Winzer Serhan *et al.*, 1997) and functional (Hayar *et al.*, 2001) in the olfactory bulb, there is relatively little known about their function in behavior. In this study, we tested the hypothesis that α -adrenoceptors have roles similar to the β -adrenoceptors in early olfactory learning. In this set of experiments we showed that activation of the $\alpha 1$ -adrenoceptors was sufficient to induce early preference learning in the rat while the $\alpha 2$ -adrenoceptor agonists did not show an effect. The α -adrenoceptors acted in a dose-dependent manner similar to the β -adrenoceptors in early odor preference learning.

This work is supported by a grant from CIHR–CEDA Regional Partnership.

Poster: Olfaction: Animal Behavior**The effect of concentration and conditioning on odorant discrimination by the honeybee**B.L. Fussnecker¹, M. Carlton¹, G. Wright² and B.H. Smith¹¹Entomology, Ohio State University, Columbus, OH, USA and ²Mathematical Biosciences Institute, Ohio State University, Columbus, OH, USA

Naturally occurring odors used by animals for mate recognition, food identification and other purposes must be detected at concentrations that vary across several orders of magnitude. Olfactory systems must therefore have the capacity to represent odors over a large range of concentrations regardless of dramatic changes in odor salience. The stability of the representation of an odor relative to other odors across concentration has not been extensively evaluated. We tested the ability of honeybees to discriminate pure odorants across a range of concentrations at and above their detection threshold. We also examined how their ability to discriminate among odors changed as a function of the number of times they had encountered an odorant in association with an appetitive reward. Our study showed that discrimination among odorants

was a function both of the concentration and the number of experiences a subject has with an odorant. We hypothesize that this arises from two separate mechanisms in the olfactory system.

Poster: Olfaction: Animal Behavior**Diltiazem administered nasally decreases food intake and attenuates weight gain in rats**T.J. Maher¹, A. Amer¹, C. Adams², W. Chen³ and K. Weinrich³¹Massachusetts College of Pharmacy and Health Sciences, Boston, MA, USA, ²Compellis Pharmaceuticals, Somerville, MA, USA and ³Longwood Pharmaceuticals, Inc., Boston, MA, USA

Energy intake is continuously influenced by many complex endogenous neurochemical systems located both centrally and peripherally, in addition to numerous external environmental stimuli, such as olfaction. As the processing of odorant information by many olfactory neurons is mediated via Ca^{2+} -currents through Ca^{2+} channels, a novel approach at influencing the ingestive behaviors of animals might therefore involve altering olfactory acuity via Ca^{2+} channel blockade. The present study tested the ability of a Ca^{2+} channel blocker, diltiazem (D), to alter food intake in rats made hyperphagic. D was delivered using the intranasal (i.n.), intraperitoneal (i.p.) and oral (p.o.) routes of administration. Male Sprague–Dawley rats maintained in a reversed-lighting environment, which had been food-deprived for 4 h at the beginning of the dark cycle, were administered different doses of D or vehicle (V) and the amount of food consumed was measured. While food intake at 1, 2 and 4 h post-D administration was significantly decreased in a dose-dependent manner after i.n. administration, neither the i.p. nor p.o. routes significantly affected food intake. In another experiment, rats were trained to eat their daily meal during the first 4 h at the onset of the dark cycle. Once acclimated to this schedule, daily treatment for 14 days with i.n. D or V prior to food introduction resulted in a dose-dependent attenuation of weight gain. Together these studies suggest that i.n. administration of D possesses significant anorectic activity that may have utility in influencing energy intake. Additional studies are needed to determine the exact mechanisms and sites of action of i.n. administered D.

Poster: Olfaction: Animal Behavior**Kv1.3-targeted gene-deletion increases metabolic function and olfactory ability**R.N. Thompson¹, R.M. Perkins¹, A.D. Parsons², M. Overton² and D. Fadool¹¹Department of Biological Science, Program in Neuroscience, Florida State University, Tallahassee, FL, USA and ²Department of Nutrition, Food, & Exercise Science, Program in Neuroscience, Florida State University, Tallahassee, FL, USA

Mice with gene-targeted deletion (KO) of the voltage-dependent K channel, Kv1.3, have smaller glomeruli and thus were behaviorally phenotyped to understand the channel's contribution to olfaction. Mice were monitored (for 8 days) in environmental chambers where data are computer-acquired every 30 s. KO animals were found to have abnormal ingestive behaviors yet equivalent daily caloric/

water intake; KOs ate more intermittently and drank larger volumes less frequently. KO animals had slightly increased metabolism and locomotor activity in the dark cycle and weighed less than age-matched wildtype mice. KO mice were not anosmic, in fact, retrieval time to recover a hidden food item was twice as fast as that observed for wildtype mice. Odor habituation trials using complex mixtures and single alcohols indicated that KO mice can discriminate molecules differing by only 1 carbon. Food-restricted mice were trained to dig for a hidden reward paired with an odorant using a two-choice paradigm to determine odorant threshold ability. KO mice performed the paired task more quickly and at concentrations 1000–10 000 fold less than that by wildtype mice. While these tasks are influenced by memory, both genotypes performed equivalently in object recognition testing and test of motivation for object exploration. This unusual set of behaviors in the Kv1.3 KO mice suggest that Kv1.3 serves additional roles beyond shaping the resting potential.

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Slide: Trigeminal Chemoreception

Lingual tactile acuity, taste perception, and the density and diameter of fungiform papillae in female subjects

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A growing body of evidence suggests that individuals who differ in taste perception differ in lingual tactile perception. To address this issue, spatial resolution acuity was estimated for 83 young adult females (52 Asians and 31 Caucasians) by their ability to examine with the tongue and identify embossed letters of the alphabet. Ratings of the magnitude of the bitterness of 6-n-propylthiouracil (PROP) were obtained to characterize subjects' taste perception. The density and diameter of fungiform papillae on the anterior tongues of the Asian subjects were measured also. Subjects who rated the bitterness of PROP as very or intensely strong (supertasters) were found to be 25% more tactually acute than subjects who rated the bitterness as moderate to strong (medium tasters) and twice as acute as subjects who rated it as barely detectable or weak (non-tasters; $P < 0.0001$). The threshold heights for letter recognition averaged 2.8, 3.5 and 5.4 mm, respectively, for the Asian subjects and 2.6, 3.2, and 5.1 mm for the Caucasian subjects. The thresholds correlated highly with subjects' ratings of bitterness ($\rho = -0.84$, $P < 0.0001$), and for the Asian subjects with the density ($\rho = -0.84$, $P < 0.0001$) and diameter ($\rho = 0.66$, $P < 0.0001$) of fungiform papillae. Mean densities varied from 54.4 cm⁻² (non-tasters) to 106.5 cm⁻² (medium tasters) to 143.7 cm⁻² (supertasters; $P < 0.0001$). These findings confirm that individuals who differ in taste (PROP) sensitivity also differ in lingual tactile acuity. Tactile and taste sensitivities covary and reflect individual differences in the density and diameter of fungiform papillae on the anterior tongue.

Slide: Trigeminal Chemoreception

Temporal integration in nasal lateralization and detection of carbon dioxide

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A number of experiments have examined the effects of stimulus-duration on supra-threshold ratings of nasal irritation, but time-concentration trading at threshold has received less attention. Accordingly, two experiments examined temporal integration in detection of nasal irritation using carbon dioxide (CO₂), an irritant with no distinct odor, as a model-stimulus. In experiment 1, subjects received air and CO₂ simultaneously, in opposite nostrils, and attempted to determine which nostril received CO₂ (lateralization). In experiment 2, subjects received air and CO₂ in successive intervals, and attempted to determine which interval included CO₂ (temporal, two-alternative, forced-choice detection). In both cases, concentration was fixed within runs, and stimulus-duration varied according to a one-up, two-down staircase to determine threshold pulse-duration. For both lateralization and detection, the shortest detectable pulse decreased as stimulus-concentration increased. Linear functions, with slopes of less than negative one, fit plots of log threshold pulse-duration versus log concentration quite well. This means more than a twofold increase in duration was needed to compensate for a twofold decrease in concentration. Therefore, as a detector of CO₂, the human nose functions as an imperfect integrator.

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Slide: Trigeminal Chemoreception

Cut-off effect in eye irritation from vapor-phase homologous acetates

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Nasal and ocular trigeminal detection of homologous volatile organic compounds seem to reach a 'cut-off' point whereby homologs larger than a certain size fail to evoke chemesthesis in acute (1–3 s) exposures. Starting with an acetate series, we began a systematic search of the homolog where the cut-off first appears for eye irritation. A glass-vessel system and a three-alternative forced-choice procedure against mineral oil blanks served to probe the eye irritation detectability of vapor from neat nonyl, decyl and dodecyl acetates at three delivery flows (2, 4 and 8 l/min) and three exposure times (6, 12 and 24 s) in four intensively tested subjects. An ANOVA showed: (i) detectability of decyl and dodecyl acetate remained close to chance for all conditions; and (ii) detectability of nonyl acetate rose with flow but was independent of exposure time. Thus, decyl acetate represents the cut-off homolog for this series. We will test whether raising the vapor concentration of the neat chemicals by increasing their temperature to 37°C enhances detection of nonyl acetate and elicits detection of decyl and dodecyl

acetate. We aim to distinguish between a physical cut-off (vapor concentration at room temperature too low to evoke detection) and a biological cut-off (molecule too large to fit a putative receptor or carrier). This difference has relevance in detection of mixtures.

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Slide: Trigeminal Chemoreception

Neural correlates of the affective processing of oral texture

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The hedonic value of food is heavily influenced by the perception of its texture. The aim of this study was to investigate brain response to oral texture preference using the H₂¹⁵O water bolus technique to measure regional cerebral blood flow (rCBF) with a Siemens H+ PET scanner while subjects ate a food with a preferred or non-preferred texture. Peanut butter (PB) was used as the stimulus because it is a food in which texture plays a strong role in preference. Ten subjects (five who strongly preferred crunchy and five who strongly preferred smooth) ate crunchy (six scans) and smooth (six scans) PB while being scanned. Comparison of rCBF obtained during eating preferred versus non-preferred (irrespective of texture) PB yielded a network of structures including the posterior insula; subcallosal, ventral and dorsal cingulate gyrus; caudal orbitofrontal cortex, anterior insula, uncus, striatum and midbrain. Since, in this case, preference was determined largely by texture, we propose that these regions form a core network in coding the subjective experience of pleasure derived from oral texture perception.

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Slide: Trigeminal Chemoreception

Fatty acids inhibit delayed rectifying K channels in isolated trigeminal neurons

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Chemosensory cues for fat have been shown to be mediated by fatty acids (FA) acting directly on subtypes of delayed rectifying K (DRK) channels in taste receptor cells (TRCs). Since it is generally believed that texture is important for oral fat recognition and that texture perception is mediated, at least in part, by trigeminal

(TG) innervation, we have examined the effects of fatty acids (0.1–10 μM) on isolated rat TG neurons using whole-cell patch clamp recording. TG ganglia were removed and individual TG neurons were isolated by enzymatic methods and placed into culture for 24–72 h. Patch recordings were made before, during and after application of a variety of fatty acids including saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) forms. Similar to our results in TRCs, PUFAs caused a reversible, time-dependent inhibition of DRK channels in TG neurons, consistent with an open-channel block leading to cell activation. In contrast to fungiform TRCs, which respond specifically to PUFAs, TG neurons were much less specific and responded to a variety of fatty acid types, including some MUFA and SFA, in a similar fashion. Thus, FA effects on DRK channels may not only mediate the taste of fat, but may also contribute to the perception of its textural properties via activation of oral TG fibers. Currently, we are using quantitative PCR to compare the expression of DRK channels in TG neurons with TRCs to determine the source of the FA specificity differences.

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Symposium: Receptors: Choosing Genes, Targeting Axons, Detecting Chemicals

Perception of chemical cues and navigation in *C. elegans*

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Behavior arises from the interplay between the environment and intrinsic properties of neurons and neural circuits. To understand how the genetics and development of the nervous system contribute to specific behaviors, we are studying olfactory system in the nematode *Caenorhabditis elegans*. *Caenorhabditis elegans* senses hundreds of different compounds, discriminates between them, and generates different behaviors in response to different odors. It is possible to define the specific neurons that generate these behaviors, since the *C. elegans* nervous system consists of just 302 neurons that have reproducible functions, morphologies and synaptic connections. Previous studies have generated an understanding of the methods by which animals detect and respond to a single sensory stimulus. In *C. elegans*, odors are detected by >1000 G-protein-coupled odorant receptors. Individual olfactory neurons express multiple receptor genes, allowing a few cells to detect many odors. A given sensory neuron is primarily dedicated to a single behavioral task, such as attraction or repulsion. We are now asking how animals navigate through complex sensory environments using multiple odors or sensory inputs. For these studies, we have focused on complex natural stimuli that should be present in the soil environment, such as different bacterial foods, natural physical stimuli, and other animals (social groups). *Caenorhabditis elegans* shows unexpected sophistication in its behavior when it faces ecologically relevant challenges like pathogenic bacteria or metabolic stress. Using the wiring diagram, we are identifying the circuits for navigation behavior and asking how sensory inputs regulate those circuits.

Symposium: Receptors: Choosing Genes, Targeting Axons, Detecting Chemicals

The biology of sweet, bitter and umami taste

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Symposium: Receptors: Choosing Genes, Targeting Axons, Detecting Chemicals

Internal representations of the olfactory world

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Olfactory perception requires the recognition of a vast repertoire of odorants in the periphery and central neural mechanisms that allow the discrimination of odors. The organization of the peripheral olfactory system appears remarkably similar in fruit flies and mammals. The convergence of like axons into discrete glomerular structures provides an anatomic map in the antennal lobe. How does the anatomic map translate into a functional map? We have developed a sensitive imaging system in the *Drosophila* brain that couples two-photon microscopy with the specific expression of the calcium-sensitive fluorescent protein, G-CaMP, to examine neural activity. At natural odor concentrations, each odor elicits a distinct and sparse pattern of activity that is conserved in different flies. We have combined Ca²⁺ imaging with electrical recordings to demonstrate the faithful propagation of the glomerular map by projection neurons that innervate the protocerebrum. The quality of an odor may therefore be reflected by defined spatial patterns of activity, first in the antennal lobe and ultimately in higher olfactory centers. We have identified a spatially invariant sensory map in the fly protocerebrum that is divergent and no longer exhibits the insular segregation of like axons observed in the antennal lobe. This organization provides the opportunity for the integration of multiple glomerular inputs by hierarchical cell assemblies in the protocerebrum.

Poster: Salt and Sour Taste

Expression of the amiloride-sensitive epithelial sodium channel in the mouse taste papillae

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It is proposed that amiloride-sensitive epithelial Na⁺ channels (ENaCs) are involved in taste signal transduction. Electrophysiological studies in C57BL mice demonstrated that responses to NaCl

are inhibited by amiloride in the chorda tympani (CT) nerve but not in the glossopharyngeal (IXth) nerve, suggesting lack of amiloride sensitivity (AS) in the posterior tongue region innervated by IXth nerve. The AS also differs among inbred mouse strains. Judging from amiloride inhibition of NaCl responses of the CT nerve, the BALB strain showed very weak AS even in the anterior tongue. In this study, by using *in situ* hybridization (ISH) and semiquantitative RT-PCR techniques, we examined expression of three subunits of ENaC (alpha, beta, gamma) in the fungiform papillae (FP), circumvallate papilla (CP) and tongue epithelial tissue without taste papillae (ET) in C57BL and BALB mice. The results demonstrated that signals for alpha subunit of ENaC were clearly detected in some spindle-shape cells in both FP and CP, whereas those for beta and gamma subunits were clearly detected in some taste cells in FP, but only slightly in CP. In the ET, all three subunits were detected. These results together with those reported by previous studies suggest that expression patterns for the three subunits of ENaC may contribute to the tongue regional difference in AS in mice. With regard to the strain difference in AS, we are examining the gene polymorphism for each subunits of ENaC between C57BL and BALB strains by sequencing analysis.

Poster: Salt and Sour Taste

Quantitative PCR analysis of the aldosterone-regulated salt transduction pathway in taste cells

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Evidence suggests that sodium salt taste transduction pathways involving the epithelial sodium channel (ENaC) are responsive to aldosterone (ALDO) via its well-documented late genomic effects (12–48 h). Using RT-PCR, we previously identified a number of early (<30 min) genomic response intermediates activated by ALDO in the peripheral taste system that ultimately alter ENaC expression and/or function. Taste receptor cells (TRCs) express NEDD4, a regulatory protein that controls ENaC expression by promoting its ubiquitination; sgk1 (serum- and glucocorticoid-regulated kinase 1), which inhibits NEDD4; CHIF (corticosteroid hormone-induced factor); and Kirsten-ras, which promotes the open state of ENaC. In addition to ENaC, RT-PCR assays demonstrate the presence of at least two other proteins involved in sodium transport in TRCs. These include NHE (N⁺-H⁺-exchanger) isoform 3 and the Na⁺/K⁺ATPase α 1. To quantify expression of mRNA levels of the proteins involved in the regulation of sodium salt taste, we used a multiplexed TaqMan-style real time qPCR assay. We have quantified expression of each of the aforementioned intermediates and effectors in the three lingual taste buds and kidney. To date, our qPCR results indicate that mRNA from taste buds express greater levels of Na⁺/K⁺ATPase α 1, NEDD4 and sgk1 compared with the other intermediates. Results from this study will be used in conjunction with physiological assays to determine the time course and magnitude of changes in the salt taste transduction pathway induced by hormonal and dietary effects.

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Poster: Salt and Sour Taste**Changes in taste receptor cell calcium modulate the amiloride-insensitive non-specific salt taste receptor**

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Regulation of the amiloride-insensitive (AI) salt taste receptor by TRC $[Ca^{2+}]_i$ was investigated by monitoring the effect of resiniferatoxin (RTX) on the rat chorda tympani (CT) taste nerve response to 100 mM NaCl + 5 μ M benzamil (Bz; a specific blocker of apical epithelial Na^+ channels) before and after treating the lingual epithelium with ionomycin (a Ca^{2+} ionophore), BAPTA (a Ca^{2+} chelator) or verruculogen (a blocker of the Ca^{2+} -activated K^+ channels). RTX increased NaCl response monotonically between 0.1 and 1 μ M. Above 1 μ M NaCl responses decreased, reaching control levels at \sim 3 μ M and the rinse levels at 10 μ M RTX. Following BAPTA treatment, RTX produced a greater increase in NaCl CT responses relative to control at all RTX concentrations, and inhibited the suppression of the benzamil-insensitive (BI) NaCl CT responses at higher RTX concentrations. Following ionomycin treatment, RTX produced a smaller increase in NaCl CT responses relative to control, and suppressed the BI-NaCl CT responses at lower concentrations of RTX. Verruculogen (1 μ M) shifted the RTX dose response curve to the right, suggesting that the inhibition of the Ca^{2+} -activated K^+ channels prevents suppression of the BI-NaCl CT responses. The results suggest that a part of the BAPTA-induced right shift of the RTX dose-response curve is due to the decrease in the activity of the Ca^{2+} -activated K^+ channels. We conclude that the AI salt taste receptor is modulated by TRC $[Ca^{2+}]_i$.

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Poster: Salt and Sour Taste**Ethanol modulates the amiloride-insensitive non-specific salt taste receptor**A.K. Vinnikova¹, V. Lyall², G.L. Heck², T.T. Phan² and J.A. Desimone²¹*Internal Medicine, Virginia Commonwealth University, Richmond, VA, USA* and ²*Physiology, Virginia Commonwealth University, Richmond, VA, USA*

The modulation of the amiloride-insensitive (AI) salt taste receptor by ethanol was investigated by monitoring the chorda tympani (CT) taste nerve responses to ethanol (0–60%) alone, to 100 mM NaCl + 5 μ M benzamil (Bz; a specific blocker of apical epithelial Na^+ channels), and to NaCl + Bz + ethanol. The CT responses were monitored in Sprague-Dawley rats, in wildtype (C57BL/6J) and vanilloid receptor-1 (VR-1) knockout (B6.129S4-Trpv1^{tm1ju1}) mice. In both rat and wildtype mouse, superfusing the tongue with solutions containing 10 mM KCl + 30–60% ethanol, increased CT response in a dose dependent manner relative to KCl baseline rinse alone. Both animals also demonstrated responses to NaCl + Bz relative to KCl. In mixtures of ethanol + NaCl + Bz, ethanol induced biphasic changes in the NaCl CT response relative to KCl + ethanol rinse solutions. Ethanol increased the magnitude of the NaCl CT response monotonically between 30 and 50% concentration. Above

50% the CT responses decreased and were significantly reduced around 60% ethanol concentration. In contrast, in the VR-1 knockout mice, no CT response was elicited following stimulation with NaCl + Bz, with ethanol alone (0–60%), or with ethanol (40–60%) + NaCl + Bz. Thus, VR-1 knockout mice demonstrate no functional AI salt taste receptor, no functional CT response to ethanol, and no ethanol salt mixture interactions. We conclude that ethanol stimulates a taste response via the AI salt taste receptor (a VR-1 variant) and also influences the response of the receptor to NaCl.

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Poster: Salt and Sour Taste**Amiloride disrupts NaCl versus KCl taste discrimination in inbred mice whether their chorda tympani nerves are amiloride sensitive or not**

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In rodent taste bud cells, NaCl is transduced via one pathway selective for Na^+ and disrupted by the lingual application of the epithelial Na^+ channel blocker amiloride, and another less cation-selective and amiloride-insensitive pathway(s). In rats, amiloride appears to render NaCl qualitatively indistinguishable from KCl in a dose-dependent fashion. In mice, amiloride has been shown to reduce chorda tympani (CT) responses to NaCl in some strains [e.g. C57BL/6J (B6)] but not others [e.g. 129, DBA/2 (D2) and BALB/c (BALB)]. We used a two-response operant task to train mice from these four strains (7 mice/strain) in a gustometer to discriminate between five-lick samples of NaCl and KCl with concentration (0.1–1 M) varied to render intensity irrelevant. Unexpectedly, the overall performance of the B6 mice was significantly lower than that of the other strains. All mice were then tested with the stimuli adulterated with a descending order of amiloride concentrations (0.1–100 μ M) with control sessions interposed. In all four strains, overall performance dropped to chance at 100 μ M amiloride and sigmoidally improved as the adulterating amiloride concentration was lowered. Performance on NaCl trials was much more affected than that on KCl trials. Thus, the ability to discriminate between NaCl and KCl depends on an amiloride-sensitive transduction pathway in the four strains tested here. Possibly, D2, 129, and BALB (and perhaps B6) mice have amiloride-sensitive taste receptor cells innervated by gustatory nerves other than the CT.

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Poster: Salt and Sour Taste**Suprathreshold intensity discrimination of NaCl in rats with chorda tympani or glossopharyngeal nerve transection**

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In rats, chorda tympani nerve (CT) transection (CTX) but not glossopharyngeal nerve (GL) transection (GLX) raises NaCl taste detection threshold, but the effect of CTX or GLX on suprathreshold intensity discrimination is unknown. We examined whether CTX or GLX in male Sprague–Dawley rats would disrupt a presurgically trained NaCl intensity discrimination in a two-lever operant task. Thirsty rats were required to correctly press one lever in response to a standard concentration and the other lever in response to higher comparison concentrations for water reward. The hit rate for comparison stimuli was adjusted for the standard false alarm rate and a logistic function was fit to the concentration-performance data. The concentrations at the curve midpoints (c) were used to compare differential sensitivity across groups. The mean c for the CTX group ($n = 4$) significantly differed from that for the sham-operated (SHAM; $n = 4$) and GLX ($n = 3$) groups postsurgically with a 0.05 M NaCl standard, though the magnitude of difference was only 0.16 \log_{10} units. The corresponding Weber Fractions (WF) were significantly higher in the CTX group (1.33) compared with the SHAM (0.64) and GLX (0.64) groups. In contrast, the c s nor WF s (CTX = 0.70; GLX = 0.67; SHAM = 0.49) significantly differed across groups when the standard was 0.1 M NaCl. These findings add to a growing list of taste-related behavior for which the GL is unnecessary, but the small effect of CTX on NaCl intensity discrimination is in contrast with its more striking effects on absolute detection.

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Poster: Salt and Sour Taste

Relative effects of transection of the gustatory branches of the 7th and 9th cranial nerves on NaCl detectability in rats

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Chorda tympani nerve (CT) transection in rats severely impairs NaCl taste detection. Higher concentrations of NaCl are nonetheless detectable suggesting that the remaining gustatory nerves can maintain some degree of salt sensibility. We used a two-response operant taste detection task to presurgically train male Sprague–Dawley rats and measure NaCl sensitivity in a gustometer. A modified descending method of limits procedure was used in which a single concentration, pitted against water in a session, was progressively lowered across test days. Logistic functions based on overall percentage correct were derived. The concentrations at the curve midpoints (c) were used to compare NaCl sensitivity before and after sham surgery (SHAM, $n = 5$), transection of both the CT and greater superficial petrosal nerve (GSP) (7X; $n = 6$), or transection of the glossopharyngeal nerve (9th) (9X, $n = 4$). The value of c did not significantly change after surgery for the SHAM and 9X groups. In contrast, the 7X surgery significantly raised c by $\sim 2.50 \log_{10}$ units, a shift greater than that reported for CT transection. These rats were still able to detect very high concentrations suggesting that the 9th or superior laryngeal nerve might support NaCl detection at these stimulus intensities, although the role of trigeminal cues cannot be dismissed. Histology is currently underway. These results suggest that the GSP contributes to NaCl sen-

sitivity in the rat and confirm the primacy of the 7th nerve relative to the 9th nerve in sensibility to NaCl.

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Poster: Salt and Sour Taste

ASIC2 is not necessary for sour taste in mice

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The acid-sensitive cation channel, ASIC2, is widely believed to be a receptor for acid (sour) taste in mammals, based on its physiological properties and expression in rat taste cells. Thus, we evaluated acid taste responses in wild type and ASIC2 knockout mice. Ca^{2+} imaging experiments were carried out using confocal microscopy on slices of circumvallate papillae to which taste stimuli were applied focally at the taste pore. Surprisingly, taste cells from ASIC2 knockout mice exhibited normal physiological responses to 20–100 mM citric acid. The incidence (number of acid-responsive cells) and response amplitude (increase in $[Ca^{2+}]_i$) were identical across the two genotypes. Hence, we explored the expression pattern of the four channels, ASIC1–4, in taste buds from both rat and mouse. Using RT-PCR, we detected expression of ASIC1 and ASIC3, but not ASIC4, in mouse and rat taste buds and non-sensory lingual epithelium. Interestingly, we found that mRNA for ASIC2 is not expressed at significant levels in mouse taste buds, although we observed its robust expression in rat taste buds. Our results indicate that ASIC2 is not required for acid taste in mice. The findings also highlight that there may be important differences in taste transduction mechanisms between mice and rats.

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Poster: Salt and Sour Taste

Cold inhibits sour taste transduction

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The taste transduction mechanism underlying sour taste remains to be elucidated. The basic mechanism is believed to be an acid-sensitive ion channel, the activation of which depolarizes the membrane potential. Membrane depolarization activates voltage-gated calcium channels (VGCCs) and allows Ca^{2+} influx, thereby increasing intracellular calcium ($[Ca^{2+}]_i$). Increased $[Ca^{2+}]_i$ presumably initiates neurotransmitter release. Many ion channels, especially acid-gated channels, are sensitive to changes in temperature. Thus, we reasoned that the temperature sensitivity of sour taste transduction might provide clues to the identity of transduction channels in acid-responsive taste cells. We examined the effect of temperature on tastant-induced changes in $[Ca^{2+}]_i$ in mouse vallate taste cells using confocal fluorescence microscopy and acutely prepared lingual slices. At 30°C, bath application of 100 mM KCl depolarized cells and activated VGCCs, resulting in increased $[Ca^{2+}]_i$ in a subpopulation of taste cells. In a subset

of these KCl-responsive cells, focal application of 100 mM citric acid produced robust increases in $[Ca^{2+}]_i$. When the bath temperature was lowered to 17°C, the response to citric acid was reduced $97 \pm 2\%$ ($n = 4$), that is, effectively abolished. In contrast, the KCl response was reduced by less than half ($43 \pm 4\%$, $n = 5$). Our results suggest that cold inhibits sour taste transduction at the initial transduction channel rather than by blocking downstream Ca^{2+} influx via VGCCs. This observation may be diagnostic of certain proton-gated channels.

Supported by grant DC00374 (S.D.R.).

Poster: Salt and Sour Taste

Neural adaptation to acid stimuli is modulated by the basolateral Na^+-H^+ exchanger-1 in fungiform taste receptor cells

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The role of Na^+-H^+ exchanger-1 (NHE-1) in neural adaptation to acidic stimuli was investigated by intracellular pH (pH_i) measurements in polarized rat fungiform TRCs and chorda tympani (CT) nerve recordings. In CO_2/HCO_3^- -free media (pH 7.4), basolateral Na^+ removal decreased taste receptor cell (TRC) pH_i and zoniporide, a specific NHE-1 blocker, inhibited the Na^+ -induced decrease in pH_i . The TRC pH_i recovery rate from NH_4Cl pulses was inhibited by basolateral zoniporide with a K_i of 0.33 μ M. Basolateral ionomycin reversibly increased TRC Ca^{2+} , resting pH_i , and the pH_i recovery rate from an NH_4Cl pulse. These effects of Ca^{2+} were blocked by zoniporide. The lingual application of zoniporide increased the magnitude of the CT responses to acetic acid and CO_2 , but not to HCl. Lingual application of ionomycin did not affect the phasic part of the CT responses to acidic stimuli, but decreased the tonic part by 50% of control over a period of ~ 1 min. This increased adaptation in the CT response was inhibited by zoniporide. Lingual application of 8-CPT-cAMP increased CT responses to HCl, but not to CO_2 and acetic acid. In the presence of cAMP, ionomycin increased sensory adaptation to HCl, CO_2 and acetic acid. Thus, cAMP and Ca^{2+} independently modulate CT responses to acidic stimuli. While cAMP enhances TRC apical H^+ entry and CT responses to strong acid, an increase in Ca^{2+} activates NHE-1, and increases neural adaptation to all acidic stimuli.

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Poster: Salt and Sour Taste

BDNF haploinsufficient mice have selective taste loss for sour

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Taste receptor cells are classified according to morphological and histochemical criteria. Type II cells express components of the phos-

pholipase C signaling pathway (Clapp *et al.*, 2004), required for bitter, sweet, and umami transduction (Zhang *et al.*, 2002). Type III cells express brain-derived neurotrophic factor (BDNF) and voltage-gated calcium channels (Medler *et al.*, 2003). A recent study suggested that voltage-gated calcium channels may be required for sour transduction (Richter *et al.*, 2003). Taken together, these findings suggest that Type III cells may mediate sour transduction. The current study tested whether mice haploinsufficient for BDNF have reduced sensitivity to sour tastants. The haploinsufficient mice utilized in these studies have a portion of the coding region of BDNF replaced by lacZ. Taste buds of these BDNF/lacZ mice are smaller than wild type littermates (Yee *et al.*, 2003). Mice were tested using a 24 h two-bottle taste preference test for the response to citric acid (1–30 mM), the artificial sweetener SC45647 (3–100 μ M) and the bitter substance denatonium benzoate (0.01–3 mM). The BDNF/lacZ mice appear to be one-half log unit less sensitive to citric acid compared with wild type animals but do not differ from wild types in sensitivity to denatonium or SC45647. These data suggest that sour taste may be specifically impacted in BDNF haploinsufficient mice and suggest that BDNF-expressing Type III taste cells are necessary for sour taste transduction.

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Poster: Gustatory Processing

The amiloride-sensitive Na-channel and NaCl-quinine mixture inhibition in the chorda tympani

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NaCl inhibits chorda tympani (CT) taste responses to quinine-HCl (QHCl) when the two compounds are presented together in a mixture (Formaker *et al.*, 1996). We tested the hypothesis that activation of the amiloride-sensitive Na^+ channel plays a role in the inhibition of QHCl responses by treating the tongue with amiloride and by substituting K^+ for Na^+ . We recorded taste responses from the CT of six golden hamsters (*Mesocricetus auratus*) before and after lingual treatment with 30 μ M amiloride. Taste stimuli were 30 mM NaCl, 30 mM QHCl, 50 mM KCl and the binary combinations of NaCl or KCl with QHCl. Responses to 500 mM NH_4Cl were used to normalize response measurements. Amiloride treatment effectively eliminated differences between KCl, NaCl, and their respective mixtures with QHCl. Without amiloride, responses to NaCl-QHCl mixtures equaled responses to NaCl alone. With amiloride, the response to the NaCl-QHCl mixture was greater than the response to either mixture component and less than the response predicted by additivity [$F(3,15) = 46.1$, $P < 0.00001$]. Responses to the KCl-QHCl and the NaCl-QHCl mixtures were equivalent following amiloride treatment and amiloride did not significantly reduce responses to KCl and QHCl. Therefore, there was no inhibition of CT responses to QHCl without activation of the amiloride-sensitive Na^+ channel. These results indicate that QHCl, KCl and NaCl may share a common peripheral transmission pathway after amiloride treatment. We are currently collecting single-fiber data to examine further the nature of these multi-fiber effects.

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Poster: Gustatory Processing**Chemical and thermal responses of gustatory neurons in the geniculate ganglion**

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We examined chemical and thermal responses of gustatory neurons ($n = 18$) in the geniculate ganglion using extracellular single-cell electrophysiology. Cells were categorized by responses to lingual stimulation with the four classic tastants—NaCl (100 mM), QHCl (20 mM), sucrose (500 mM) and citric acid (10 mM)—at a baseline temperature of 25°C. Responses to temperature change of 1°C/15 s ($\pm 15^\circ\text{C}$) also were measured. Finally, responses to the four tastants were evaluated after adaptation to 40°C and to 10°C. We recorded from one Na-specialist (5.6%), one QHCl-best (5.6%), two acid-best (11.1%) and 14 Na-generalists (77.8%). Temperature change affected firing in only 3/18 cells (16.7%) and only when cooled from 40° to 25°C. Specifically, two Na-generalists increased firing by 1.1 spikes/s (sps) during cooling and the QHCl-best cell increased by 2.9 sps. After 3–5 min adaptation to 10°C, responses to all chemical stimuli decreased in all cells, with the responses to the best stimulus reduced by 2.5 ± 0.3 sps. The most robust decrease in activity occurred in the QHCl-best cell (-4.9 sps); acid best cells decreased firing by -3.9 sps. In contrast, after 3–5 min adaptation to 40°C, responses increased to the best stimulus in 10/16 cells tested (62.5%): 2/2 acid-best ($+1.9$ sps) and 8/13 Na-generalists ($+2.0 \pm 0.4$ sps). Responses decreased or were not affected in the remaining six cells: 5/13 Na-generalists (-0.8 ± 0.5 sps) and the QHCl-best cell (-1.5 sps). Thus, temperature differentially affects responses of geniculate ganglion neurons to taste stimuli.

Supported by NIH grant DC 04875.

Poster: Gustatory Processing**Feeding related peptides: involvement with the gustatory system in the brain of goldfish (*Carassius auratus*)**G. Huesa¹ and T. Finger²¹*University of Colorado Health Sciences Center, Denver, CO, USA and*²*Cell and Developmental Biology, University of Colorado Health Sciences Center, Denver, CO, USA*

Melanin concentrating hormone (MCH) and hypocretins (Hcr) — also called orexins — are peptidergic neurotransmitters expressed primarily in neurons located in the lateral hypothalamus. This region is known to have a critical role in control of feeding and these peptides actively participate in the modulation (stimulation) of food intake. To test whether these orexigenic peptidergic systems are involved in the gustatory information processing we examined their distribution in gustatory nuclei of the CNS of goldfish. For this purpose we employed immunocytochemical techniques using polyclonal antisera directed against MCH or Hcr2. The reaction was developed with diaminobenzidine to enhance the intensity of the labeling. The distribution of these two peptides was similar to those described in mammals, with cell bodies in the hypothalamic

region and fibers projecting rostrally and caudally. Hcr2 immunoreactive fibers are located mainly in preoptic areas, hypothalamus, a few mesencephalic regions and the dorsal horn in the spinal cord, but are scarce in taste related brainstem nuclei. In contrast, MCH fibers were present in many encephalic regions, including the vagal gustatory lobe. These MCH fibers occur mainly in layer IV of the sensory zone of the vagal lobe, where many primary gustatory afferents terminate. In addition, occasional MCH fibers could be observed crossing the sensory zone, in layer IX and also in fiber and motor layers. These results support the concept that MCH but not Hypocretins may act as a central modulator of gustatory processing in goldfish.

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Poster: Gustatory Processing**Expression of neurotransmitter receptors within the nucleus of the solitary tract of the hamster**

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The nucleus of the solitary tract (NST) processes taste information from the periphery that is modulated by descending projections from several areas of the forebrain. Glutamate receptors (GluR) mediate peripheral excitation of all taste-responsive NST neurons: both AMPA/kainate and NMDA antagonists block driven activity. Subsets of these neurons are excited by microinjection of substance P and inhibited by local infusion of γ -aminobutyric acid (GABA) or met-enkephalin. To further delineate the neural circuitry involved in these modulatory effects, we are examining the expression of a number of neurotransmitter receptors in the NST. Serial coronal or horizontal 50 μm sections through the hamster brainstem were processed for immunoreactivity to antibodies directed against μ - and δ -opioid receptors, glutamate receptor subtypes GluR-1, -3 and -4 (AMPA) and GluR-6 (kainate), the tachykinin receptor NK1 and the GABA_A receptor. A given brain was processed using the ABC method for one of these eight antibodies. Cells within the gustatory region of the NST expressed GluR-1, -3, -4 or -6, the NK1 or the GABA_A receptor. Afferent fibers of the VIIth and IXth nerves entering the solitary tract expressed the μ -opioid receptor, whereas the δ -opioid receptor was seen in cells of the NST, including the gustatory zone. This pattern of expression would suggest a substrate for both pre- and postsynaptic inhibition of gustatory neurons. Further work will examine receptor expression in physiologically characterized NST neurons.

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Poster: Gustatory Processing**Paired pulse facilitation and depression observed in taste cells in the rat nucleus of the solitary tract following glossopharyngeal nerve stimulation**

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Evoked responses from paired pulse stimulation of the glossopharyngeal (GP) nerve was studied in taste responsive cells in the nucleus of the solitary tract (NTS) of anesthetized rats. Adult male rats were anesthetized with urethane (1.4 g/kg) and Nembutal (25 mg/kg), and prepared for unilateral electrical stimulation of the lingual branch of the GP nerve and for electrophysiological recording from the ipsilateral NTS. Once a taste-responsive cell was isolated, responses to taste stimuli (0.1 M NaCl, 0.5 M sucrose, 0.01 M quinine-HCl, 0.01 M HCl) and electrical stimulation of the GP nerve were recorded. Up to 100 pairs of pulses were delivered to the GP nerve at various interpulse intervals and responses from cells were recorded. For each cell, the threshold for stimulation of the nerve that evoked a response was determined and a slightly higher current level was used for subsequent stimulation. Electrical pulses were 0.2 ms in duration and the maximum current level was 1.5 mA. Thus far, both paired pulse facilitation and depression have been demonstrated in taste cells in the NTS in response to stimulation of the GP. Much like paired pulse depression, which has been previously demonstrated with stimulation of the chorda tympani nerve, paired pulse facilitation and depression in the GP may be mechanisms by which cells integrate incoming information, modify the responses of other cells or enable the system to increase contrast among taste stimuli.

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Poster: Gustatory Processing

Single taste-responsive neurons in the nucleus of solitary tract project axons to both parabrachial and hypoglossal nuclei: an in-vivo intracellular recording and labeling study

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We examined the axonal projections of individual taste-responsive neurons of the nucleus of the solitary tract (NST) of hamsters using intracellular recording and labeling. The activity of NST neurons was recorded intracellularly to determine their intrinsic firing patterns and their responses to lingual stimulation with anodal current and with sucrose, NaCl, citric acid and quinine. Recorded cells were labeled with 2% biocytin (500 ms, 1 na, 1.4 Hz, 6–40 min); a minimum of 2.5 h was imposed between cell labeling and perfusion to permit axonal transport. Hamsters were given lethal injections of Nembutal and perfused with chilled 4% paraformaldehyde. Brains were removed, refrigerated in fixative overnight, and sectioned at 100 μ m in a horizontal or sagittal plane. Sections were incubated in ABC and reacted with DAB to reveal the labeled cell and counterstained with cytochrome oxidase to visualize brainstem nuclei and tracts. To date, we have injected six cells (three multipolar, two elongate, one bipolar-like) and traced the axonal projections to medial and lateral divisions of the parabrachial nuclei (PbN) for three cells. In two cells, NST axons projected to both the PbN and the hypoglossal nucleus. NST axons contained bouton-like swellings along their axonal pathway as well as in their terminal fields. These results suggest that a single class of NST cells can provide output to three or more separate synaptic targets.

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Poster: Gustatory Processing

Taste-evoked responses in the nucleus of the solitary tract of C57BL/6ByJ and 129P3/J mice

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Although mice offer several advantages for examining the role of genetic factors in taste sensation, gustatory responses have been recorded only from the periphery in this species. In the present experiment, the properties of cells in the first central gustatory relay, the nucleus of the solitary tract (NST), were determined in male C57BL/6ByJ (B6) and 129P3/J (129) mice. Animals were anesthetized, a surgery was performed to expose the surface of the brainstem, and neural activity was measured using glass micropipettes. When the activity of a single neuron was isolated, a stimulus array that included 100 mM NaCl, 500 mM sucrose, 10 mM HCl and 20 mM quinine-HCl was applied to the tongue and oral cavity. Taste-responsive units were found at coordinates relative to obex that correspond to the location of the rostral NST in mouse neuroanatomical atlases. We recorded the activity of 18 single cells in B6 mice and 13 cells in 129 mice. Responses to sucrose were significantly larger in B6 mice. In both strains, the properties of cells were similar to those reported previously for rats, including breadth-of-tuning values that averaged 0.8. This represents a large increase over breadth-of-tuning values reported previously for peripheral gustatory neurons in mice, and it suggests that peripheral fibers with different response profiles converge onto the same NST neurons in these animals. The larger neural response to sucrose in B6 mice may underlie their higher preference for this compound in long-term tests.

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Poster: Gustatory Processing

The effect of flow rate on the temporal structure of a taste response recorded from the nucleus of the solitary tract in the rat

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Recent work has suggested that taste quality may be encoded by the temporal structure of initial 2 s of response (Di Lorenzo and Victor, 2003) in the nucleus of the solitary tract (NTS). Data from Smith and Bealer (1975) in the whole chorda tympani nerve (CT) in the hamster suggest that this interval may also contain information about stimulus flow rate. Here, the effects of flow rate on the temporal structure of taste responses in the NTS of anesthetized rats were examined. Taste responses to high (5 ml/s) and low (3.0 ml/s) flow rates of NaCl (0.1 M) were recorded from taste responsive cells in the rostral nucleus of the solitary tract (NTS). Each trial consisted of 10 s spontaneous activity, 10 s distilled water, 5 s NaCl, 5 s post-stimulus pause and a 20 s water rinse. The inter-stimulus interval was 2 min. Low and high flow rates were alternated, and multiple replications were presented. Results indicated that approximately half of the taste cells also showed evidence of tactile responsiveness. Approximately half of the cells showed identical response

to NaCl presented at high and low flow rates. A minority of cells showed an enhanced phasic response related to the high flow rate, similar to results in the hamster CT. Another subset of cells showed the opposite effect. Results indicate that flow rate is encoded within taste responses of NTS cells and that some cells may be more sensitive to flow rate than others.

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Poster: Gustatory Processing

Gusto-salivary reflex circuits in rat brainstem

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Neural circuits underlying taste-salivary reflexes have been studied in rat brainstem slices. Parasympathetic neurons innervating the lingual (von Ebner) and parotid salivary glands were retrogradely labeled by application of fluorescent Alexofluor dextran to either the glossopharyngeal or otic ganglion and whole cell patch clamp recordings made from the labeled neurons. Neurons were first visualized with fluorescent illumination and once a neuron was selected for recording, it was imaged using differential interference contrast infrared microscopy. The electrode also contained Lucifer yellow (LY) to fill the recorded neuron for subsequent identification. After recording, the LY filled neurons were imaged using a confocal microscope, reconstructed and morphometrically analyzed. Successful recordings have been made from 86 neurons. Based on morphometric measurements neurons innervating the parotid gland are significantly larger than those innervating the lingual glands. Using various current injection protocols two major groups of neurons have been defined based on biophysical and repetitive discharge characteristics. The majority of the neurons have an action potential discharge pattern that is modified by the interaction of excitatory and inhibitory synaptic input, while the minority of neurons transmit the afferent input pattern unchanged. Since all post-synaptic potentials recorded from the parasympathetic neurons were a complex mixtures of excitatory and inhibitory potentials considerable processing of afferent taste input occurs to determine the output pattern of discharges transmitted by the secretomotor neurons.

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Poster: Gustatory Processing

Alcohol activates a sucrose-responsive gustatory neural pathway

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A strong association exists between the intake of alcohol and sweet-tasting substances. The neural mechanisms underlying this relationship are unknown, although recent data suggest that gustatory factors are involved. Here, we explored the role of taste receptors and CNS circuits for sugar taste in the gustatory processing of ethanol. Taste responses to ethanol (3, 5, 10, 15, 25 and 40% v/v) and stimuli of different taste qualities (e.g. sucrose, NaCl, HCl and quinine-

HCl) were recorded from neurons of the nucleus of the solitary tract in anesthetized rats prior to and following oral application of the sweet receptor blocker gurmarin. The magnitude of ethanol-evoked activity was compared between sucrose-responsive (S_1 , $n = 21$) and -unresponsive (S_0 , $n = 20$) neurons and the central neural representation of ethanol taste was explored using multivariate analysis. Ethanol produced robust concentration-dependent responses in S_1 neurons that were dramatically larger than those in S_0 cells ($P_s \leq 0.02$). Gurmarin treatment selectively and similarly inhibited ethanol and sucrose responses ($P_s \leq 0.01$). Across-neuron patterns of response to ethanol were most similar to those evoked by sucrose (multiple $r = +0.89$), becoming increasingly more so as the ethanol concentration was raised. Results implicate taste receptors for sucrose as candidate receptors for ethanol and reveal that alcohol and sugar taste are represented similarly by activity in the CNS. These findings have important implications for the sensory and hedonic properties of alcohol.

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Poster: Gustatory Processing

Characterization of rat orbitofrontal cortical neurons during *ad libitum* drinking of liquid rewards

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In gustatory physiology, the orbitofrontal cortex (OFC) contains neurons that have roles in tongue movements, satiety, and other motivational outcomes involved in obtaining rewards. There is, however, a paucity of information regarding the nature of the OFC responses obtained from freely moving rats licking to obtain a reward. To this end we have chronically implanted bundles of electrodes in rat OFC while they were free to drink from a sipper tube water or a sucrose solution. 172 single unit responses were characterized. A cross-correlogram analysis between licking and neural activity revealed that 17% of the responses faithfully followed the licking frequency (~7 Hz). Of these oscillating neuronal responses, 24% responded either more actively or with a different morphology when drinking sucrose than water, probably reflecting the differential reward values of these stimuli. To obtain a better understanding of the neuronal activity involved in the initiation of a licking bout, peri-event histograms were constructed using the onset of the first lick after at least a 1 s pause. Four other morphologically distinct types of responses were identified. Relative to the initiation of a drinking bout: two types began firing before (anticipatory), one type decreased firing and the other increased firing. In summary, five types of OFC neurons have been identified. Two, as expected, are related to the anticipation of a reward; others however are related to licking, and to the nature of the reward.

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Poster: Olfactory CNS Physiology and Coding

Ontogeny of sensory-evoked responses in rat amygdala

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The amygdala plays a critical role in emotion and memory. Recent work has suggested that adult reactions to emotional and/or stressful stimuli can be shaped by the effects of early experience on amygdala functional ontogeny. However, while ontogeny of amygdala neuron phenotype and neuroanatomy have been described, very few descriptions of amygdala physiology and function during the postnatal and adolescent period exist. As a first step toward understanding how early experiences shape amygdala function, the present study examined amygdala single-unit activity and responsiveness to sensory input during postnatal development. Single-unit and local field potential (LFP) activity were recorded in amygdala nuclei (primarily basolateral) of urethane-anesthetized, Long-Evans hooded rats. Rats were aged postnatal day (PN) 10 to adult (>PN70). Spontaneous activity, odor-evoked and footshock-evoked activity were determined for each cell using peri-stimulus time histograms. Spontaneous activity increased and evoked response latency decreased with age. While both odor-evoked and footshock-evoked responses could be observed at all ages, the temporal structure of these responses changed dramatically over the age range tested. Odor-evoked LFP's showed strong oscillations in the high beta band in adults, however the primary frequency of these oscillations shifted to lower ranges in younger animals toward the theta range at PN10. Given the hypothesized importance of temporal structure in stimulus encoding, these developmental changes may be indicative of a slow postnatal emergence of mature sensory processing by the rat amygdala, perhaps contributing to the sensitivity of this structure to early experiences.

Poster: Olfactory CNS Physiology and Coding

Facilitation of main olfactory input to the medial amygdala and medial preoptic area by gonadotropin-releasing hormone

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Sensory signals received during mating activate brain regions along the vomeronasal pathway and the medial preoptic area. These activated regions contain cell bodies and fibers of gonadotropin-releasing hormone (GnRH) neurons, suggesting a possible relationship between chemosensory input and GnRH. Chemosensory input can be detected by the vomeronasal organ and/or the main olfactory system. The consequences of vomeronasal organ removal (VNX) are most apparent in sexually naive animals. Experienced, but not inexperienced, male hamsters can use main olfactory input to maintain mating after VNX, suggesting that with experience, neural circuits acquire the ability to use main olfactory information as the essential chemosensory input for mating. GnRH has also been shown to restore mating behavior in naive VNX male hamsters. GnRH may facilitate transfer of olfactory information to the medial amygdala and medial preoptic area in naive intact and VNX males. Electrical stimulation of the main olfactory bulb activates neurons in anterior and posterior medial amygdala and increases expression of Fos-related antigens. Initial experiments indicate an icv injection of GnRH into the lateral ventricle increases activation in medial amygdala and medial preoptic area. These experiments examine the effect of GnRH on chemosensory information transfer via the main olfactory pathway to these regions that

are involved in mating behavior driven by either chemosensory pathway.

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Poster: Olfactory CNS Physiology and Coding

Odor responses of cerebral lobe neurons

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Functional information as to how odors are processed at more central neural levels than the olfactory bulb (OB) is fragmentary at best in mammals and nonexistent in teleosts. The long-term goal is to determine similarities and differences in how odor information arriving via the olfactory tracts is processed within the cerebral lobes (CL) of the channel catfish compared with that in the OB. Odor-responsive neurons were located in caudo-medial and lateral regions of the CL as predicted from previous forebrain anatomical investigations (Finger, 1975; Bass, 1981). Recordings were performed *in vivo* with metal-filled glass microelectrodes. Tested were bile salts (taurocholic and tauroolithocholic acids) and amino acids (L-methionine and L-arginine), effective odor stimuli in channel catfish (Nikonov and Caprio, 2001). Sixty-four CL neurons responded to the odor stimulation; 45 were excited and 19 were suppressed (12 by amino acids and seven by bile salts). Fifteen units located medially in the CL were excited by bile salts and were unresponsive to amino acids. Twenty-six of the 30 excitatory units located more laterally responded to 0.1–100 μ M amino acid; four additional units within this region along the OB midline responded to bile salts. The medial-lateral distinction between excitatory responses to bile salts and amino acids reflects a similar topographical organization within the OB. Ongoing experiments will determine if an odotopic map as observed in the OB (Nikonov and Caprio, 2001) exists or is altered within the CL and whether the response properties of CL neurons are different from those of OB neurons to the same odors.

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Poster: Olfactory CNS Physiology and Coding

Odor responsiveness of the olfactory cortex and residual tyrosine hydroxylase activity in the olfactory bulb in the CNGA2 knockout mouse

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Mice deficient for subunit A2 of the cyclic nucleotide-gated (CNG) channel respond to select odorants (see Restrepo *et al.*, this meeting). We examined the presence of alternate transduction pathways and the potential role of necklace glomeruli, which receive axons from neurons expressing the cGMP pathway (Juifls *et al.*, 1997, *Proc Natl Acad Sci USA* 94: 3388–3395) and remain active in CNGA2 KO mice (Baker *et al.*, 1999, *J. Neurosci.* 19: 9313–9321). Immunoreactivity to the cGMP-stimulated phosphodiesterase (PDE2), tyrosine hydroxylase (TH) and odor-induced Fos was used to visualize necklace glomeruli, levels of glomerular activity

and activation respectively. We found that the TH expression in juxtglomerular cells surrounding PDE2-positive necklace glomeruli is dependent on afferent input as unilateral naris occlusion dramatically reduced the TH expression in CNGA2 KO mice. Importantly, exposure CNGA2 KO mice to a mixture of putative pheromones (2-heptanone and 2,5-dimethylpyrazine) increased odor-evoked Fos induction in juxtglomerular cells of some necklace and regular glomeruli. Further, we observed Fos expression in a substantially larger number of cells in both the anterior olfactory nucleus (AON) and piriform cortex in CNGA2 knockout mice compared with the same regions from mice exposed to fresh air. These experiments provide evidence for alternate transduction pathways in the main olfactory system in mice.

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Poster: Olfactory CNS Physiology and Coding

Synaptic mechanisms contributing to olfactory cortical adaptation

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Anterior piriform cortex (aPCX) neurons rapidly filter repetitive odor stimuli despite relatively maintained input from mitral cells. This cortical adaptation is correlated with short-term depression of afferent synapses *in vivo*. The purpose of this study was to elucidate mechanisms underlying this non-associative plasticity using *in vivo* and *in vitro* preparations and to determine its role in cortical odor adaptation. We have previously described our *in vitro* stimulation results. In particular, we have discovered that the longer lasting portion (~120 s) of the *in vitro* short-term synaptic depression can be blocked pharmacologically. Following 50 s of simulated odor stimulation, either the metabotropic glutamate II/III antagonist CPPG (500 μ M) or the β -adrenergic receptor agonist isoproterenol (20 μ M) can decrease the duration of synaptic depression. More recently we have discovered that, *in vivo*, both adaptation of odor responses in the β (15–35 Hz) spectral range and the associated synaptic depression following a 50 s exposure to a mixture of odorants can also be blocked by intracortical infusion of CPPG (2.5 mM). Currently, we are extending this work to behavioral habituation using heart rate orienting response habituation as a measure of cortical adaptation. Prior to adaptation of the heart rate orienting response, 3 μ l of 2.5 mM CPPG or vehicle will be infused into aPCX bilaterally. Results from the behavioral paradigm will assist in clarifying the independent roles of receptor adaptation and cortical adaptation in behavioral habituation to odors.

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Slide: Olfactory CNS Physiology and Coding

Rapid odor change and brain electrical activity

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Some odors produce increased activity in areas of the brain often associated with language even though the task demands do not ex-

PLICITLY involve language. Visual and auditory stimuli which change rapidly tend to produce more left temporal lobe activity than stimuli that change slowly. If odor mixtures are transduced in such a way that each component produces high temporal overlap with the other components, this should effectively be construed as rapid stimulus change and lead to more left temporal lobe responses. To test this hypothesis, 10 undergraduate students served as subjects in a chemosensory event-related potential (CSERP) study. Three odors, phenethyl alcohol, vanillin, and citral were presented in binary pairs via a constant flow olfactometer and stimulus administration was triggered by subjects' inhalation. The stimuli were presented in two different ways. Given the members of the binary odor pair, A and B, one condition (Slow) required subjects to smell a sequence of A followed by B. Both A and B lasted 300 ms and were counterbalanced. In the Fast condition, the sequence was A–B–A–B, where each stimulus lasted 150 ms and was counterbalanced. In both cases, each odor lasted a total of 300 ms and the total stimulus administration lasted 600 ms. CSERP data were collected from 80 scalp locations. Analysis revealed that brain activity differed between the two types of stimulus administration. Subsequent analysis with LORETA indicated that the left temporal lobe was more active in the Fast condition supporting the hypothesis that temporal overlap in odor mixtures leads to more left temporal lobe activity.

Poster: Olfactory CNS Physiology and Coding

PET activations during smelling of androstenone: osmics versus anosmics

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We used positron emission tomography to evaluate the neural correlates involved in perception of the endogenous steroid androstenone. Our aim was to investigate whether differences exist in brain activations between subjects with high sensitivity (low thresholds) for androstenone and those with a specific anosmia. We screened 87 subjects using a staircase procedure to establish individual thresholds. Twelve subjects unable to detect the steroid in its crystal form were selected for an anosmic group, and 12 subjects capable of consistently detecting a 1.4×10^{-7} M solution of androstenone comprised an osmic group. Subjects were scanned during passive smelling of phenyl ethyl alcohol (PEA), pyridine, androstenone (AND) or the diluant (for baseline). Our first analysis compared brain responses of the two groups to two of these stimuli: PEA and AND versus baseline. Activations for PEA were observed in olfactory regions, including orbitofrontal and piriform cortex, in both subject groups. However, osmics and anosmics differed in their response to AND. Osmics showed more activity in the dorso-medial area of the left parietal cortex and had no significant olfactory activations, whereas anosmics showed more frontal activations in the right dorsomedial and orbitofrontal regions. The results for PEA are consistent with other studies of olfactory processing, showing activations in olfactory cortices, whereas the AND results suggest differential function in the human brain related to individual sensitivity to androstenone. Implications of the parietal lobe activations will be discussed.

Supported by Canadian Institutes of Health Research.

Poster: Olfactory CNS Physiology and Coding**Functional neuroimaging of odor imagery**

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We used positron emission tomography to investigate brain regions associated with odor imagery. We compared changes in brain activation during active imagination of odors versus those during nonspecific expectation of olfactory stimuli, and during experience of physically presented versus imagined odors. Sixty-seven healthy volunteers were screened for their odor imagery (with a paradigm developed in a previous study), and 12 of them, selected as 'good odor imagers', participated in the neuroimaging study. Comparison of odor imagery with general expectation of odors revealed activation in the left primary olfactory cortex (POC) including piriform cortex, as well as in the insula bilaterally and the left posterior orbitofrontal cortex (OFC). On the other hand, comparison of odor perception with an odorless baseline resulted in increased activation bilaterally in the piriform and insular cortex and in the right posterior OFC. Results of a conjunction analysis revealing regions of common activation during odor perception and imagery were convergent with the results obtained by the subtraction method: left piriform cortex and bilateral insula were activated both when smells were perceived and imagined. Findings also suggested a differential role of the right versus left posterior OFC in perceptual versus imaginal olfactory processing: whereas the right OFC was activated during odor perception, the same region of the left hemisphere was activated during odor imagery. Overall, the findings indicated that neural networks engaged during odor perception and imagery partially overlap.

Supported by Canadian Institutes of Health Research.

Slide: Olfactory CNS Physiology and Coding**Neural substrates underlying mental imagery of pleasant and unpleasant smells**M. Bensafi¹, J.A. Porter², R.M. Khan¹, J. Mainland¹, B. Johnson³, C. Zelano⁴ and N. Sobel¹¹Neuroscience, University of California, Berkeley, Berkeley, CA, USA,²Psychology, University of California, Berkeley, Berkeley, CA, USA,³Bioengineering, University of California, Berkeley, Berkeley, CA, USA and⁴Biophysics, UC Berkeley, Berkeley, CA, USA

Patterns of neural activity in orbito-frontal cortex (OFC) reflect odor valence: the medial OFC responds preferentially to pleasant odors, whereas the lateral OFC responds preferentially to unpleasant odors. Whether this dissociation exists during odor imagery in the absence of any odorants is unknown. Here, we approached this question using fMRI (4 T). Sixteen subjects (eight female) were tested in an event-related design with foveal trial types, each repeated 22 times ($T_R = 500$ ms, ISI = 30 s). In three sensory trial types, a pleasant odor (strawberry), an unpleasant odor (rotten eggs) and a non-odorized control were presented by air dilution olfactometer. In two imagery trial types, no odorants were presented, but subjects were asked to imagine the same smells (strawberry, rotten eggs). Replicating previous reports, initial analysis revealed a dissociation during perception whereby strawberry induced a significant

activation in medial OFC, whereas rotten eggs induced an increase in lateral OFC ($P < 0.05$). Furthermore, as in perception, imagining an unpleasant smell induced a significant activation in lateral OFC ($P < 0.05$). Whether olfactory imagery exists is still a matter of controversy. That the hedonic-specific pattern of neural activity during imagery mimicked that during perception lends support to the existence of odor imagery.

Slide: Olfactory CNS Physiology and Coding**Brain activation to the invigorating and relaxing odors**J. Wang¹, Q.X. Yang¹, P.J. Eslinger², M.B. Smith¹, R. Ansari³, J. Behan³ and A. Richardson³¹Pennsylvania State University College of Medicine, Hershey, PA, USA,²Neurology, Pennsylvania State University College of Medicine,³Technical Affairs, Quest International Fragrance Company, Mount Olive, NJ, USA

The neural mechanism of how olfaction affects emotions and moods remains largely unknown. We used fMRI to investigate olfactory activations by two types of commercially popular fragrances with distinctive emotional attributes that are invigorating (peppermint, types ST1221 and STP0654, Quest International) and relaxing (lavender, types SC1139 and PD1861, Quest International). Twenty-five normal non-smoking young adults received olfactory functional MRI (fMRI) with one of three pairs of fragrances on a 3 T MRI system. Statistical parametric maps of each group were generated using one-sample *t*-test ($P < 0.001$). Another group of 20 normal adults smelled and evaluated the three pairs of fragrances out of the magnet. All six fragrances activated the primary olfactory system in the brain. In addition, all six fragrances elicited patterns of widespread brain activation involving mainly frontal, temporal, insular and parietal cortices that mediate emotion, attention, sensorimotor and cognitive processes. However, the activation maps elicited by the six fragrances exhibited distinctive features. The invigorating fragrances stimulated more widespread and larger activation than the relaxing ones did. On the other hand, the activation maps produced by fragrances with similar affective attributes are similar.

Thanks for support from Quest International Fragrance Company.

Poster: Unicellular Chemoreception**GPI anchored receptors in chemosensory transduction**

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Glycosyl phosphatidylinositol (GPI) anchored proteins are surface proteins that are tethered to the cell surface. They serve as receptors of ligands in axon guidance and T cell activation among other functions. In *Paramecium* they function in chemoreception. We have shown through disruption of anchoring using an antisense expression to PIG-A (an enzyme in the first step in anchor synthesis) that chemoresponses to folate and glutamate are almost eliminated. Disruption of the last enzyme of anchor synthesis with an RNAi expression vector also disrupted chemoresponse, but not as

profoundly. The genes for the first and last enzymes in the pathway were cloned using homology PCR, and with the sequencing of the *Paramecium* genome, we have identified the sequences of the other pathway enzymes. We have examined the distribution of the GPI anchored proteins on the cell surface and found that they are not evenly distributed across the surface, but rather they cluster near the bases of the cilia, where another chemosensory transduction component (the plasma membrane calcium pump, PMCA) is also found. We show this distribution through confocal and deconvolution microscopy using antibodies to the folate chemoreceptor, 5'-nucleotidase, GPI anchored surface antigens, tubulin and the PMCA. The *Paramecium* genome project has provided us with sequences for putative GPI anchored folate receptors and our preliminary results using RNAi-feeding support the hypothesis that at least one of these proteins functions in folate chemoresponse.

Supported by NIH DC00721, GM59988 and the Vermont Cancer Center.

Poster: Unicellular Chemoreception

Lipid rafts in chemosensory transduction

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Lipid rafts are lipid domains high in glycosphingolipids and cholesterol that serve as platforms for organizing signal transduction components such as GPI anchored proteins, G-proteins (large and small), serpentine receptors, receptor and non-receptor tyrosine kinases. Proteins in lipid rafts are insoluble in cold Triton X-100 and are found in low density fractions when the Triton insoluble fractions are separated in sucrose or Optiprep gradients. We have found that *Paramecium* surface membranes can be separated in Optiprep gradients into fractions by density, and correlated with ganglioside and cholesterol levels. Proteins such as the putative folate chemoreceptor (and other GPI anchored proteins such as 5'-nucleotidase and surface antigens), G-beta, and the plasma membrane calcium pump separate into the light density fractions, supporting the existence of lipid rafts that organize these proteins in signaling. When we remove cholesterol from the membranes of living cells using methyl-beta-cyclodextrin, we find that chemoresponse to folate is significantly reduced, implying that raft organization is important for chemoresponse. We are also using cholera toxin to localize gangliosides as markers of rafts on the cell surface, and asking whether removal of cholesterol changes the distribution of signaling proteins in Optiprep gradients and on the cell surface.

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Poster: Unicellular Chemoreception

Plasma membrane calcium pumps functioning in chemical sensing

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The plasma membrane calcium pumps (PMCA) play a role in sensory transduction in *Paramecium* by sustaining the hyperpolarizing conductance that is initiated by a K conductance upon stimulus application. We identified 4 PMCA isoforms through homology PCR, and found that three were expressed. The *Paramecium* genome project shows that there are up to four more related sequences that probably code for additional PMCA. To explore the roles of these PMCA we have begun to overexpress them with and without epitope tags to localize them on the cell surface, and found that overexpression disrupts calcium homeostasis in the cell. The cells' swimming patterns are dependent upon the intraciliary calcium levels, and overexpression of the isoforms 2 and 3 cause cells to swim backward for long periods of time, consistent with high intraciliary calcium. We have also found that the calcium conductance of the voltage gated calcium channels is significantly changed in cells overexpressing the isoforms 2 and 3, consistent with abnormally high intracellular calcium and defective calcium homeostasis. This has allowed us to systematically over express the isoforms and ask which chemoresponse is affected. We find that isoform 2 overexpression primarily affects folate chemoresponse, while isoform 3 over expression affects chemoresponse to folate, glutamate, acetate, all of which function through different signal transduction pathways, but all of which we believe to include the PMCA.

Supported by DC 00721 and Vermont Cancer Center.

Poster: Unicellular Chemoreception

The ryanodine receptor antagonist dantrolene alters swimming behavior and causes mortality in *Paramecium tetraurelia*

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Paramecium tetraurelia are unicellular eukaryotes with excitable ciliated membranes that use calcium as a regulator of membrane potential. Several critical *Paramecium* functions are known to be mediated by calcium such as chemoresponse and exocytosis. Because caffeine and 4-chloro-*m*-cresol stimulation of *Paramecium* results in an increase in intracellular calcium concentration (Klauke *et al.*, 2000, *J. Membrane Biol.* 174: 141–156), it is possible that a calcium channel similar to the ryanodine receptors found in skeletal muscle may be involved in calcium mobilization. We examine the effects of the ryanodine receptor antagonist, dantrolene, on *P. tetraurelia*. Dantrolene was toxic when applied in doses normally used in mammalian systems in the presence of magnesium. It is possible that magnesium, which also is a ryanodine receptor inhibitor, amplifies the effect of dantrolene on ryanodine-like receptors in *Paramecium* resulting in toxicity. Also of interest is the lack of protection from dantrolene-mediated toxicity in the mutant eccentric, which has a defective magnesium conductance. Initial experiments indicate that dantrolene does not affect the ability of caffeine to increase intracellular calcium levels. Dantrolene does however, significantly reduce the backward swimming time of *Paramecium* exposed to depolarizing concentrations of potassium. Chemosensory behavior in T-maze assays to the attractants biotin and acetate was not altered by dantrolene treatment in potassium solutions.

This work was supported by grant J-651 from the Jeffress Memorial Trust and a VMI research grant in aid.

Poster: Clinical Evaluation and Consumer Research**Computerized history of olfactory dysfunction**H. Cornelia¹, B.N. Landis², J. Frasnelli¹ and T. Hummel¹¹ORL, University of Dresden Medical School, Dresden, Germany and ²ORL, University Hospital of Geneva, Geneva, Switzerland

The patient's/subject's history is an integral part of investigations on human olfactory sensitivity. Numerous ways are being employed to obtain information from subjects/patients. Based on Filemaker Developer 6.0 (R), the current exercise aimed to produce a relatively short, computerized questionnaire which allows subjects/patients to enter their data directly into a database in a controlled fashion. Further, a separate section is available for the examiner to enter data with regard to drug intake, qualitative olfactory dysfunction, results of chemosensory testing, results from nasal endoscopy, treatment etc. A different set of questions is available for return visits. In addition, the present approach combines this database with software for the computerized testing of olfactory thresholds (triple-forced choice, single staircase), odor discrimination (triple forced choice, 16 triplets) and odor identification (multiple forced choice, 16 items). In a clinical context, the database allows to create a quick overview across changes of olfactory function over time. It is hoped that this approach will eventually lead to more complete information from subjects/patients. Further, it is hoped that this approach will help to standardise information that is obtained at different centers.

Poster: Clinical Evaluation and Consumer Research**Clinical test of olfaction based upon a MEMS-microvalve olfactometer**

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We describe the development of a clinical test for the assessment of olfactory function which incorporates state-of-the-art Micro-Electro-Mechanical Systems (MEMS) microvalves. These miniature, discrete devices form the basis of the MEMS olfactometer, a relatively small, inexpensive device capable of producing 40 or more discrete odor stimuli. Moreover, the MEMS olfactometer will have the capacity for generating the stimuli necessary for a large number of trials (>100). This capability can be replenished by replacing inexpensive odor cartridges. The initial test selected for development is an odor identification test, the paradigm most frequently used to assess olfactory function. The test, called the OLFAC (OLfactory Function Assessment by Computerized Testing), will be enhanced by inclusion of pictures, along with words, in the description of test item choices. The presentation of the test items, along with scoring of the test, and recording of all pertinent data will be totally computerized. Norms for the test will be developed and compared with standardized norms already available for other commercial tests. Finally, the test will be enhanced to run on the WWW as a Web application. Once a MEMS olfactometer has been attached to a computer linked to the WWW, administration of the tests can be authorized over the Web. Anonymous data can also be collected via the Web for inclusion into a centralized database. This test will provide a comprehensive and sensitive system for evaluating the

sense of smell at a lower cost while providing greater utility than current tests.

Supported by NIH grant DC 06369.

Poster: Clinical Evaluation and Consumer Research**Heart rate changes during odorant administration: promotion of 'cool-down' and recovery in college athletes**

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An often under-addressed aspect of athletic training, and even casual exercise, is the proper amount of time for 'cool-down' and recovery. However, when an ample recovery period is not available, the likelihood of injury and overtraining increases while athletic performance decreases. Previous research has shown that odorants can affect one's mood, motivation and task performance. Moreover, peppermint odor is linked to enhanced athletic performance, while jasmine odor is a proven sleep aid. These unique odorant characteristics led to their inclusion within the present experiment in an attempt to determine whether jasmine and peppermint odors can enhance athletic recovery. In a within-subjects design, 20 athletes performed a modified version of the Bruce Stress Test Protocol on a treadmill for 15 min and then completed push-ups until exhaustion. Following 10 min of 'cool-down' stretching in a peppermint, jasmine or no-odor condition, physiological data were recorded and the participant completed questionnaires related to workload demands and mood. In addition, level of vigor was rated over the following 12 h. Jasmine odor significantly reduced athletes' heart rate following the 'cool-down' period compared with the non-odorized control condition. Such a finding supports the hypothesis that odorants may have a substantial role in naturally and safely expediting recovery from physical exertion.

This study was funded by a grant from NASA to B.R.

Poster: Clinical Evaluation and Consumer Research**Effects of peppermint odor administration on augmenting basketball performance during game play**B. Raudenbush¹, J. Smith¹, K. Graham¹ and A. Mc Cune²¹Psychology, Wheeling Jesuit University, Wheeling, WV, USA and²Physical Therapy, Wheeling Jesuit University, Wheeling, WV, USA

Previous research indicates that inhaling peppermint odor prior to and during athletic activity increases strength, speed and endurance. It has also been found to reduce fatigue, perceived effort and perceived frustration, and increase levels of vigor and motivation. However, assessment of peppermint odor efficacy has yet to be performed during actual physical game play. The present study was designed to assess whether the degree to which athletes inhale peppermint odor affects such aspects as motivation, energy, fatigue, reaction time, confidence and performance during the course of a basketball season. Male and female Division II basketball players were provided with a peppermint inhaler (Peak Performance™ Sports Inhaler™) for use during practice and game play. Level of inhalant use constituted group composition for data analysis.

Higher levels of inhalant use were associated with increased motivation, energy, speed, alertness, reaction time, confidence and strength. Levels of fatigue and frustration were lower in the high-use group. In addition, athletes' ratings of their competitive advantage over opponents and ratings of overall performance were enhanced. Implications are particularly salient in regards to augmenting a variety of factors related to athletic performance using an all-natural, non-pharmacological ergogenic aide.

Poster: Clinical Evaluation and Consumer Research

Effects of beverage flavor on athletic performance, mood, and workload

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Previous research indicates that the administration of peppermint odor can augment athletic performance and mood, and decrease workload demands. The present study extended those findings by evaluating athletic performance and physiological changes during the administration of flavored beverages. Utilizing a within-subjects design, athletes performed a 15 min modified treadmill stress test. At 3 min intervals, 50 ml of beverage (peppermint water, unadulterated water or Gatorade sports drink) were consumed. In the control condition, no beverage was consumed. Pre- and post-testing physiological measurements were taken (blood pressure, pulse, oxygen concentration). In addition, ratings of mood (via the Profile of Mood States) and workload (via the NASA Task Load Index) were completed. No physiological changes were noted, however, both the peppermint and Gatorade sports drink conditions lead to greater ratings of personal performance and increased mood. These results provide additional support for the implementation of non-pharmacological methods to increase an athlete's performance and mood during exercise and/or competition.

This study was funded by a grant from NASA to B.R.

Poster: Clinical Evaluation and Consumer Research

Olfactory loss following intranasal zinc gluconate

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Beneficial zinc absorption takes place via enteral, parenteral, or cutaneous routes. Direct application to the olfactory epithelium, however, has been reported to cause loss of smell. Intranasal zinc gluconate has recently been recommended as a treatment for the common cold. Severe posttreatment hyposmia and anosmia have been observed. The case report of a typical patient will be presented and analyzed in detail, followed by a series of patients with severe hyposmia or anosmia following the use of intranasal zinc gluconate in both of the two currently available commercial products. While interindividual variation in drug response and drug effect is apparent, the loss may be permanent. The mechanism of olfactory loss is thought to be the direct effect of the divalent zinc ion on the olfactory receptor cell.

Poster: Clinical Evaluation and Consumer Research

Chemosensory changes in estrogen receptor positive breast carcinoma: a case report

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Abstract: despite reduction in appetite in patients with cancer, physicians rarely assess chemosensory function. A patient with estrogen receptor positive (ER+) breast carcinoma presents with chemosensory complaints. Case presentation: a 55-year-old female, presented with 7 months of taste distortion. She initially noted a phantageusia of 'Windex', which changed to a soapy, metallic sensation, localized to the posterior tongue and upper lip. Drinking water improved the phantageusia, and consuming sweets, milk, vinegar or acidic foods worsened it. Taste threshold testing revealed hypogeusia to bitter, salt and sour. Six weeks after evaluation, she underwent modified radical mastectomy for ER (+) breast carcinoma. Immediately thereafter, the phantageusia resolved and her perception of sweet, salty and sour taste intensified. Repeat chemosensory testing 1 month later on no medications, prior to any chemotherapy, demonstrated marked improvement for all taste modalities except salt, which remained unchanged. Discussion: possible origins for gustatory deficits include remote effects of carcinoma, counter-stimulation from circulating tumor-induced hematogenous tastes, tumor released hematogenous synergistic tastes, taste bud regeneration inhibition, zinc deficiency, elevated calcium or lactate levels, dry mucous membranes, and lack of interest and motivation. Chemosensory evaluation is warranted in patients who present with ER (+) breast carcinoma.

Poster: Clinical Evaluation and Consumer Research

Odorants at the World Trade Center disaster site: analysis and psychological impact

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Odors present during a traumatic event may become associated with internal and external aspects of the experience, and, in turn, can trigger an emotional or stress response when encountered at a later time. Knowledge of the odor-causing molecules present at disaster sites is central to developing a synthetic odor-mimic, which can be used to prospectively educate rescue workers and to desensitize those who have already developed odor-stress associations. The distinct and pervasive odors lingering in the vicinity of lower Manhattan for weeks following 9-11 were prime candidates for eliciting odor-mediated 'flashbacks' among worker and residents. To identify and describe the quality of the odorants contributing to this unique, but complex, smell, four of the authors rated the sensory attributes and collected air samples using Tedlar bags and SPME 'field units' at two sites surrounding the collapsed World Trade Center towers. Using GC-olfactometry, the individuals who experienced the odors at the site evaluated the odorants as they emerged from the GC, being particularly careful to identify

odorants that could be linked to the characteristic odor of the site. Many of the characteristic odorants were linked to specific compounds (e.g. 'smokey' from guaiacol; 'sour/musty' from C7–C9 acids) or small groups of compounds ('musty/burnt' and irritating/vinegar-like from a combination of butyrolactone/benzocyanide/triethylenediamine) which are commercially available and may be used to reconstitute the characteristic odor.

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Poster: Clinical Evaluation and Consumer Research

Odor conditioning and the stress response

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The present study evaluated the degree to which an emotional state (i.e. stress or relaxation) initially experienced in the presence of a novel odor could later be elicited by the odor alone. Evaluations of both subjective (self-reported stress, health symptoms and mood) and objective (salivary cortisol) indices of stress demonstrated the efficacy of the Trier Social Stress Test (TSST) as a stress manipulation. Further, salivary cortisol levels revealed that subjects who were administered the TSST in the presence of a novel odor were more stressed upon re-exposure to that odor than they were upon re-exposure to a different odor that had been paired with relaxation instructions. This apparent ability to induce stress below the level of awareness and its relevance to odor-conditioned stress responses as a potential mechanism underlying persistent aftereffects of trauma and odor-associated syndromes, such as multiple chemical sensitivity, will be discussed.

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Poster: Clinical Evaluation and Consumer Research

The impact of malingering on three measures of olfaction

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Experts on the sense of smell are occasionally asked to evaluate olfaction in forensic cases. Since it has been estimated that the faking of a physical injury, sensory or cognitive loss (known as malingering) occurs in as many as one out of six of these cases, it is valuable to have a measure of olfaction that is resistant to malingering. The Sniff Magnitude Test (SMT) is a newly developed measure of olfactory function that uses sniffing responses to assess the sense of smell. The SMT may be resistant to malingering because it has low difficulty and at the same time it is not obvious to a typical patient how an olfactory loss is feigned. A total of 120 subjects were randomly assigned into four groups: a control group, which received the standard administration of each olfactory test; Malingering Group 1, which received instruction to fake an olfactory loss but was not provided further instructions; Malingering Group 2, which was told to fake a loss and was given informa-

tion about what each test measures; and Malingering Group 3, which was told to fake a loss and was coached on how to appear anosmic on each of the three tests. The SMT was compared with two traditional tests- PEA odor threshold and performance on the UPSIT. Results are discussed in terms of the impact of test information on feigning an olfactory loss and the resistance of each test to malingering.

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Poster: Clinical Evaluation and Consumer Research

Unilateral olfactory thresholds in a chemosensory clinical population

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Although unilateral (UNI) testing of olfactory threshold sensitivity is routinely performed in many chemosensory clinics to supplement bilateral (BI) tests, few studies have addressed the usefulness of these additional measures in either characterizing individual patients or providing insight into general characteristics of olfactory dysfunction. We report findings from 195 non-anosmic patients presenting to the Monell-Jefferson Taste & Smell Clinic who underwent both BI and UNI tests of thresholds for phenylethyl alcohol (PEA); BI and UNI thresholds for pyridine (PYR) were also obtained from a subset ($n = 73$). Some 37.4% of patients showed a left (L)–right (R) difference of at least one log step in PEA threshold concentration (v/v), but only 54.8% of those also showed a directionally consistent L–R difference in PYR thresholds. The presence of a UNI difference was not related to either degree of bilateral dysfunction or etiology; however, UNI testing did enable the identification of an olfactory problem in 15 patients whose BI testing yielded no evidence of abnormality. In contrast to what has been reported in largely non-clinical populations, patients' best UNI thresholds for PEA were significantly poorer than their BI thresholds. Interestingly, however, this effect interacted with the presence of a L–R difference, being evident only in patients with comparable L–R thresholds. Thus bilateral facilitation of olfactory threshold sensitivity may occur in dysfunction when the two nostrils are similarly affected.

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Poster: Clinical Evaluation and Consumer Research

Clinical evaluation of the sniff magnitude test

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The Sniff Magnitude Test (SMT) is a newly developed olfactory measure that examines sniff patterns to assess smell function. Its

utility has been assessed in children, university students, and older adults where it has proven to be a reliable measure that is strongly correlated with the UPSIT and odor threshold tests. This project examined the use of the SMT in a clinical setting. Forty-four otolaryngology patients completed the UPSIT and SMT during a visit to an ENT clinic in Cincinnati, OH. Patients who came to the clinic for olfactory complaints were compared with patients that came in for sinus-related problems and patients with hearing-related complaints. It was predicted that the olfactory complaint group would perform more poorly than the sinus and hearing groups on both the UPSIT and SMT. Patients with complaints about the sense of smell performed significantly worse on both the UPSIT and SMT compared with the sinus patients [$F(1,30) = 9.89, P < 0.005$ and $F(1,33) = 9.90, P < 0.005$, respectively]. This was also the case for the participants who had hearing complaints [$F(1,26) = 25.083, P < 0.001$ and $F(1,29) = 12.36, P < 0.001$, respectively]. There was no difference between the sinus and hearing patients on the two olfactory tests. These results demonstrate the utility of the SMT in a clinical setting and support additional studies of its use in other clinical conditions where olfactory deficits are a concern.

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Poster: Clinical Evaluation and Consumer Research

The influence of odor pleasantness and irritation on the Sniff Magnitude Test

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The Sniff Magnitude Test (SMT) measures olfactory function based on the reduction of the size of a sniff in response to an odor. In the past, malodors have been successfully utilized to trigger sniff reduction. Experiments were conducted to assess the use of pleasant odors and to address concerns about possible irritation associated with the odorants being used with the SMT. In total, 119 university students were tested in three experiments. The stimuli included one malodor (methylthiobutyrate or MTB) and two pleasant odors, strawberry flavor (STR) and isoamyl acetate (AA). In Experiment 1, STR (1%v/v) suppressed sniffs by 37%. STR was then compared with MTB (3% v/v) and the results indicated that MTB suppressed sniffs by 46%, similar to but significantly more than STR ($P < 0.05$). In Experiment 2, assessment of sniff magnitude to MTB (1% v/v) and AA (1% v/v) yielded results similar to the STR. While sniff suppression to AA was effective (44%), participants suppress more to the MTB stimulus (51%, $P < 0.05$). Experiment 3 assessed irritation and possible trigeminal sensitivity to MTB and AA. This was achieved using a nasal lateralization technique. It was demonstrated that neither MTB nor AA possess trigeminal components at the concentrations tested. The results from these studies support the use of both pleasant and unpleasant odors in the SMT and also allay fears about the potentially confounding role that trigeminal stimulation plays in the test.

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Slide: Clinical Evaluation and Consumer Research

Influences of age and sex on a microencapsulated odor memory test

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While influences of such variables as age and sex are well established for most standardized tests of odor identification and detection, this is not the case for tests of odor memory. In this study, 231 non-smoking men and women, ranging in age from 10 to 68 years, were administered a standardized 12-item delayed match-to-sample micro-encapsulated odor memory test (OMT). Anosmics were excluded from the study. Each participant was asked to smell a target odor after its release from a micro-encapsulated odorant pad and then, after a delay interval of 10, 30 or 60 s, to pick the target from a similarly presented set of four odors, three of which were foils. Backward counting by threes was required during the delay intervals in an effort to minimize semantic rehearsal. Overall OMT scores were higher for women than for men, and decreased, in each sex, as a function of age in a manner similar to the age-related decline observed in tests of odor identification and detection. Performance did not change as a function of delay interval. A significant correlation between the overall OMT test scores and scores on the University of Pennsylvania Smell Identification Test were observed for women, but not for men, in accord with the notion that women may be more likely to employ semantic cues in their strategies to remember odors. The findings are discussed in light of the complexities of the construct of odor memory.

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Poster: Clinical Evaluation and Consumer Research

Use, handling and assessment of perfumes depending on brand name and packing

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The experiment investigates how the image of a perfume influences its assessment and handling.

Three perfumes with different images, designed for different age groups, were tested. In a pilot study 130 subjects rated the general image of 10 different well known perfumes (e.g. age and style of users, wish to buy it). In the main experiment Chanel 5, Naomagic, Kölnisch Wasser were used. Each perfume was tested in its own bottle and packing and in the bottles of the other two. As a result we had nine experimental conditions. A total of 225 women assessed the perfumes in three conditions in random order on bipolar rating scales while they were videotaped. The handling of the packing and

the bottle were analysed (e.g. for duration of touching). Four ways of smelling the perfume were categorized for behavioural analysis: applying on a paper strip, applying on skin, smelling the bottle and smelling the cap. Trials using Kölnisch Wasser were the shortest. The odor of the perfumes (Naomagic, Kölnisch Wasser and Chanel 5) in the bottle of Naomagic were fanned for much longer. The handling of the packing of Naomagic was longer than of Kölnisch Wasser. Kölnisch Wasser was rated as moderate pleasant. It was significantly rated as more pleasant in the packing of Naomagic and Chanel 5 than in its own package.

Slide: Odorant Receptors & Transduction

Organization of chemosensory signaling components in lipid rafts

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Glycosyl phosphatidylinositol (GPI) anchored proteins are tethered to the cell surface and function as receptors that generally activate tyrosine kinases to initiate signal transduction pathways in axon guidance or T-cell activation, among other functions. These proteins are characteristic of lipid rafts, lipid domains that are enriched in glycosphingolipids and cholesterol and signal transduction proteins. At least one *Paramecium* receptor for folate is GPI anchored and likely to be in the lipid rafts. Another component of the signal transduction pathway, the plasma membrane calcium pump (PMCA), is enriched in lipid rafts in other systems, and shows the hallmarks of lipid raft association in *Paramecium*. Fluorescence microscopy shows that the GPI anchored proteins, including the folate receptor, co-localize with PMCA and basal bodies, consistent with the localization of these proteins at the bases of cilia. We used cholera toxin to localize the gangliosides in rafts. In a separate approach to lipid rafts, we used gradients to separate membrane fractions by density, with lipid rafts and their proteins characteristically populating the lighter fractions. Raft fractions are those with gangliosides, cholesterol and GPI anchored proteins. There are light density fractions of the *Paramecium* membranes that include G-proteins, the folate receptor, other GPI anchored proteins and the PMCA. In a third approach, we removed cholesterol from the whole cell membranes and noted that the cells are defective in their chemoresponse to folate. The varied approaches help us to examine the role of lipid rafts and other Triton X-100 insoluble components, such as the cytoskeleton, in chemoresponse.

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Slide: Odorant Receptors & Transduction

DNA-based fluorescent chemosensors for direct detection of volatile compounds in an artificial nose

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As reported previously (White *et al.*, 2002, AChemS XXIV), we are developing a portable artificial olfactory system to detect and iden-

tify volatile compounds in the ambient environment. In a manner based directly on animal behavior, the device actively samples air via pulsatile sniffing. Air samples are drawn over an array of broadly responsive, optically based chemical sensors, which are used to detect and discriminate odorants in the sample. Device function thus depends directly on the sensitivity and diversity of the sensors in the array. Biopolymers, particularly nucleic acids, are attractive for building sensors of great variety and wide utility. This is because of the tremendous combinatorial complexity made possible by constructing them from sequences of just a few building blocks. We have found that dye-labeled DNA sensors, dried onto a substrate, differentially respond to volatile chemical compounds. To our knowledge, this is the first time that solid phase DNA-based sensors have been used for directly detecting small, vapor phase molecules. Furthermore, our experiments indicate that Cy3-labeled, single-strand DNA sequences of similar length but different base sequence can respond differently to the same odorant set. These observations suggests that 'sensor discovery' via high throughput screening of large-scale DNA-based sensor libraries may be possible, thereby providing a rapid means to easily identify sensors that are optimized for defined odorant detection tasks.

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Slide: Odorant Receptors & Transduction

Making sense of olfaction through molecular modeling

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We used the MembStruk first principles computational technique to predict the three-dimensional (3-D) structure of 10 mouse olfactory receptors (S6, S18, S19, S25, S46, S50, S1, MOR-EV, MOR-EG and MI7) for which experimental odorant recognition profiles are available. We used the HierDock method to scan each predicted OR structure for potential odorant binding site(s), and to calculate binding energies of each odorant in these binding sites. The calculated binding affinity profiles correctly identify the chemical classes recognized experimentally by each OR, validating the predicted 3-D structures and binding sites. Correlation between calculated binding affinity and elicited response is also found within each chemical class. However, the cutoff response/no-response is not always well defined. For each of the 10 ORs, the binding site is located between TMs 3–6, with contributions from EC loops 2 and 3. In particular, we find six residue positions in TM3 and TM6 to be consistently involved in odorant binding. These positions are consistent with mutation data on ligand binding for other GPCRs and sequence hypervariability studies for ORs. Amino acid patterns associated with the recognition of chemical classes were defined using the predicted binding modes. These sequence fingerprints were used to probe the alignment of 869 OR sequences from the mouse genome in order to identify other ORs matching each fingerprint.

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Slide: Odorant Receptors & Transduction

The multifaceted receptors of the human nose

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Human olfactory receptor (OR) genes are highly multifaceted, as manifested at several different levels. This is documented in the updated Human Olfactory Receptor Data Exploratorium (HORDE version 40, <http://bip.weizmann.ac.il/HORDE/>), which contains 853 entries. One dimension of diversity spans a continuum between intact ORs and definite OR pseudogenes. To probe the undecided cases, we have devised methods to identify subtle deviations from shared motifs. Positional departures of initiation and stop codons may indicate inactive ORs. Second generation rhodopsin-based homology models provide new clues to sequence positions essential for functionally intact structure, including helix-kinking residues. A new algorithm, based on paralog-ortholog comparisons for three mammalian species (human, mouse and dog), allowed us to identify potential odorant contact residues. These harbor maximal intra-species variability, but also constitute additional targets for inactivating mutations. Another dimension of diversity is inter-individual variability, generated by single nucleotide polymorphisms that occur at pseudogenizing sequence positions. This results in a personal genetic 'bar-code', whereby every human individual has a different combination of intact and inactive ORs. These 'segregating pseudogenes' are likely key determinants of specific anosmia. Finally, OR gene diversity is probed at the 5' untranslated exon sequences by computer-based and experimental approaches. This may shed light on presumed roles of upstream segments in OR messenger RNA stability and translation.

Slide: Odorant Receptors & Transduction

Molecules that regulate translocation and function of mammalian odorant receptors

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Progress in understanding olfactory coding requires knowing ligand specificity of odorant receptors (ORs). It has been difficult to examine ligand-binding specificity of mammalian ORs in heterologous cells, mainly due to poor plasma membrane trafficking of ORs in these cells. Using a set of cDNA clones that are expressed by olfactory neurons, we have screened and found three genes that induce cell surface expression of ORs when expressed in HEK293T cells. We named them REEP1, for Receptor Expression Enhancing Protein 1, and RTP1 and 2, for Receptor Transporting Protein 1 and 2, respectively. All three proteins contain a single putative transmembrane domain and they are specifically expressed by the olfactory neurons in the olfactory epithelium. These proteins are associated with OR proteins, and enhance activation of OR by odorants. Using a cell line expressing these genes, we have identified new mouse ORs responding to aliphatic odorants. Our results suggest that REEP1, RTP1 and RTP2 have important roles in trafficking of ORs and provide an efficient system to investigate OR-odorant interaction.

Slide: Odorant Receptors & Transduction

The role of odor receptors in odor coding

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We are undertaking a large-scale analysis of *Drosophila* odor receptors. The *Or* gene family contains ~60 members, which are expressed in at least 35 functional classes of olfactory receptor neurons (ORNs). We are examining the functions of odor receptors using an 'empty' *ab3A* antennal ORN ($\Delta ab3A$) that lacks its endogenous odor receptors as an *in vivo* expression system. Individual receptors are introduced into the empty ORN ($\Delta ab3A:OrX$) and odor response is assayed electrophysiologically. We are establishing a receptor-to-neuron map by matching the odor response spectra of $\Delta ab3A:OrX$ ORNs to the odor response spectra of wild-type ORNs. We have found that the odor receptor dictates not only the odor response spectrum, but also the signaling mode (excitation versus inhibition) and the response dynamics of the ORN in which it is expressed. Different receptors, when expressed in the same ORN and given the same odorant stimulus, can confer responses that differ in signaling mode. Moreover, an individual receptor can confer responses of different modes to different odorants in the same ORN. The results thus show that odor receptors contribute to multiple aspects of odor coding in *Drosophila* ORNs, and they suggest a model for odor receptor transduction. Not only can multiple receptors function in an individual ORN, but an individual receptor can function in multiple ORNs, suggesting a broad compatibility among receptors and ORNs. Finally, we express two odor receptors from the malaria vector mosquito *Anopheles gambiae* in *Drosophila* and find that the female-specific receptor AgOr1 responds to 4-methylphenol, a component of human sweat.

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Slide: Odorant Receptors & Transduction

The hOR17-4 signaling system—one receptor, dual capacity

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A repertoire of mammalian olfactory receptor (OR) genes are predominantly expressed in spermatozoa. One of these receptors, named hOR17-4, is attributed an important role in human sperm physiology by mediating directed chemotactic movement *in vitro*. The underlying molecular mechanisms transducing OR ligand binding into flagellar motion remain obscure. Here, we show that sperm OR activation is coupled to a membrane-bound adenylyl cyclase (mAC). Odorant-induced Ca²⁺ signaling, chemotaxis, and chemokinesis are completely abolished by a specific inhibitor of mACs. Identifying membrane proteins in human sperm via mass spectrometry, we show expression of specific receptors, G-proteins and mACs. Their spatial distribution patterns largely correspond to the spatiotemporal character of odorant Ca²⁺ signals. Thus, our data link mAC activation to human sperm chemotaxis. Whether sperm ORs are functionally restricted to

reproductive issues or additionally perform their 'conventional' task in olfaction is a longstanding question. Therefore, we also investigate hOR17-4 expression in human olfactory tissue adopting. Our results suggest that the same human OR protein is similarly utilized to fulfill chemosensory functions in such diverse cell types as spermatozoa and olfactory sensory neurons.

Slide: Odorant Receptors & Transduction

Mechanisms of odor receptor gene choice

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Little is known about how individual neurons select which *Or* genes to express. The *Or* gene family in *Drosophila* consists of ~60 members, most of which are expressed either in the antenna or the maxillary palp, but not both. We have used bioinformatics to identify *cis*-regulatory elements important for *Or* gene choice in the maxillary palp. Among several motifs that are over-represented upstream of *Or* genes, one, CTA(N)₉TAA, is found within 500 bp upstream of *Or* genes expressed in the maxillary palp. We have found by mutational analysis that this motif is necessary for expression of *Or* genes in this organ. A second over-represented motif among maxillary palp genes is CTTATAA, and we have found evidence that this motif is a negative regulatory element that mediates repression of palp *Or* genes in the antenna. What information specifies the particular neuron class in which a receptor is expressed? A pair of *Or* genes, *Or85e* and *Or33c*, is co-expressed in the pb2A neurons of the palp. We have identified three motifs shared between the upstream sequences of these two co-regulated genes, but not by other palp genes. To identify neuron-specific regulatory motifs for the remaining palp *Or* genes, we have compared the *Or* upstream regions with those of their *D. pseudoobscura* orthologs. This approach has identified several additional gene-specific conserved elements. A second mechanism is used to achieve co-regulation of two genes in another neuron, pb2B. Two clustered *Or* genes, *Or46a* and *Or46b*, are transcribed as a bicistronic message in this neuron. We are testing the activity of a putative IRES, and the functional significance of the co-expression.

Slide: Odorant Receptors & Transduction

Regulation of odorant receptor expression

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The expression of a single odorant receptor (OR) in each olfactory neuron from a repertoire of >1000 genes is essential for odor coding and axonal targeting. The genes encoding these receptors each possess a simple genomic structure and in several cases, small DNA segments surrounding the transcription initiation sites are sufficient to direct expression of reporters in a pattern that mimics the endogenous genes. One remarkable aspect of this gene regulation is that each olfactory neuron expresses OR protein from only one allele of this large, dispersed gene family. We have used targeted transgen-

esis, insertion of defined DNA constructs at specific location in the genome to identify a new role for OR protein as an essential regulator in the establishment of mono-allelic OR expression. OR-promoter driven reporters expresses in a receptor-like pattern, but unlike a native OR, are co-expressed with an additional OR allele. Expression of a functional OR from the identical promoter eliminates expression of other OR alleles. The presence of an untranslatable OR coding sequence in the mRNA is insufficient to exclude expression of a second OR. Together, these data identify the OR protein as a critical element in a feedback pathway that regulates odorant receptor selection. Current efforts in the laboratory are focused on elucidating the nature of the feedback signal and the mechanisms that lead to selective receptor expression.

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Symposium: The Ins and Outs of Sensory Cilia

What the *Chlamydomonas* flagellum is teaching us about sensory cilia

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Both motile cilia and non-motile cilia, including mammalian primary cilia, are sensory organelles that display receptors and relay signals about the extracellular environment to the cell body. The unicellular, biflagellate green alga *Chlamydomonas reinhardtii* has provided an essential foundation for understanding this important ciliary function. A notable example is the recent discovery and characterization of intraflagellar transport (IFT) in *Chlamydomonas*. IFT is a process in which flagellar precursors are actively moved into the flagellum and out to its tip, where axonemal assembly occurs; disruption of this process blocks flagellar assembly. Genetic and biochemical studies in *Chlamydomonas* have elucidated the molecular motors and other components of the IFT system; all of these proteins have homologues in other ciliated organisms, including *Caenorhabditis elegans*, *Drosophila melanogaster* and mammals. Because IFT is necessary for ciliary assembly, mutation of a gene encoding a mouse homologue of a *Chlamydomonas* IFT protein disrupts assembly of primary cilia, providing a valuable tool for testing the function of these widespread organelles (see G. Pazour, this meeting). Although primary cilia cannot be isolated, *Chlamydomonas* flagella can be readily purified in amounts sufficient for biochemical analysis. An ongoing proteomic analysis of the *Chlamydomonas* flagellum is revealing numerous conserved proteins that are likely to be involved in sensory processes. The mammalian homologues of these proteins are prime candidates for carrying out sensory reception and signal transduction in the primary cilium.

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Symposium: The Ins and Outs of Sensory Cilia

Probing the function of mammalian primary cilia by analysis of the Tg737 mouse

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Most mammalian cells have a non-motile primary cilium projecting from their surface. The importance of these organelles to mammalian health and development has been highlighted by recent analysis of the Tg737 mouse. This mouse has a mutation in the gene encoding the IFT88 subunit of the intraflagellar transport particle (Pazour *et al.*, *J. Cell Biol.* 151: 709–718) and develops polycystic kidney disease (PKD) (Moyer *et al.*, *Science* 262: 1329–1333) and other disorders. The Tg737 mutation causes ciliary assembly defects in the kidney and other organs. These ciliary defects are likely to be the primary cause underlying the PKD and other diseases seen in the animal. We hypothesize that primary cilia are serving as organizing centers for sensory receptors and signaling proteins. In support of this, we and others have shown that the polycystins are localized on kidney primary cilia. The polycystins are membrane proteins thought to sense the state of the kidney epithelium and control proliferation and differentiation of these cells. Polycystin mutations cause excess cell proliferation in kidney nephrons resulting in adult onset PKD in humans. The ciliary assembly defect probably causes PKD by disrupting the localization of the polycystins. It is likely that all primary cilia serve similar functions in organizing receptors and pathways to monitor extracellular parameters that are important to the cell's physiology.

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Symposium: The Ins and Outs of Sensory Cilia

Intraflagellar transport motors

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The assembly and function of sensory cilia depends upon intraflagellar transport (IFT), the bidirectional transport of macromolecular complexes called IFT-particles, along axonemal microtubules (MTs) between the base of the cilium and the distal tip. We are studying the role of IFT in the formation and function of the sensory cilia on the endings of chemosensory neurons within the nematode, *Caenorhabditis elegans*. These cilia serve as specialized compartments for concentrating the sensory signaling machinery that detects chemical cues in the environment and thereby play important roles in chemosensory behaviour. Our hypothesis is that IFT-particles contribute to ciliary function by carrying key components of the ciliary axoneme, the signaling machinery, and possibly signals themselves, between the base and the tip of the cilium, and that the transport of these particles depends upon the action of two anterograde motors, kinesin-II and OSM-3-kinesin and a retrograde motor, IFT-dynein. We will report our recent progress in using light microscopy, biochemistry, genomics and genetics to dissect the protein machinery that drives IFT in this system.

Poster: Olfactory Development

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

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Peripheral olfactory systems of insects such as moths, bees, mosquitoes and flies 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 and gene expression activities in the imaginal disc and early developing antenna to identify events which lead to the patterning of the adult antenna and the specific expression of genes (ORs, OBPs) by distinct classes of olfactory sensilla. This study focuses on the antennal disc; disc growth occurs during the final larval instar from a ring of tissue surrounding the base of the larval antenna. We have characterized the spatial patterns of disc growth using an antibody against phosphorylated histone H3 (mitotic marker) and have characterized temporal and spatial specific expression of transcription factors Broad (metamorphosis) and distal-less (pattern) and the signaling protein Notch. Prior to pupation, the disc elongates and everts; we have shown this process is regulated by ecdysteroid hormones, and have observed a subsequent decline in total DNA content suggesting apoptotic events associated with this restructuring. These studies are establishing a foundation for identifying early events leading to the selection of specific chemosensory phenotypes of adult olfactory sensilla.

Poster: Olfactory Development

Ig-family cell adhesion molecules in the developing antennal lobe of the moth *Manduca sexta*

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Establishment of the adult antennal (olfactory) lobe of *Manduca sexta* requires numerous choices regarding pathfinding, migration, fasciculation, and branching by olfactory receptor cells, glia, and antennal lobe neurons. We are investigating the roles played by cell adhesion molecules in this process. Using an antibody to *Manduca* neuroglian, a homolog of vertebrate L1, we find that receptor cell axons express neuroglian specifically in the axonal sorting zone and in the nerve layer surrounding the glomerular layer in the lobe; expression is notably absent in the glomerular neuropil. Neuropil-associated glia also label, and do so even in the absence of afferent axons. This suggests the possibility of a homophilic interaction between neuroglian molecules on receptor cell axons and antennal-lobe glia. Following sorting of axons into new fascicles, we find that only some fascicles are labeled, suggesting targeting-related specificity, as has been seen for fasciclin II. In a search for additional cell adhesion molecules, we have used an antibody against the Ig-domain of human NCAM and obtained strong labeling of antennal lobe neurons and glia, but not axons. Western blots using this antibody demonstrate a prominent band at 125 kDa, similar in size to vertebrate NCAM-120 and OCAM and distinguishing this protein from the other Ig-containing molecules known to occur in *Manduca*, fasciclin II and neuroglian.

Supported by NIH DC2004598.

Poster: Olfactory Development**Olfactory receptor cells of transsexually grafted female antennae determine odor responses of output neurons in the antennal lobe of male *Manduca sexta***

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The antennal lobe (AL) of the moth *Manduca sexta* contains a small number of sexually dimorphic glomeruli: the macroglomerular complex (MGC) in males and the large female glomeruli (LFGs) in females. The role of olfactory receptor cells (ORCs) in the formation of these glomeruli was demonstrated by transsexual transplantation experiments: sensory axons of a grafted male antenna induce formation of an MGC in a host female, while sensory axons of a grafted female antenna induce formation of LFGs in a host male. The odor-response properties of the induced glomeruli, however, are unknown. Using intracellular recording and staining techniques, we studied the properties of output neurons (PNs) in males with a grafted female antenna. To produce these gynandromorphs, one antennal disk from a second-day fifth-instar larva was excised and replaced by the corresponding disk from a female donor. Twenty-two percent of animals had a normal female antenna ($n = 7$) and an antennal nerve innervating the AL. We found that PNs arborizing in the induced LFGs (like latLFG-PNs in normal females) were more sensitive to (+) than to (-)linalool ($n = 3$, 2 animals). Although not morphologically identified, we found three more presumptive PNs with the same odor specificity. The morphology of PNs innervating glomeruli outside the induced LFGs was similar to that of PNs in normal females ($n = 4$). One of these PNs arborizes in a glomerulus next to the induced latLFG, and did not respond to antennal stimulation with either (+) or (-)linalool. These preliminary results are consistent with the hypothesis that ORCs confer specific odor-tuning characteristics to their glomerular targets.

Supported by NIH/PEW.

Poster: Olfactory Development**NO-mediated signaling from olfactory receptors to peripheral nerve glia in the moth olfactory pathway**L.A. Oland¹, N.J. Gibson² and L.P. Tolbert²¹A.R.L. Division of Neurobiology, University of Arizona, Tucson, AZ, USA and ²Neurobiology, University of Arizona, Tucson, AZ, USA

In the developing olfactory (antennal) nerve of the moth *Manduca sexta*, the glia that populate the nerve arise in the antenna and migrate along the nerve. They reach the CNS/PNS interface region of the nerve several stages after the first axons have arrived and begin to ensheath bundles of axons in the nerve. In recent *in vitro* studies, antennal nerve (AN) glia were found to advance readily along glial processes, and ORN axons were found to induce the AN glia to form multicellular arrays (Tucker and Tolbert, 2003). Array formation did not require direct ORN-AN glia contact, but occurred only in glia in close proximity to the axons, suggesting that the axons release a short-range diffusible signal. Evidence from previous studies of nitric oxide (NO) in developing *Manduca* indicated that

a Ca^{2+} -dependent NO synthase is strongly expressed in the antenna (Nighorn *et al.*, 1998), that the ORN axons express NO synthase during stages of axon ingrowth when the AN glia are populating the nerve (Gibson and Nighorn, 2000), and that NO regulates AN glial migration (Gibson *et al.*, 2001). Current experiments test whether the formation of arrays by AN glia *in vitro* is a consequence of NO release by ORN axons. In co-cultures of ORN neurons and PN glial cells, glial arrays can be reduced by a NOS inhibitor. In cultures of AN glia only, NO donors can induce glial chaining. Our initial results suggest that NO signaling from the ORN axons may be involved in the normal developmental processes of AN glia elongation and migration.

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Poster: Olfactory Development**Expression profiles of genes regulated by thyroid hormone in the nose of *Xenopus laevis***

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In *Xenopus*, metamorphosis is initiated by rising levels of endogenous thyroid hormone (T_3). During metamorphosis from tadpole to frog, essentially every organ/tissue is remodeled and many new structures must be formed. The nose of *Xenopus* tadpoles contains two areas of sensory epithelium. At metamorphosis, one area is completely remodeled and a new, third area of sensory epithelium is formed *de novo*. Apoptosis, cell proliferation, remodeling of the extracellular matrix and cell differentiation all take place during this transformation. Previously by our laboratory, >125 gene fragments associated with metamorphosis in the frog nose were isolated and identified using representational difference analysis, including gene fragments known to be associated with all of the above cellular processes. In this study, 56 of those genes were subjected to real time quantitative RT-PCR at five different stages of development throughout metamorphosis. We found the expression levels of several of the T_3 early response genes, including *Gene 12*, transcription factors (*nuclear factor I-B*, *TH1bZIP*, *TRB*) and an extracellular matrix remodeling enzyme (*stromelysin 3*), were elevated 3- to 57-fold in response to 48 h of T_3 treatment, and expression remained elevated throughout metamorphic climax. The elevated levels of expression were visualized by *in situ* hybridization. Therefore, these genes, which are known to participate in remodeling other tissues undergoing metamorphosis, also aid in remodeling the nasal capsule.

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Poster: Olfactory Development***De novo* DNA methyl transferases and methyl DNA binding domain proteins in olfactory neurogenesis**

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Epigenetic Regulation (Silencing) of mammalian genomic DNA occurs when *de novo* methyltransferases (DNMTs) methylate sites in the mammalian genome to form targets for methyl-CpG binding domain proteins (MBDs). MBDs form repressor complexes that can directly repress promoters or modify chromatin structure by recruitment of histone deacetylases (HDACs). In the mouse olfactory epithelium, DNMT1, 2, 3a and 3b are expressed in the OE from embryonic development through adulthood. DNMT3a and 3b expression is maximal in developing ORNs at peaks of neurogenesis. DNMT3b is expressed in presumptive globose basal cells, sustentacular cells and cells migrating from the basal layer to the sustentacular layer in the E16.5 OE. DNMT3b is primarily expressed (with PCNA) in actively proliferating cells and may thus play a role in ORN progenitor cell cycle exit or neuronal fate choice. DNMT3b+/PCNA+ cells migrating from the basal layer are NST negative and likely represent an alternative lineage to the neuronal lineage. DNMT3a is expressed in post-mitotic immature ORNs and a subset of mature ORNs and may play a role in lineage restriction and induction of terminal ORN differentiation in non-proliferating neuro-precursors. Expression of HDAC2 is induced at the earliest stages of neuronal differentiation and is down-regulated at terminal differentiation of ORNs. A small percentage of DNMT3b positive cells begin to express HDAC2, while the majority of DNMT3a positive cells express HDAC2. This continuum of HDAC2 expression suggests that it may be appropriately expressed to mediate gene silencing responses to DNA methylation catalyzed by both DNMT3b and DNMT3a.

Poster: Olfactory Development

Mechanisms by which BDNF and Ngf act and interact with atrial natriuretic peptide type-C to promote proliferation or differentiation of olfactory neuronal precursors

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Both BDNF and NGF promote the proliferation of a subset of olfactory neuronal precursor cells. Concurrent exposure to atrial natriuretic peptide type-C (CNP) inhibits this proliferation and promotes differentiation. Immunocytochemical analysis of cell cycle regulatory proteins shows that both BDNF and NGF increase levels of cyclin D1 and cdk4, although the mechanisms behind these increases differ. NGF also increases p27. Addition of CNP with neurotrophin inhibits increases in cyclin D1 and p27 while alternatively increasing levels of a variety of cell cycle inhibitory proteins, including p21. The profile and time course of this inhibitor expression varies between BDNF and NGF. Analysis of the mechanisms behind changes in cyclin D1 and p21 shows that inhibition of the MAPK pathway blocks alterations in cyclin D1 levels in response to either neurotrophins or neurotrophins with CNP. CNP, however, does not promote Erk1/2 phosphorylation, suggesting that it may alter the specificity of MEK1/2 signaling. Other signal transduction pathways are responsible for alterations in p21. Inclusion of cycloheximide results in an increase in p21 levels in response to neurotrophins and an increase in cyclin D1 levels in response to neurotrophins with CNP. These results are mimicked by application of 26S proteasome inhibitors. This suggests that the switch between proliferation and differentiation is primarily regulated

through reciprocal degradation of inhibitory or progressive cell cycle proteins with a requirement for protein synthesis.

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Poster: Olfactory Development

Adult olfactory progenitor cells give rise to both neurons and non-neuronal cells in culture

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Recent data, including transplantation of FACS-sorted cells, indicate that the adult olfactory epithelium (OE) retains multipotent progenitors (MPPs) among the globose basal cell (GBC) population, which can be activated when reconstitution of the OE requires replacement of both neurons and non-neuronal cells. Not much is known about the regulation of progenitor cell capacity, and *in vitro* studies of multipotency are warranted. Dissociated epithelial cells were taken from mice exposed to MeBr gas (MeBr-OE) 40 h prior to use. Harvested cells were plated either on uncoated or matrigel-coated surfaces and grown in complex, serum-rich medium or defined, serum-free medium. In serum-rich medium, regardless of coating, MeBr-OE gives rise to cultures that are heterogeneous: constituent cells, in aggregate, display the phenotypic characteristics of neurons [TuJ-1(+)], sustentacular cells [cytokeratin (CK)-18(+)] and horizontal basal cells [CK-5 or 14(+)]. TuJ-1(+) cells grow in a scattered fashion, while CK-expressing, epithelioid cells cluster together tightly. In serum-free medium, MeBr-OE generates only CK-18 or CK-5/14(+) epithelioid islands. Our GBC markers GBC-2 and GBC-3 stain some, but not all, cells in the islands. Interestingly, cells grown on matrigel, regardless of serum, generate spheres that project above the surface to the dish. The ability to assay and manipulate adult MPPs *in vitro* will give us a better understanding of the mechanisms that regulate proliferation and differentiation, and may lead, eventually, to therapeutic applications.

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Slide: Olfactory Development

Studies of olfactory cell lineage and differentiation using an *in vitro* neurosphere model and time-lapse videomicroscopy

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The mammalian olfactory neuroepithelium possesses a unique progenitor cell as it avidly supports neurogenesis throughout adulthood and is capable of reconstituting cells of neuronal and non-neuronal lineages after injury. Most evidence suggests that this progenitor resides in the GBC layer and, using retroviral lineage tracing, Huard *et al.* (1998) found evidence supporting a multipotent progenitor capable of making GBCs, HBCs and sustentacular cells. To identify this cell *in vitro* we developed a culture system of olfactory progenitor cells. Turbinate tissue was taken from 48-h-old Wistar rat pups and putative progenitors enriched from a preparation

of dissociated olfactory neurons (Cunningham *et al.*, 1999). Clonal neurospheres formed from individual motile cells and made progeny of neuronal and sustentacular classes, based on immunocytochemical analysis for GBC-1, Olf-1, G_{olf}, Type 3 adenylyl cyclase and SUS-4. Double-labeling immunocytochemistry of young spheres revealed cellular heterogeneity, consistent with lineage specification occurring early in sphere development. Spheres passaged and generated secondary spheres although at lesser frequency than CNS neurospheres generated from forebrain. Our data are consistent with our having isolated *in vitro* a progenitor predicted to exist by Huard *et al.* (1998). Our system of clonal neurospheres provides a model of progenitor cell development that will allow better understanding of the mechanisms underlying olfactory neurogenesis.

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Slide: Olfactory Development

Stochastic yet biased expression of multiple Dscam splice variants by individual cells

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The *Drosophila Dscam* gene is essential for axon guidance and has 38 016 possible alternative splice forms. This extraordinary diversity can potentially be used to distinguish cells. We have analyzed the *Dscam* mRNA isoforms expressed by different cell types and individual cells. The choice of splice variants expressed is regulated both spatially and temporally. Different subtypes of photoreceptors express broad yet distinctive spectra of *Dscam* isoforms. Single cell RT-PCR documented that individual cells express at least several different *Dscam* isoforms and allowed an estimation of the diversity that is present. For example, we estimate that each R3/R4 photoreceptor cell expresses 14–50 distinct mRNAs chosen from the spectrum of thousands of splice variants distinctive of its cell type. Thus, every cell's *Dscam* repertoire is different from those of its neighbors, providing a potential mechanism for the generation of unique cell identity in the nervous system and elsewhere. Prior studies by Zipursky and colleagues indicate an important role for the *Dscam* gene in controlling the proper targeting of olfactory receptor neurons. We are currently assessing the importance of the expression of distinct alternative splice forms by different olfactory neurons in axon targeting specificity.

Poster: Olfactory Development

A putative role for MHCI in the axonal targeting of the mouse olfactory system

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The ability to smell offers a remarkable solution to the daunting challenge of discriminating between diverse chemical molecules. Investigation into the cellular and molecular mechanisms of mammalian olfaction has established the olfactory bulb as a primary processing unit that organizes the signaling from olfactory sensory

neurons into a topographical sensory map. However, the molecular mechanisms establishing this sensory map remain to be elucidated. In the current study, we investigate the role that major histocompatibility complex class I molecules (MHCI) play in organizing this sensory map. Towards this end, we have used immunohistochemistry to demonstrate the expression of MHCI molecules in the main olfactory bulb (MOB). Additionally, we have examined mice that are severely deficient in the expression of MHCI on the surfaces of cells (*TAP*^{-/-}). By crossing these mice with a strain of mice that co-expresses Tau-LacZ with the P2 olfactory receptor, we can visualize the axonal projection patterns of the P2 olfactory sensory neurons in a background deficient for the expression of MHCI molecules. After mapping the P2 glomeruli in the MOB of both wild-type and *TAP*^{-/-} mice, we have uncovered subtle but statistically significant differences in the location and number of P2 glomeruli.

Poster: Olfactory Development

Cell types expressing OMP in the olfactory epithelium of larval zebrafish

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The olfactory epithelium of zebrafish contained ciliated, microvillar and crypt-type sensory neurons. While there appears to be some overlap in specificity across the cell types the ciliated OSNs appear to respond preferentially to bile salts and the microvillar cells to amino acids. Recently, Celik *et al.* (2002, *Eur. J. Neurosci.* 15: 798) reported the expression of GFP under the zebrafish OMP promoter was found primarily in ciliate OSNs but also found in cells with shorter and stouter dendrites, presumably microvillar cells. In the current investigation we produced a genetic construct with the zebrafish OMP promoter driving expression of eYFP to quantitatively examine the distribution of eYFP in the OSN populations of larval zebrafish. The zOMP promoter was cloned from genomic DNA using primers described by Celik *et al.*, ligated into an pEYFP vector (Clontech). The vector was subsequently linearized and injected into one-cell-stage zebrafish embryos. OMP promoter driven expression of eYFP was noted by 48 h. The transiently transfected embryos were reared for 48–72 h post-fertilization, fixed and processed for whole embryo immunocytochemistry using the anti-GFP antibody. After immunostaining the embryos were embedded in Eponate plastic and sectioned for electron microscopy. Preliminary counts indicate that the majority of the eYFP expressing cells are ciliated OSNs but confirm the expression was also observed in some microvillar OSNs. Until a stable transgenic line is produced we cannot provide reliable estimates of the proportions of ciliated or microvillar OSNs expressing.

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Poster: Olfactory Development

Tyrosine hydroxylase-like immunoreactive cells in the olfactory tracts of goldfish

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Goldfish possess a substantial population of tyrosine hydroxylase (TH)-like-immunoreactive cells within the olfactory tracts (OT) as well as TH-like-ir cells in the olfactory bulbs (OB). The TH-like-ir neurons in the OB have round cell bodies and large round nuclei with a single dendritic process extending towards the margin of the OB. These neurons were identified as a subset of granule cells (Alonso *et al.*, 1989). The nature and origin of the TH-like-ir cells in the OT are unknown. The aim of the present study was to clarify whether the TH-like-ir cells are newly generated cells which migrate into the OB or whether they are more mature cells that are stationary in the OT. Goldfish was chosen as a model since they have long OTs, and ample information is available as to anatomy, physiology and behavior. BrdU-injections were used to visualize newly generated cells; and antibodies used to characterize the nature of the cells in question. The TH-like-ir cells in the OT are similar in shape to those in the OB, however, the tract cells are bipolar, extending one long process towards the OB and one towards the telencephalon. The majority of these cells occur in the medial OT, fewer in the lateral OT. One day after injection, BrdU-positive nuclei occur along the medial and lateral OTs. Six days after BrdU injection, labeled nuclei are present at the whole length of the OTs and also in the OB. Further experiments are underway to test whether the TH-positive cells of the OT are equivalent to the rostral migratory stream of rodents.

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Slide: Olfactory Development

Postnatal changes in the rat modified glomerular complex: a quantitative cytochrome oxidase study

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The modified glomerular complex (MGC) has been described as a set of glomeruli on the dorsomedial side of the rat olfactory bulb (OB), associated with suckling behavior in the neonate. We further explored the morphometric and anatomical features of MGC during postnatal development, using coronal series of cytochrome oxidase (CO)-stained sections of the OB at postnatal ages of 1, 3, 10 and 25 days and analyzed with regard to lateral and medial distribution of the glomeruli, their size and number, and the anterior-posterior (AP) length of MGC. The MGC stained intensely for CO even in the newborn animal, compared with main OB glomeruli. In addition to the originally described medial complex, a lateral complex was also noted, particularly in rat pups 3 days and older. This complex had fewer glomeruli and was shorter than the medial complex, but its glomeruli were similar in size and stain intensity. Anteriorly the lateral and medial portions appear to fuse along the midline, forming an asymmetrical horseshoe structure. Total glomeruli number in the entire complex (medial and lateral) increased postnatally, from ~15 at birth to ~20 at 3 days, 40 at 10 days and ~70 at day 25. During the same period, mean glomerular diameter increased from ~60 μ m at birth to ~85 μ m in the weanling. These changes were accompanied by marked increases in total glomerular volume. Results indicate that MGC is well developed at birth, but continues to develop postnatally in terms of number and size of its

glomeruli, indicating possible olfactory functions of this complex in the weanling and older animals.

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Poster: Odor Activation and Modulation

OMP is a modulator of Ca²⁺ clearance processes in mouse olfactory receptor neurons

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Ca²⁺ entry into olfactory receptor neurons (ORNs) is a well-studied event in chemosensory transduction. However, restoration of intracellular Ca²⁺ levels to the pre-stimulus level is less well understood. Recent behavioral and electrophysiological analyses of OMP-null mice led us to hypothesize that OMP participates in the recovery phase of olfactory signal-transduction. To investigate this we compared Ca²⁺ transients in ORNs of wild-type and OMP-null mice by activating various pathways to increase the cytoplasmic [Ca²⁺]. KCl, 3-isobutyl-1-methylxanthine (IBMX) and caffeine were used to induce Ca²⁺ influx from voltage-gated Ca²⁺ channels, cyclic-nucleotide gated channels and intracellular Ca²⁺ stores, respectively. Confocal laser images were recorded from dendritic knobs of mouse ORNs loaded with the Ca²⁺-indicator dye fluo-4 AM. In OMP-nulls in response to IBMX, the rate of Ca²⁺ entry to reach peak was 5-fold slower, and to achieve half-recovery to basal level was 4-fold slower compared with controls. Following KCl depolarization, the OMP-null mice showed a 2-fold delay in the half-recovery time of the Ca²⁺ peak. Similar results were obtained following Ca²⁺ store depletion by stimulating ryanodine receptors with caffeine. These results suggest that the cytoplasmic Ca²⁺ clearance of ORNs is significantly impaired in the OMP-null mice. These data imply that OMP is a modulator of Ca²⁺ extrusion or sequestration processes in ORNs. Characterization of the mechanism by which OMP modulates Ca²⁺ flux in ORNs is under investigation.

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Poster: Odor Activation and Modulation

OMP: a cautionary tale of a gene within a gene

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Expression of the olfactory marker protein gene (*Omp*) is very highly restricted to mature olfactory receptor neurons and vomeronasal receptor neurons in vertebrates. A related cDNA has been cloned from the snail *Eobania vermiculata* (Mazzatenta *et al.*, 2002), suggesting that OMP is of broad phylogenetic distribution, distant evolutionary origin and functional significance. The detailed characterization of the OMP gene and protein has made it a desirable locus for genetic manipulation in the olfactory system. The *Omp* locus, including the intronless coding region for OMP as well as all its regulatory elements, spans ~12 kb in the rodent genome. To

our surprise, our recent *in silico* analysis of this genetic region has revealed that the mouse *Omp* locus is entirely contained within an ~16 kb intron of the gene encoding the Calpain 5 (*Capn5*) protease. The *Omp* and *Capn5* genes are similarly organized in the human, rat and *Fugu* genomes. These analyses beg a critical question: do the observed phenotypic alterations that result from genetic manipulations of the *Omp* locus reflect (i) a direct and accurate result of these manipulations or (ii) an alteration in the splicing and/or expression pattern of *Capn5*? We will present data that address these concerns.

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Poster: Odor Activation and Modulation

Response profiles and narrowing selectivity of olfactory receptor neurons of *Xenopus laevis* tadpoles

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In olfactory receptor neurons (ORNs) of aquatic animals amino acids have been shown to be potent stimuli. Here we report on calcium imaging experiments in slices of the olfactory mucosa of *Xenopus laevis* tadpoles. We were able to determine the response profiles of 283 ORNs to 19 amino acids, where one profile comprises the responses of one ORN to 19 amino acids. Of the 283 response profiles, 204 differed from each other. Thirty-six response spectra occurred more than once, i.e. there were 36 classes of ORNs identically responding to the 19 amino acids. The number of ORNs that formed a class ranged from 2 to 13. Shape and duration of amino acid-elicited $[Ca^{2+}]_i$ transients showed a high degree of similarity upon repeated stimulation with the same amino acid. Different amino acids, however, in some cases led to clearly distinguishable calcium responses in individual ORNs. Furthermore, ORNs clearly appeared to gain selectivity over time, i.e. ORNs of later developmental stages responded to less amino acids than ORNs of earlier stages.

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Poster: Odor Activation and Modulation

Responses of olfactory receptor neurons lacking spontaneous activity to amino acid stimuli in black bullhead catfish (*Ameiurus melas*)

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The cilia and microvilli of olfactory receptor neurons (ORNs) of freshwater fish are directly exposed to water containing few ions. We showed that non-spontaneously active ORNs respond to amino acid stimuli even when exposed for several hours to highly purified water ($R > 18.2 M\Omega cm$). Using extracellular platinum black electrodes, we recorded concentration dependent responses to 10 amino acid stimuli: L-nVal, L-Met, L-Ala, L-Leu, L-Ser, L-Lys, L-Val, L-Arg, L-Ile and L-Pro at 10^{-4} M. Five non-spontaneously active ORNs responded to L-nVal (lowest threshold, 10^{-7} M) and L-Met only, and two ORNs

responded to L-nVal and L-Val only. The only completely specialist cell responded to L-Ala. Additional extracellular recording sites were selected by moving the electrode and searching for a response to L-nVal. At 15 such locations, responses to other amino acids were recorded. Responses to L-Met were observed at 13, to L-Ala at 9, to L-Ser at 9, to L-Leu at 7, to L-Val at 4, to L-Ile at 4, to L-Arg at 4, to L-Lys at 3 and to L-Pro at 2 locations. All recording locations were equivalent and situated along lamellae 2–7. The number of locations where responses to specific amino acid stimuli were observed correlates highly with the magnitude of the EOG responses (Spearman correlation, $R = 0.91$; $P < 0.1\%$) to the same amino acids. This relationship indicates that the receptor potentials of the ORNs, which were the source of the action potentials, add up to the EOG response. It remains to be answered if these responses originate from specialist ORNs or whether a portion of the responses derives from ORNs that respond to several amino acids.

Poster: Odor Activation and Modulation

Characterization of stimulus-elicited calcium changes in isolated bird olfactory neurons

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To understand bird olfaction, it is important to characterize the peripheral olfactory system of a representative bird species. A previous study (Lalloue *et al.*, 2003, *Chem. Senses* 28: 729–737) demonstrated that the embryonic chick olfactory epithelium is capable of responding to odorants. This study determined the properties of stimulus-elicited calcium changes in acutely isolated olfactory receptor neurons (ORNs) of the chick. ORNs were isolated from day 18–21 embryonic chicks by dissection and enzymatic dissociation, and tested for odorant sensitivity using ratiometric calcium imaging techniques. We tested single ORN responses to odorant mixtures and quantified their single odorant sensitivity, response breadth and concentration–response function, and the pharmacology of odorant signaling. Chick ORNs display properties similar to those found in other vertebrate species (i.e. rodents, humans, salamanders): they respond to odors with increases in $[Ca^{2+}]_i$ mediated by a Ca^{2+} influx, they possess voltage-gated calcium channels (determined by responsiveness to K^+ depolarization) and odorant responses are reversibly inhibited by biochemical inhibitors of second messenger signaling. Thus it appears that there are common features of odorant signaling shared by a large variety of vertebrate species.

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Poster: Odor Activation and Modulation

Effects of GnRH on tiger salamander olfactory receptor neuron responses to amino acids

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To assess the role of the terminal nerve in olfaction we tested the hypothesis that gonadotropin-releasing hormone (GnRH) alters olfactory receptor neuron (ORN) responses to odorants. Recordings were made from acutely isolated ORNs of tiger salamanders using the loose patch-clamp technique and yielded data for 60 ORNs. L-Glutamate and L-alanine singly and as a mixture (AA) were used as stimuli. Application of the AA mixture (both amino acids) evoked hyperpolarizing responses in 41% of ORNs and depolarizing responses in 12% of ORNs; 47% of ORNs did not respond to the AA mixture. A hyperpolarizing response was dominant when L-alanine alone was used. L-Glutamate evoked only hyperpolarizing responses. GnRH modulated chemosensory responses of ORNs. GnRH increased the amplitude of hyperpolarizing responses to the AA mixture and single amino acids. The effect of GnRH on depolarization could not be assessed due to few cells responding with depolarization. Of the 60 neurons tested, 28 did not show a response to the AA mixture or individual amino acids. In these ORNs application of GnRH with AAs induced the appearance of excitatory or inhibitory responses. Application of GnRH alone never induced responses in ORNs. These results suggest that GnRH can modulate odorant sensitivity of ORNs and may play a role in governing the balance of information flow to the olfactory bulb.

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Poster: Odor Activation and Modulation

Gonadotropin-releasing hormone modulates K⁺ currents in tiger salamander olfactory receptor neurons

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We have previously shown that gonadotropin-releasing hormone (GnRH), a peptide present in the terminal nerve, modulates the voltage-dependent Na⁺ current and general odorant responses in salamanders. In this study, we used whole-cell patch clamp recordings to investigate the effects of GnRH on K⁺ currents in isolated olfactory receptor neurons from tiger salamanders (*Ambystoma tigrinum*). First, we demonstrated that bath application of 10 μM GnRH onto olfactory receptor neurons suppresses outward currents in 42% of cells tested. We are now determining which outward currents are affected by GnRH. Substituting Ba²⁺ for Ca²⁺ to block activation of Ca²⁺-dependent currents, we found that application of GnRH decreases the magnitude of Ca²⁺-independent outward currents by ~50% in 60% of the cells examined. We then blocked the transient K⁺ current ('A current') by adding 5 mM 4-aminopyrine (4-AP) to the bath in addition to Ba²⁺, and found that GnRH suppresses the magnitude of the delayed rectifier K⁺ current in 39% of the cells tested. We are now investigating the effects, if any, of GnRH on the transient K⁺ current and on the Ca²⁺-dependent K⁺ current. In addition to the suppressive effects described here, we have found that ~10% of the cells tested display an increase in the overall outward current during GnRH application. These results demonstrate that GnRH modulates voltage-activated K⁺ currents. Overall, terminal nerve modulation may play an important role in peripheral olfactory signal transduction.

This study was conducted in accordance with PHS guidelines, and supported by NSF (IBN 9982934) and NIH (DC05366).

Poster: Odor Activation and Modulation

Putative reproductive pheromones in the round goby, *Neogobius melanostomus*: biosynthesis and olfactory mucosal responses

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Previous studies indicate that, in the round goby *Neogobius melanostomus*, the reproductively mature male releases a pheromone that attracts ripe females. These studies furthermore suggest that the pheromone may be a steroid (more specifically a 5-β-reduced androgen) produced by specialized glandular tissue in the testes. In the present study, *in vitro* incubation of the testes converted [³H]androstenedione into (in order of abundance): 5-β-androstan-3-α-ol-11,17-dione (11-ketoetiocholanolone, 11K-ETIO); 11K-ETIO sulfate (11K-ETIO-s); 11-ketotestosterone; 5-β-androstan-3-α-ol-17-one (etiocholanolone, ETIO); 11-β-hydroxyandrostenedione; ETIO sulfate; and testosterone. 11K-ETIO and 11K-ETIO-s appear to be novel compounds in teleost gonads. While olfactory potency of 11K-ETIO-s has not been tested, the response threshold concentration was 0.1 nM for the 11K-ETIO and ETIO sulfate, when olfactory mucosal activity was measured by electro-olfactogram (EOG). The other compounds elicited olfactory mucosal responses at concentrations >0.1 nM. For 11K-ETIO, ETIO sulfate and 11-ketotestosterone; EOG response magnitudes in ripe females were significantly greater than in non-ovulated females. In addition, the fact that the carbon A ring of 11K-ETIO and 11K-ETIO sulfate have a 5-β-configuration (already linked with olfactory sensitivity and behavior induction in two species of gobies) makes these likely candidates pheromones in the round goby.

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Poster: Odor Activation and Modulation

The rabbit mammary pheromone elicits responses in the main olfactory epithelium of newborn rabbits and rats

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As previously described newborn rabbits rely for survival on a pheromone released from the doe's nipples (Hudson and Distel, 1983). The pheromone inducing suckling behavior in rabbit pups has been identified in rabbit milk as a single compound, namely trans-2-methyl-2-butenal (2MB2), and acted behaviorally species specific failing to induce suckling behavior in rat pups (Schaal *et al.*, 2003). Using an air dilution olfactometer we recorded electro-olfactograms (EOG) on 18 different sites of the mucosa of the nasal septum and turbinates of newborn rabbits and rats from postnatal

days 2–11. We tested 2MB2, three structurally related odorants, one with similar odor quality and three unrelated general odorants. Most recording sites of the septal and turbinate mucosa of rabbit and rat were responsive to all eight odorants. All stimuli elicited typical EOGs with a rapid rising phase and a slower decline. Amplitudes to a given stimulus at a given recording site varied from animal to animal and were not age dependent. Topographic pattern for maximum sensitivity of 2MB2 were different in all individuals. 2MB2 was the most potent stimulus only in 30% of the instances tested. All recording sites sensitive to 2MB2 responded also to several other test odorants. This indicates that the olfactory mucosa of newborn rabbit and rat exhibited neither regions of specific selectivity nor a general increase in sensitivity to 2MB2.

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Poster: Environment and Human Olfaction

Children's preferences for tobacco odor: effects of maternal smoking and mood states

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Ongoing research in our laboratory focuses on children's hedonic judgments of and preferences for odors as a function of the context of early experience. Age-appropriate, game-like tasks that were fun for children and minimized impact of language development were used to examine responses of 3- to 9-year-old children ($n = 296$) and their mothers to a variety of odors ranging in hedonic valence and familiarity, one of which was tobacco smoke. Parental smoking, as well as the mood state of the parent, as determined by standard tests (e.g. Beck Depression Inventory), influenced children's preference for tobacco odors. The data revealed that women who smoked liked the tobacco odor significantly more than non-smoking women. Likewise, children whose parents smoked were significantly more likely to prefer the odor of cigarette relative to neutral odors when compared with children of non-smokers. This preference for the tobacco odor was significantly less pronounced in children of smoking mothers who were depressed when compared with children of non-depressed smoking mothers. Depressed smoking mothers also scored significantly higher on the POMS Tension, Fatigue, Anger and Confusion and lower on the vigor scales when compared with non-depressed smoking mothers. These findings suggest that children's preferences for the odor of tobacco smoke are related to the emotional context in which their mothers experience tobacco.

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Poster: Environment and Human Olfaction

Androstadienone exposure modulates mood ratings but not behavior in women

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Exposure to the endogenous steroid androstadienone has repeatedly been shown to modulate women's feeling of being focused

in a positive direction (Lundström *et al.*, 2003). The aim of the current study was to investigate whether exposure to a subthreshold concentration of androstadienone would also facilitate behavioral performance in an attention task. Thirty-seven women participated in a double-blind within-group experiment where they performed a 20 min tracking task measuring sustained attention. Either a male or a female ran the experiment. The experimental substance contained 250 μ M androstadienone. Both the experimental and control substances were masked by eugenol in order not to be perceptually discriminable. Effects on mood variables as well as attraction ratings of male faces were measured. Exposure to androstadienone modulated participant's mood in that they felt more focused, social, happy and less irritated. Interactions between test substance and sex of experimenter indicated that the presence of a male experimenter enhanced some of the effects in a positive direction. However, no effects on attention performance or attraction ratings were found.

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Poster: Environment and Human Olfaction

Smelling a partner's clothing during periods of separation: prevalence and function

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Scattered reports in both the scientific and popular literature, as well as personal anecdotes, suggest that it is not uncommon for persons to deliberately smell their sexual partner's clothing, especially when separated. To our knowledge, there has been no documentation of the frequency of this behavior. We asked undergraduate men and women who were, or had ever been, in a committed heterosexual relationship if they had ever deliberately smelled their partner's clothing, or had slept with an article of their partner's clothing, during periods of separation. A large majority of women had done one of these behaviors at least once; men reported doing it much less. We discuss possible functions of this behavior. It is possible individuals may be evaluating their partner's MHC or other signals of health provided by odor, but that would not explain why the behavior occurs in the partner's absence. Attractiveness of a partner's odor may derive from its value in signaling the fitness benefits provided by a mate's presence. The sex difference may reflect the fact that women especially benefit from the protection provided by a mate, or the greater importance women place on their partner's odor (Herz and Cahill, 1997), or the greater choosiness of women in mate selection (Buss and Schmitt, 1993).

Poster: Environment and Human Olfaction

Human olfactory detections of social and non-social chemosignals

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Olfaction is important for the survival of many animals and used for detection of a wide range of social and nonsocial information. Research in animals (Petrucci *et al.*, 1999) suggests that the mechanisms involved in processing smells serving different functions (e.g. food versus reproduction) may be different. Previous research shows that humans can recognize individuals, distinguish between emotional states (Chen and Haviland-Jones, 2000; Chen and McClintock, in preparation), and make fine discriminations between various nonsocial smells, although few has examined olfactory sensitivities to different types of social and nonsocial smells within the same study. In this study, we investigate and compare human olfactory sensitivities to a variety of social and nonsocial chemosignals. Thirty-two college-aged adults were asked to identify themselves and their roommate, and to distinguish between different emotional states based on the smell of sweat. Their sensitivities to nonsocial smells were also assessed using established clinical tests (threshold and odor identification tests). Over three-fourths of the subjects identified themselves in a well-controlled three-item-forced-choice task (chance = 33%, $P < 0.0000004$), a somewhat smaller percentage (59%) identified their roommate ($P < 0.004$). Olfactory recognition of individuals is not related to subjects' report of self consciousness or sensitivity to sensory stimuli in general. Their sensitivities to emotional and nonsocial smells will also be reported.

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Poster: Environment and Human Olfaction

The effect of human emotional chemosignals on task performance

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Previous research shows that affective chemosignals can be distinguished based on their olfactory cues (Chen and Haviland-Jones, 2000; Chen and McClintock, in preparation), although little is known about their impact on behavior. In this double-blind study, we examine the effect of emotional chemosignals on task performance. Following McClintock's hypothesis (1999) that human chemosignals may serve the function of modulating ongoing behavior, we predict that the smell of fear would bias subjects towards attending to potentially threatening stimuli more so than would the smell of a neutral state. College-aged women performed a word-association task on a computer while they were exposed to either underarm sweat collected from people when they were in fear or from the same people when they were calm and feeling neutral. Subjects' reaction time to the words, their self-reported mood, and their autonomic nervous system responses (heart rate, skin conductance, respiration) were recorded. Preliminary analyses based on 28 subjects showed a significant smell condition effect depending on whether the words were related to one another and whether the prime was threatening or not ($P = 0.011$, partial $\eta^2 = 0.23$). In one smell condition, subjects were faster at detecting related over unrelated words when the prime was threatening ($P = 0.043$, partial $\eta^2 = 0.30$), but showed no difference when the prime was neutral. In the other smell condition, the opposite was found.

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Poster: Environment and Human Olfaction

Olfactory cuing of emotional events

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The aim of this research was to examine olfactory cuing of emotional and neutral events. In study 1, subjects were exposed to a series of photo slides depicting an emotional event (car accident) along with a congruent odor (i.e. petrol) or no odor. Five days later participants were asked to recall details of the event. Results indicated that both central details and circumstantial information of the emotional event were better retained following olfactory cuing. In study 2, we explored the role of olfactory congruency and retention interval on recall proficiency of emotional events. Subjects were presented with series of photo slides showing either emotional or neutral events in conjunction with a context congruent odor, a context incongruent odor, or no odor. We expected that olfactory cuing in general enhance recall performance as compared with the no odor condition, and that memory for emotional events with olfactory congruent processing will be better than that for non-congruent processing. Results will be presented both with respect to memory performance (e.g. number of details, accuracy), assessed 24 h and eight days after exposure across all cue formats, and experiential measures (e.g. arousal, intensity).

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Poster: Environment and Human Olfaction

Processing of odorous information is influenced by gustatory stimulation

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Objectives: taste and smell interact. The aim of this study was to examine this interaction using gustatory and olfactory stimuli applied at the same time, which were both contextually related and unrelated. Methods: thirty-two, young, healthy normosmic subjects (16 men, 16 women) took part in two randomized test sessions. In session 1 olfactory event-related potentials (ERP) were elicited using vanillin (pleasant), in session 2 chemo-somatosensory ERP were elicited using CO₂ (40% v/v; stinging, unpleasant), both sessions with 60 stimuli each. During ERP recordings four conditions (each during 15 stimuli) were additionally applied: subjects had either nothing in their mouth (condition 1), an empty taste dispenser (condition 2), a dispenser filled with sucrose (condition 3) or citric acid (condition 4). Subjects rated stimulus intensity of vanillin and CO₂. Results: during the 'sweet condition' (condition 3) largest ERP amplitudes were obtained for vanillin while CO₂ produced the smallest amplitudes ($P = 0.025$). This was the other way around for the 'sour condition' (condition 4). Moreover, during the sweet condition the latency P1 was shorter than in the sour condition while for CO₂ it was the other way around. Conclusions:

application of a sweet taste significantly enhanced the early ERP components elicited by vanillin which is a contextually related stimulus. In contrast, response amplitudes to CO₂-stimulation were specifically amplified through gustatory stimulation with the contextually related sour stimulus. This indicates that gustatory stimuli may act as specific primers of odorous impressions.

Poster: Environment and Human Olfaction

Perithreshold not suprathreshold exposure increases sensitivity to odors

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Repeated threshold test exposures to perithreshold odorants can lead to dramatic sensitivity increases among young women, (Dalton *et al.*, 2002), even when the target odorant is superimposed on a suprathreshold 'background' odorant (Dalton *et al.*, 2003). To evaluate whether this phenomenon is concentration-dependent such that perithreshold stimulation is required to induce sensitization, nine subjects (five females, 18–24 years) were exposed to suprathreshold concentrations of benzaldehyde or citralva in the process of determining Weber fractions. After multiple sessions, absolute odor detection thresholds were measured for experimental and control odors. In contrast to prior studies, we found no difference in sensitivity changes between males and females: sensitivity to both control and experimental odors increased up to one order of magnitude for both groups. The same subjects were later exposed to perithreshold concentrations of one of the experimental odors over 10 sessions. Under these conditions, females increased sensitivity by almost three orders of magnitude, whereas males increased sensitivity by only slightly more than one. This suggests that attention to suprathreshold stimulation was insufficient to elicit sensitization and that perithreshold stimuli may be required.

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Poster: Environment and Human Olfaction

Odor perception and judged probabilities of health risk

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The purpose of the present study was to determine how intuitive notions about the significance of odor from a chemical source combine with rational, scientific information to form risk judgements in individuals. Subjects were given information pertaining to an imaginary odor, including the following benchmarks: the concentration (in parts per million) at which the odor can be detected, the concentration at which the odor is considered harmless, and the concentration at which the odor is considered harmful. In addition to this information, three samples of 1-butanol at varying concentrations were provided to correspond to these benchmarks of the imaginary odor. The order of the benchmarks was different for each of three groups tested. Armed with this information, subjects were asked to rate the level of risk associated with exposure to several concentrations of the imaginary odor that fell above and below the benchmarks provided. Half of the subjects were given a tight

range of concentrations to rate while the other half were given a wider range of concentrations. Results show that participants consistently perceived the chemical as being more harmful after it exceeded the level of odor detection than when it was below the level of detection, regardless of whether odor perception occurred above or below levels associated with toxicity.

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Poster: Trigeminal Chemoreceptors

P2X-receptor expression and their contribution to chemosensation in trigeminal neurons

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The facial innervation pattern of trigeminal nerve fibres comprises the innervation of the nasal epithelium, where free trigeminal nerve endings contribute to detection and discrimination of chemical stimuli including odorants. The signal transduction mechanisms in sensory nerve endings underlying perception of chemical stimuli remain widely uncovered. Here, we characterized trigeminal ATP-activated P2X-receptors in cultured rat trigeminal neurons and investigated their role in chemoperception. We identified a new subpopulation of neurons lacking typical nociceptive characteristics and expressing homomeric P2X₂-receptors. Using a certain group of chemicals known as trigeminal stimuli we found no direct activation of trigeminal neurons, but a modulation of P2X₂-receptor mediated currents. In contrast, P2X₃-receptor mediated currents of nociceptive trigeminal neurons remained unaffected by the tested chemicals. Therefore, we assume a functional role of the newly identified subpopulation in chemodetection of certain trigeminal stimuli.

Poster: Trigeminal Chemoreceptors

Single nucleotide polymorphisms in the capsaicin receptor: relationship to chemo-sensory performance in a pilot sample

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The capsaicin or vanilloid (VR1/TRPV1) receptor responds to multiple noxious stimuli, including H⁺, heat, and capsaicin and its analogs. We recently observed marked inter-individual variation in subjective rating of capsaicin solution applied topically to the nasal mucosa in human subjects, and hypothesized that sequence variations in the TRPV1 gene could be responsible for this phenomenon. To test this hypothesis, we sampled genomic DNA from 10 subjects (four females, six males; age range 24–56 years), all of whom had undergone trigeminal threshold testing with CO₂ (an acid producer) and six of whom had given suprathreshold irritation ratings to nasal capsaicin challenge. We sequenced five coding regions with known single nucleotide polymorphisms (SNPs) in the TRPV1 gene in each of the genomic DNA samples. Three of these SNPs resulted in a non-synonymous change in amino acid sequence of TRPV1. We found polymorphism in two of these non-synonymous SNPs.

However, neither of these polymorphisms correlated with either capsaicin suprathreshold rating or CO₂ threshold performance of the 10 subjects. The only obvious chemosensory correlation was heterozygosity in one SNP resulting in a synonymous change in TRPV1 sequence in one subject with unusually low ratings of capsaicin irritancy. In this small pilot sample we demonstrated the feasibility of comparing chemosensory performance with sequence variation in the TRPV1 receptor gene. However, our results suggest that none of the five SNPs initially examined predicts inter-individual differences in capsaicin and CO₂ perception.

Poster: Trigeminal Chemoreceptors

Capsaicin self-sensitization in cultured trigeminal neurons

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Sensitization of peripheral somatosensory neurons is one means by the amplification of the system can be modulated in certain cases of tissue damage and pathology. It is well known, for instance, that bradykinin (an endogenous algogen released on tissue injury) sensitizes somatosensory neurons to heat and capsaicin (CAP). When CAP is periodically applied to the oral mucosa at short intervals, typically 1 min between stimuli, the reported irritation increased over time and has been termed sensitization (Green, 1991). Studies in which cultured trigeminal or dorsal root ganglion neurons have been exposed to repeated stimulation with CAP have only reported desensitization of subsequent responses to CAP stimulation. Because of this, it has been most tempting to attribute the observed increases in the intensity of sensory irritation to either a slow increase in the peripheral tissue concentration of CAP or to sensitization of central processes. To examine the peripheral basis of CAP self-sensitization, intracellular calcium responses of fura2-loaded rat trigeminal neurons were measured. Tested at concentrations lower (50 nM–1 μM) than previously tested (1–10 μM), CAP induced self-sensitization in a portion of neurons. At 1 μM, repeated stimulation with CAP only induced the same amplitude or decremented responses. Between 50 and 300 nM CAP, up to 46.2% of CAP-sensitive neurons exhibited sensitization. This suggests that a balance between sensitizing and desensitizing processes occurs in trigeminal neurons with the desensitizing processes having a higher threshold. Although narrow in concentration range, this demonstrates that peripheral self-sensitization by CAP does take place.

Poster: Trigeminal Chemoreceptors

The effect of VR1 blockers on peripheral trigeminal nerve responses to irritants

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The vanilloid receptor (VR1, aka TRPV1), present on trigeminal nerve fibers, is a non-selective cation channel with high permeability for divalent cations. VR1 responds to heat, acids and capsaicin but

its specific role in trigeminal stimulation by irritants has not been well studied. Our study seeks to further investigate the role of VR1 in peripheral trigeminal nerve responses. Neural recordings were obtained from the ethmoid nerves of adult rats in response to irritants delivered in solution to the nasal cavity. The ethmoid nerve responded to capsaicin, propionic acid, cyclohexanone and nicotine. When these compounds were presented with ruthenium red, an inhibitor of Ca²⁺ transport through membrane channels, there was a decreased response to capsaicin, propionic acid and cyclohexanone, but no change in the response to nicotine. When these compounds were presented with capsazepine, a competitive VR1 inhibitor, there was a decreased response to capsaicin and cyclohexanone, but no change in the response to nicotine and propionic acid. These data indicate that while capsaicin, cyclohexanone and propionic acid may at least partially exert their effect through VR1, nicotine does not. Additionally, these data show that while both ruthenium red and capsazepine work to block VR1 receptors, they do so in different ways, as exhibited by their effect on the propionic acid response. Future work will look at additional compounds and receptor blockers to better understand the mechanisms of trigeminal nerve nociception.

Poster: Trigeminal Chemoreceptors

Persistence of nasal solitary chemoreceptor cells after neonatal capsaicin treatment

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Our recent studies (e.g. Finger *et al.*, 2003, *Proc Natl Acad Sci USA*) show that nasal trigeminal chemosensitivity in mice and rats is mediated in part by solitary chemosensory cells (SCCs) distributed throughout much of the nasal respiratory epithelium. These SCCs express gustducin and T2R (bitter) taste receptors, and synapse onto peptidergic (substance P/CGRP) fibers of the trigeminal nerve that densely innervate the receptor cells. Administration of capsaicin to neonatal rat pups (0.1 ml of a 1% capsaicin solution, 50 mg/kg) destroys the peptidergic trigeminal ganglion cells and effectively eliminates the trigeminal neural response to most irritants. These experiments were designed to test whether capsaicin-mediated elimination of trigeminal peptidergic innervation reduced or eliminated the nasal SCCs, i.e. whether nasal SCCs are dependent on innervation. Two to four months following neonatal injection of capsaicin, rats were anesthetized and prepared for electrophysiology. In three of three desensitized animals, the ethmoid nerve showed no responses to cycloheximide solution applied to the nasal mucosa—a solution that evokes robust activity in normal animals. Following recordings, these and other desensitized rats were perfused with 4% paraformaldehyde and prepared for immunocytochemistry. Despite nearly total elimination of CGRP-immunoreactive nerve fibers, the gustducin-expressing nasal SCCs remained. Sparse non-peptidergic innervation of the epithelium remained (as assessed with PGP antisera) but these remaining nerve fibers did not form close, embracing contacts with the SCCs.

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Poster: Trigeminal Chemoreceptors**Patterns of variation in the behavioral responses of rats to irritants after neonatal capsaicin treatment**

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Administration of capsaicin to neonatal rat pups has been shown to produce adults with decreased trigeminal sensitivity to many irritants. This observed loss in chemosensitivity presumably occurs through the elimination of the vanilloid receptor-expressive cell population of the trigeminal ganglion, and it was hypothesized that the degree of desensitization in individual adults would vary depending on the efficacy of the initial capsaicin treatment. To test this hypothesis, a behavioral screen was developed to examine inter-animal variability in aversion responses to irritants. Adult rats injected with either capsaicin or a control solution as neonates were presented with a series of eight irritating/non-irritating chemical stimuli. Each rat's response within the first few seconds of encounter with the stimulus source was then scored using a scale to measure aversive and/or favorable responses. Neonatal capsaicin treatment was found to result in varying degrees and differing patterns of desensitization to the five irritants tested. While most of the capsaicin-injected rats displayed diminished aversion reactions when compared with control animals, a few (3 of 13) were found to be more sensitive than the least sensitive control animal. Among the most desensitized animals, sensitivity to acetic acid remained intact whereas sensitivity to nicotine, cyclohexanone, amyl acetate, and ethanol were diminished. Capsaicin treatment had no effect on the behavioral response to non-irritating stimuli. These findings show that neonatal administration of capsaicin does not guarantee a desensitized-adult state, and may result in varying degrees of desensitization.

Poster: Trigeminal Chemoreceptors**Topographical differences in the sensitivity of the intranasal trigeminal system**

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Preliminary data indicate that the nasal mucosa shows a differential responsiveness to trigeminal stimuli depending on the site of stimulation. Aim of the present study was the comparison of the sensitivity of two different areas of the respiratory epithelium to mechanical (air puffs) and chemosensory (gaseous CO₂) stimuli. Specifically, stimuli were applied to the anterior portion of the nasal cavity and to the pharyngeal area. Responses were quantified using psychophysical (intensity) and electrophysiological (event related potentials—ERPs) techniques. For all measured parameters, a significant interaction between 'location' and 'stimulus' emerged, indicating that (i) CO₂ evoked ERP of higher amplitude and higher intensity ratings when applied to the anterior portion of the nasal cavity compared with pharyngeal stimulation; and (ii) mechanical stimuli elicited larger responses when presented to the pharynx than to the anterior nasal cavity. These findings suggest that the nasal mucosa does not exhibit a homogeneously distributed sensitivity,

but that, in addition to the olfactory mucosa, there are areas with specific sensory function. These topographical differences in intranasal sensitivity may play a role in terms of differences between the ortho- and retronasal perception of odors.

Poster: Trigeminal Chemoreceptors**Viral tracing of murine trigeminal neurons innervating the nasal cavity**N. Damann¹, B. Klupp², T.C. Mettenleiter², H. Hatt¹ and C. Wetzel¹¹*Cell Physiology, Ruhr-University, Bochum, Germany and*²*Friedrich-Loeffler-Institute, Bundesforschungsanstalt für Viruskrankeheiten der Tiere, Insel Riems, Germany*

The trigeminal nerve is the major mediator of sensations from the mammalian head and comprises neurons that transduce mechanical, thermal and chemical stimuli. Thereby single neurons mediate sensory input from selective areas of the head (meninges, cornea and conjunctiva of the eyes, facial skin, mucous membranes of the oral and nasal cavities). Differential physiological features of peripheral neurons depending on their function and area of innervation remain largely unclear. Viral tracing was performed to identify trigeminal neurons that mediate information from the murine nasal cavity. After application of high titered Pseudorabies virus (PrV) into the nose, markerprotein and immunohistochemistry based investigations were carried out. Paraffin embedded sections and whole mount preparations were used to describe viral spread. Histochemical investigations revealed an ipsilateral spread via the ophthalmic division of the trigeminal nerve to the gasserian ganglion (GG). Infected GFP labeled ganglion neurons could also be identified after dissociation and plating allowing activity measurement of individual identified neurons in primary cell culture. PrV constitutes a powerful tool to perform rapid tracing of the murine trigeminal system and affords the possibility to selectively label neurons innervating the nose. Electrophysiological and calcium imaging based characterization of these neurons concerning their specificity for selected chemical compounds shall be performed in future research.

Poster: Trigeminal Chemoreceptors**Gender differences and nasal integration studies performed using an ocular exposure device for detection of irritation thresholds: the t.i.d.e. system**

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Ocular irritation from low-level volatile chemicals is one of the most common complaints in occupational and residential environments. In contrast with techniques available for nasal irritation assessment, however, there are few standardized methods available for measuring ocular irritation thresholds. Based on the lateralization technique originally developed for nasal irritation thresholds, we have developed an instrument that can deliver controlled concentrations of a chemical vapor to one eye and a blank stimulus to the other without producing mechanical somatosensory stimulation that might alter ocular thresholds for chemicals. Initial results

suggest that this system can be very useful for objective measurement of ocular irritation, that simultaneous nasal stimulation affects ocular thresholds and that females have a lower ocular threshold upon integration than males. Results also indicate that while there is a wide variation of ocular irritation thresholds among individuals, vapor concentrations required to initiate conjunctival irritation can be substantially lower than those encountered in occupational settings.

Poster: Trigeminal Chemoreceptors

Lateralization of chemosensory stimuli: effects of olfactory function, age and gender on trigeminally mediated sensations

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The present investigation aimed to compare trigeminal nasal function of anosmic and hyposmic patients to healthy controls. Further, we aimed to study effects of age and gender on trigeminally mediated sensations following intranasal chemosensory stimulation. Participants were 35 patients with olfactory dysfunction ($n = 13$: functional anosmia; $n = 22$: hyposmia; age 28–69 years). Their results were compared with 17 normosmic subjects (age 28–82 years). Olfactory function was assessed using the ‘Sniffin’ Sticks’ test kit (butanol odor threshold, odor discrimination, odor identification). The subjects’ ability to lateralize odors was investigated for benzaldehyde and eucalyptol. Patients with olfactory dysfunction had lower scores in the lateralization task than controls ($P < 0.001$) indicating decreased trigeminal sensitivity. Among anosmic patients scores were not different in relation to different causes of olfactory dysfunction ($P < 0.29$). When investigating normosmic subjects only, no gender-related difference was apparent for lateralization scores. However, older subjects had lower scores than younger ones ($P < 0.01$). In conclusion, results of the present study indicate that patients with olfactory dysfunctions have lower trigeminal sensitivity compared with normosmic controls. Analyses additionally indicate a correlation between olfactory and trigeminal sensitivity.

Poster: Functional Organization of the Gustatory System

Ion substitution affects the lingual surface potential in humans

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We have demonstrated in humans that Na^+ evokes a lingual surface potential (LSP) and that amiloride inhibits a portion of the Na^+ -evoked LSP in some but not all subjects (*J. Neurophysiol.* 90: 2060, 2003). To further characterize electrophysiologic aspects of the hu-

man tongue we examined whether ion substitutes influence the LSP. Initial studies employed K^+ and Li^+ , because Li^+ transits Na^+ -selective pathways, e.g. ENaC, while K^+ does not readily transit such pathways. The LSP response to K^+ and Li^+ depended on whether the individual’s Na^+ -evoked LSP was inhibited by amiloride. In subjects who manifested amiloride sensitivity, K^+ substitution reduced the LSP, while Li^+ ’s effect was similar to that of Na^+ . In these subjects, after addition of amiloride to solutions, the Na^+ - and Li^+ -evoked LSPs were reduced and were similar to that of K^+ . In subjects who had no amiloride sensitivity, the effect of K^+ on the LSP was similar to those of Na^+ and Li^+ . In other studies gluconate and sulfate replaced Cl^- . While the substitute anions increased the Na^+ -evoked LSP, the sulfate effect was ~3-fold greater than that of gluconate. The effects of the anion substitutes were not influenced by amiloride or the subject’s sensitivity to amiloride. These data complement our previous observation that some but not all individuals manifest amiloride sensitivity of their Na^+ -evoked LSP. These data also indicate that the amiloride sensitive Na^+ pathway allows Li^+ to transit, but excludes K^+ , consistent with the presence of ENaC in a subset of humans. Anions also influence the Na^+ -evoked LSP, but they do so independently of ENaC.

Poster: Functional Organization of the Gustatory System

Expression of delayed rectifying K channels in taste cells of obesity-prone and -resistant rats

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Direct fatty acid-mediated inhibition of delayed rectifying potassium (DRK) channels, specifically those in the *Shaker* subfamily (Kv1), has been proposed as a taste transduction mechanism for dietary fat. Our previous work demonstrated that fungiform TRCs responded only to *cis*-polyunsaturated fatty acids (PUFAs), while those found in the foliate and vallate papillae, responded to both PUFAs and a subset of monounsaturated fatty acids. In addition we demonstrated that TRC sensitivity to PUFAs was inversely correlated with dietary fat preference. Patch clamp recording has shown that TRCs in Osborne–Mendel (OM) rats, which show a marked preference for dietary fat and become obese when placed on a high fat diet, are much less sensitive to PUFAs than are TRCs in S5B/Pl (S5B) rats that tend to avoid fat and remain lean even on a high fat diet. Our hypothesis is that TRCs in S5B rats express a greater ratio of fatty acid-sensitive to fatty acid-insensitive DRK channels than do those in OM rats. To determine if this difference in PUFA responsiveness is correlated with expression levels of DRK channels, we used real-time quantitative PCR to measure expression levels of DRK channels in taste buds from OM and S5B rats. Consistent with DRK current densities, which are twice as great in OM rats than in S5B rats, TRCs from OM rats exhibit greater expression of DRK channels. Specifically, the Kv2 (*Shab*) and Kv3 (*Shaw*) DRK channel families, which may represent the PUFA-insensitive DRK channels, are much more highly expressed in OM rats than in S5B rats consistent with our hypothesis.

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Poster: Functional Organization of the Gustatory System

The pore-forming antibiotic nystatin inhibits taste cell K currents in perforated patch recordings

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Nystatin and amphotericin B, pore-forming antibiotics commonly used in perforated patch clamp recording, have been shown to inhibit and increase the rate of inactivation of the delayed rectifying K channel Kv1.3 when applied to the intracellular face of the membrane (Hahn *et al.*, 1996, *Neuropharmacology* 35: 895). Since it is not known whether these antibiotics can gain access to the intracellular face and affect K currents in the perforated patch configuration, we investigated this possibility in taste receptor cells (TRCs), which we have shown by RT-PCR and immunocytochemistry to express Kv1.3 as well as other delayed rectifying K channels. We found that nystatin, but not amphotericin, caused roughly a 40% inhibition of delayed rectifying K currents in TRCs at 20 min. and increased the rate of inactivation several fold. This difference between nystatin and amphotericin may be due to the fact that the IC₅₀ of amphotericin's effects on Kv1.3 is ~18 times higher than that of nystatin, while the concentrations of amphotericin we found necessary to gain good electrical access to the TRCs was only 2–3 times higher than the concentrations of nystatin. Thus, one should exert caution when using pore-forming antibiotics in perforated patch recordings because of possible effects on ionic currents.

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Poster: Functional Organization of the Gustatory System

The cellular and molecular basis for water taste in mice

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Recent studies have begun characterizing water (*hypoosmotic stimuli*) responses in the peripheral gustatory system in rat. Increases in activation were associated with rapid and reversible changes in cell volume. Such rapid changes in cell volume may be due to aquaporin channels (AQP) previously identified in taste receptor cells (TRC). We have begun examining this response in two inbred mice strains (C57/6ByJ and 129X/SvJ) using whole cell patch clamp recording. Approximately half of the fungiform TRCs in each strain responded to a moderately hypoosmotic solution (255 mOsm) with reversible increases in conductance, presumably due to activation of a volume-sensitive Cl channel. Immunocytochemistry showed apical expression of AQP5 in isolated fungiform TRCs in both strains. We have also examined how these mice respond behaviorally to changes in solution osmolarity using mannitol (believed to be tasteless) to alter tonicity. Twenty-four-hour three-bottle intake tests assessed preferences for several concentrations of mannitol (55–330 mM) to distilled water. As concentration (osmolarity) in-

creased, we found decreased mannitol preference. There were also significant strain differences at lower concentrations. Preference scores for mannitol were lower for the B6 than 129 mice. Therefore, mice respond to changes in osmolarity both behaviorally and at the cellular level. In addition, expression of water channels, particularly the apically expressed AQP5, may play an important role in the initial transduction events. Current studies are addressing the link between AQP expression and water responsiveness in mice.

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Poster: Functional Organization of the Gustatory System

Monitoring T1R taste receptor dimerization

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T1Rs are taste cell-expressed type 3 G-protein-coupled receptors (GPCRs). Dimerization of T1R receptors has been inferred, but not actually shown. We have used BRET (bioluminescence resonance energy transfer) assay to demonstrate T1R receptor dimerization. BRET2 assay is based on energy transfer between fusion proteins containing *Renilla* luciferase (Rluc) and green fluorescent protein (GFP).

Human T1R1 (hT1R1), hT1R2 and hT1R3 coding regions were subcloned into Rluc and GFP vectors. HEK-293T cells were co-transfected with all pairs of hT1R-Rluc and hT1R-GFP plasmids. Significant BRET signals were observed in cells expressing hT1R1 and T1R3 pairs, hT1R2 and hT1R3 pairs, but not hT1R1 and hT1R2 pairs. Saturation curves were constructed for each dimer pair. BRET signals were largely independent of receptor density indicating that they came from dimerization and not random collision events. BRET competition assays with untagged hT1R3 reduced the dimer BRET signal, confirming the specificity of the interactions. We generated multiple hT1R3 mutants to investigate the effects of disulfide bonds on dimer formation and ligand binding.

Poster: Functional Organization of the Gustatory System

Expression of RGS in taste bud cells

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Bitter, sweet and umami taste stimuli are detected by G-protein coupled receptors. The activation of these receptors in turn stimulates heterotrimeric G-proteins, and triggers signal transduction cascades, eventually leading to a change in cell membrane potential and the release of neurotransmitters onto afferent axons. Recently, we and others have isolated several key taste transduction molecules. However, the mechanisms underlying the termination and

desensitization of taste responses remain to be determined. To identify proteins that are involved in taste signal processing and peripheral coding, we isolated individual taste receptor cells, amplified single cell transcriptomes and constructed single taste cell cDNA libraries. By subtractively screening these libraries, we isolated a number of genes that are selectively expressed in taste cells. Among them is a regulator of G-protein signaling (RGS) protein, which presumably serves as a negative regulator of heterotrimeric G-protein mediated signaling pathways by stimulating GTPase activity of the α subunits. *In situ* hybridization showed that this RGS is expressed in a subset of taste receptor cells. Further analysis of its interaction with taste signaling molecules in these taste cells could provide novel insights into our understanding of taste response processing and termination at the taste end organs.

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Poster: Functional Organization of the Gustatory System

Do taste cells that utilize the PLC signaling pathway also express voltage-gated calcium channels?

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It has been well established that bitter, sweet, and umami taste stimuli are coupled to G-proteins that activate the PLC signaling pathway (Zhang *et al.*, 2003, *Cell*). This pathway increases intracellular Ca^{2+} via release from intracellular stores and store operated influx (Ogura *et al.*, 2002, *J. Neurophys.*). Recently, we showed that a subpopulation of these cells, the bitter-responsive, gustducin-expressing cells, lacks voltage-gated Ca^{2+} channels (Medler *et al.*, 2003, *J. Neurosci.*). However, it is not known if lack of voltage-gated Ca^{2+} channels is a characteristic of all taste cells that use this pathway. In this study we used Ca^{2+} imaging to determine if any PLC-responsive cells respond to depolarization with an increase in intracellular Ca^{2+} . We found that cells that respond to a PLC activator rarely show increases in intracellular Ca^{2+} when depolarized with KCl (55 mM). In the cells that did respond to both, the response to the PLC activator was not robust. These data suggest either some overlap between excitable cells and the PLC β 2 signaling pathway or the presence of multiple PLC isoforms in taste cells. Further work is needed to clarify these results.

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Poster: Functional Organization of the Gustatory System

Taste response and molecular expression of receptor cells of the mouse fungiform papillae

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In taste bud cells, expression of various molecules concerned in taste reception was detected by various molecular biological techniques. Here, we investigated taste responses of receptor cells showing action potentials and mRNA expression of taste related genes in these cells at the same time. Using loose patch technique, we recorded increases in firing frequency from taste bud cells tested for taste stimuli (NaCl, saccharin, HCl, quinine-HCl). Two types of NaCl responding cells exist: one is amiloride-sensitive and the other amiloride-insensitive. In some cells, responses to MSG were enhanced when MSG was mixed with IMP. Responses to sweet substances in some receptor cells were suppressed by apical treatment of gurmarin and recovered after apical application of β -cyclodextrin. Of 68 cells responding to taste stimuli, 40 (59%) responded to one, 24 (35%) to two and 4 (6%) to three of four taste stimuli. The entropy value presenting the breadth of responsiveness was 0.213 ± 0.252 , which was close to that for the nerve fibers. These results suggest that taste cells generating action potentials have response characteristics to taste stimuli that are comparable to those for nerve fibers. After recording of taste response, single receptor cell was withdrawn from a taste bud and checked mRNA expression of taste related genes such as T1R3 by RT-PCR method. Our preliminary data indicate that a taste cell responding to sweet stimuli expressed T1R3 and gustducin mRNA. Thus, this technique might be useful to examine molecular expression in receptor cells responding to taste stimuli.

Poster: Functional Organization of the Gustatory System

The A blood group antigen is expressed by a unique subset of taste bud cells

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Although mammalian taste cell types differ across species, most investigators recognize at least two classes of elongated, spindle-shaped cells: Type I (dark) and Type II (light) cells, based on their relative electron densities when stained with uranyl acetate. These cell types differ markedly in their morphology in transverse sections through the taste bud. A third cell type (Type III), which is electron lucent and round in transverse section like a Type II cell (and therefore a subtype of 'light' cell), was first described in rabbit foliate taste buds as possessing synaptic connections with nerve fibers. Type III cells have also been described in rat and mouse vallate taste buds on the basis of specific antigen expression and ultrastructural similarity to rabbit Type III cells. Here we examine rat and mouse taste buds for immunocytochemical expression of several markers that delineate differences among light cell types, which, unlike Type I cells, are heterogeneous in their expression patterns. NCAM is expressed on a subset of Type III cells and α -gustducin on a subset of Type II cells, only some of which also express the Lewis^b blood group antigen. The blood group A antigen co-localizes with α -gustducin on some cells (which do not express Lewis^b) and not others but does not label any NCAM- or PGP 9.5-positive cells. Combined with the work of others showing subtypes of Type III cells expressing combinations of PGP 9.5, 5-HT and/or NCAM, these data provide additional evidence for the molecular complexity of mammalian taste bud cells.

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Poster: Functional Organization of the Gustatory System

Proliferation of lingual macrophages after unilateral denervation of fungiform taste buds

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Within days after unilateral chorda tympani nerve (CT) section, activated ED1+ macrophages are increased on both the sectioned and intact sides of the tongue. Activated macrophages release a variety of cytokines and growth factors, which are proposed to affect sodium taste function after neural injury. We hypothesized that local proliferation of ED1+ macrophages accounts for the dramatic increase observed after nerve section. SD specified pathogen-free rats received unilateral CT section or sham section on day 0. On day 1 or 2 post-section, rats were given an injection of BrdU (50 mg/kg i.p.) 6 h prior to sacrifice. Paraffin sections were processed for double immunofluorescent staining with antibodies to BrdU and ED1. As previously observed, there was an increase in ED1+ macrophages at both day 1 and day 2 post-sectioning. However, while BrdU+ cells were also numerous throughout tongue, few cells were double-positive. Therefore, ED1+ macrophages do not appear to proliferate in the lingual environment in response to denervation. We next examined whether the increase in ED1+ macrophages might be due to proliferation of resting, resident ED2+ macrophages that convert to an activated phenotype. Yet there were also few ED2+/BrdU+ cells after nerve section, suggesting that proliferation of ED2+ macrophages does not account for the increased ED1+ population at these time points. We are currently examining the possibility that circulating ED1+ cells enter the tongue in response to denervation. Resolving this issue will increase our understanding of potential immune mechanisms that regulate peripheral taste function.

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Slide: Beidler Colloquium on Taste Transduction

The mammalian amiloride-insensitive non-specific salt taste receptor is a vanilloid receptor-1 variant

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The amiloride-insensitive (AI) non-specific salt taste receptor is the predominant transducer of salt taste in some mammalian species, including humans. The physiological, pharmacological and biochemical properties of the AI salt taste receptor were investigated by RT-PCR, direct measurement of unilateral apical Na⁺ fluxes in polarized rat fungiform taste receptor cells (TRCs), and chorda tympani (CT) nerve recordings to lingual stimulation with NaCl, KCl, NH₄ Cl and CaCl₂, in both the rat model and the vanilloid receptor-1 (VR-1) knockout mouse model. We report that the AI salt taste receptor is a constitutively active non-selective cation channel derived from the *VR-1* gene. It accounts for all of the AI CT

response to Na⁺ salts and part of the response to K⁺, NH₄⁺ and Ca²⁺ salts. It is activated by vanilloids (resiniferatoxin and capsaicin) and temperature (>38°C), and is inhibited by VR-1 antagonists (capsazepine and SB-366791). In the presence of vanilloids, external H⁺ and ATP lower the temperature threshold of the channel. This allows for increased salt taste sensitivity without an increase in temperature. VR-1 knockout mice demonstrate no functional AI salt taste receptor and no salt taste sensitivity to vanilloids and temperature. We conclude that the mammalian AI non-specific salt taste receptor is a VR-1 variant.

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Slide: Beidler Colloquium on Taste Transduction

Residual responses to bitter, sweet and umami compounds in TRPM5 knockout mice

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To determine the role of TRPM5 in taste responses *in vivo*, we generated TRPM5 knockout (KO) mice by homologous recombination in ES cells. Two-bottle preference tests of wildtype (WT) versus TRPM5 KO mice showed reduced, but not abolished, responses of the KO mice to the bitter compounds quinine and denatonium. By this test the TRPM5 KO mice were indifferent to concentrations of quinine and denatonium up to 1 and 10 mM, respectively, then showed strong avoidance to higher concentrations. A brief access test using a gustometer confirmed that high concentrations of denatonium were aversive to the KO mice. The KO mice were indifferent to low concentrations of denatonium and to all concentrations of quinine. With sweet compounds, two bottle preference tests of TRPM5 KO mice showed reduced preference for sucrose and the artificial sweetener SC45647. However, in the brief access test, TRPM5 KO mice were indifferent to these compounds. Post-ingestive effects (two bottle preference test) and/or lower sensitivity (gustometer) may affect the behavioral responses elicited by these behavioral tests and explain this apparent discrepancy. Gustatory nerve recordings are in progress to further investigate this. The TRPM5 KO mice showed residual preference for 100 and 300 mM monosodium glutamate (MSG; umami) by two-bottle preference test, but not by lickometer. Both behavioral tests showed that the TRPM5 KO mice avoid 1 M MSG. The behavioral responses to HCl elicited by either test were identical in the KO and WT animals. Together, these results indicate that TRPM5 is an important component of the taste response to bitter, sweet and umami compounds, but that additional TRPM5-independent response pathways exist.

Slide: Beidler Colloquium on Taste Transduction

Dual regulation of the taste transduction ion channel TRPM5 by Ca²⁺ and PIP₂

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The transduction of taste is a fundamental process that allows animals to discriminate nutritious substances from those that are noxious. Three taste modalities, bitter, sweet and amino acid, are mediated by G-protein coupled receptors. Recent evidence suggest that these receptors signal through a common transduction cascade: receptors activate phospholipase C (PLC), leading to a breakdown of phosphatidylinositol-4,5-bisphosphate (PIP₂) into diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP₃), which causes release of Ca²⁺ from intracellular stores. The ion channel, TRPM5, is essential for transduction of bitter, sweet and amino acid tastes, but the mechanisms which it is activated are not well understood. We find that when expressed heterologously in HEK 293, TRPM5 forms an outwardly rectifying cation channel that is activated downstream of Gq-coupled membrane receptors. In excised patches from CHOK1 cells expressing TRPM5, channel activity was induced by micromolar concentrations of intracellular Ca²⁺, but not by DAG or IP₃. Sustained exposure to Ca²⁺ desensitized TRPM5 channels. Application of the membrane phospholipid, PIP₂ enhanced the current following desensitization, but not before desensitization, suggesting that loss of PIP₂ may underlie desensitization. These data allow us to propose a model for G-protein-coupled taste transduction in which Ca²⁺ serves as the second messenger linking receptor signaling to membrane depolarization.

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Slide: Beidler Colloquium on Taste Transduction

Deorphanization and functional snp analysis of TAS2R bitter taste receptors

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Taste sensitivities to different bitter compounds vary between individuals. The most prominent example for genetically determined taste differences is the bitter taste of phenylthiocarbamide (PTC) and 6-n-propylthiouracil (PROP). A recently published study (Kim *et al.*, *Science*, 299: 1221–1225) showed a good correlation between the human PTC taster and non-taster phenotype and three single nucleotide polymorphisms (SNPs) in the *hTAS2R38* gene, suggesting that these SNPs might cause the individual taste differences for PROP, PTC, and various other chemically related bitter compounds. Using functional expression and calcium imaging (Bufe *et al.*, *Nat. Genet.*, 32: 397–401) we demonstrated that the *hTAS2R38* taster variant can be activated by PTC. In addition, we identified ligands for four other human bitter taste receptors *hTAS2R10*, *hTAS2R16*, *hTAS2R43* and *hTAS2R44*. Analysis of the human genome SNP database and own sequencing efforts so far revealed 14 non-conserved SNPs in the coding region of these receptors. Using site directed mutagenesis we created corresponding receptor variants and are studying their effects in our heterologous expression system. Initial results revealed that several of the *hTAS2R38* SNPs altered the receptor function.

Symposium: Olfaction and Neurodegenerative Disorders

Olfaction and neurodegenerative disorders

R. Doty

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Numerous major neurological disorders are associated with olfactory dysfunction. Indeed, the first clinical sign of such diseases as Alzheimer's disease and idiopathic Parkinson's disease appears to be decreased olfactory function. In the case of Parkinson's disease, the prevalence of smell loss is greater than the prevalence of tremor, and is essentially equivalent to that of the other major motoric signs of the disease. Importantly, not all neurological or neurodegenerative disorders are associated with smell loss. Hence, olfactory testing can aid in the differential diagnosis of a number of disorders commonly confused on initial presentation, including Alzheimer's disease versus major affective disorder, and Parkinson's disease versus progressive supranuclear palsy. The olfactory evaluation of at-risk individuals, including relatives, may prove to be of considerable clinical value for initiating neuroprotective therapy early in disease development. This symposium provides an up-to-date overview of relationships between olfactory function and four major neurological diseases. The participants and discussants represent active researchers in this field, and bring a variety of perspectives and a wealth of information to this topic.

Symposium: Olfaction and Neurodegenerative Disorders

Longitudinal evaluation of olfactory function in Alzheimer's disease

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We evaluated the predictive utility of olfactory deficits in patients with minimal to mild cognitive impairment (MMCI). One hundred and fifty outpatients presenting to a Memory Disorders Center were recruited and followed at 6 month intervals. Sixty-three group-matched controls were followed annually. Patients had a mean (\pm SD) age of 67 ± 9.8 years and mean education of 15 ± 4.2 years. Mean age for controls was 65 ± 9.3 years and mean education was 16 ± 2.6 years. UPSIT scores were lower in patients (mean 31 ± 6.4) than controls (mean 34 ± 4.2). Baseline UPSIT scores were lower in converters to Alzheimer's Disease (AD; $n = 35$, mean 26 ± 8.2) than non-converters (mean 33 ± 4.7) (mean follow-up = 39 months). In Cox regression analyses, low olfaction scores predicted AD (Wald $\chi^2 = 15.9$, $P < 0.0001$). The UPSIT score remained a significant predictor ($P < 0.0003$, relative risk = 0.9 per UPSIT point change) even when age, sex and education were included as covariates, and when MMS was included as a covariate. ApoE e4 genotype did not differ between non-converters (22%) and converters (28%). UPSIT scores were correlated with right hippocampal volume ($r = 0.20$, $P < 0.03$). In Cox regression analyses, low olfaction scores predicted outcome (Wald $\chi^2 = 10.6$, $P < 0.002$, relative risk = 0.92 per point change) even when hippocampal volume, age and gender were included.

Olfactory deficits predicted conversion to AD in patients with MMCI, even after controlling for demographic, cognitive, and brain volumetric predictors. In addition, odor-induced fMRI activation is also being examined in controls and AD and MMCI patients.

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Symposium: Olfaction and Neurodegenerative Disorders

Olfactory system dysfunction in schizophrenia

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Psychophysical studies of olfaction have documented impairments in odor detection thresholds, identification, memory and hedonics in patients with schizophrenia. Similar deficits exist in non-ill family members. Despite evidence of behavioral impairment, there is little evidence that the neuroanatomical or neurophysiological substrates of olfaction are abnormal. We have conducted a series of studies examining the structural and functional integrity of the olfactory system in patients and their first-degree relatives. (i) Using acoustic rhinometry, we have shown a reduction of the left-posterior nasal cavity in male patients. (ii) Using high-resolution MRI, we found bilateral olfactory bulb volume reductions in patients, and unilateral right-sided reductions in family members. (iii) Following parcellation of the anterior ventromedial temporal lobe into perirhinal, entorhinal and temporal polar regions, patients exhibited selective gray matter volume reductions in the perirhinal and entorhinal areas bilaterally. (iv) Using air-dilution olfactometry, both patients and non-ill family members were observed to exhibit dose-dependent abnormalities in the olfactory evoked potential. There are thus primary structural and functional abnormalities in the olfactory system, which underlie the behavioral deficits seen in schizophrenia. These abnormalities appear to denote an endophenotypic genetic vulnerability marker, rather than an index of either clinical disease status or treatment. A greater understanding of the neurobiology of these olfactory deficits could offer clues to both the basic neuropathology and the genetic precursors of this disorder.

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Symposium: Olfaction and Neurodegenerative Disorders

Olfactory dysfunction in multiple sclerosis

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For many years it was assumed that multiple sclerosis (MS) was unaccompanied by olfactory dysfunction. While several early studies, including a pioneering study by Ansari in 1976, failed to find any olfactory deficits in this disease, more recent studies from our group have clearly demonstrated that some MS patients exhibit olfactory loss. In 1984 we reported that 23% of a group of 31 MS patients

exhibited smell dysfunction, as measured by the University of Pennsylvania Smell Identification Test (UPSIT). A decade later we presented clinical cases in which smell loss was a presenting sign of MS. In a subsequent series of studies in the late 1990s, we demonstrated that such loss was correlated with the number of MS-related plaques in subtemporal and subfrontal brain regions, but not in other brain regions. Moreover, we showed that olfactory function waxes and wanes longitudinally in accordance with the number of active plaques in these brain regions. In this presentation, I review these studies, as well as initial results of a 5-year-long study of 73 MS patients and controls, in which a range of sensory and neuropsychological tests were administered, including ones tapping olfactory (i.e. odor identification, detection and discrimination), gustatory (e.g. regional taste identification), auditory (e.g. pure tone thresholds, competing words, auditory figure-ground), vestibular (e.g. Rhomberg eyes open and closed tasks), and cognitive (e.g. Wechsler Memory Scale-Revised; National Adult Reading Test; Wisconsin Card Sorting Test) functions.

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Symposium: Olfaction and Neurodegenerative Disorders

Olfaction in Parkinsonism

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There has been gradual increase of interest in olfaction since it was realized that anosmia was a common feature of idiopathic Parkinson's disease (IPD) and Alzheimer-type dementia (AD). It is an intriguing possibility that the first sign of a disorder hitherto regarded as one of movement or cognition may be that of disturbed smell sense. In this review of parkinsonian syndromes the following observations can be made: (i) olfactory dysfunction is frequent and often severe in IPD and may precede motor signs of IPD; (ii) normal smell identification in IPD is rare and should prompt review of diagnosis unless the patient is female with tremor dominant disease; (iii) olfactory impairment in suspected progressive supranuclear palsy or corticobasal degeneration is atypical and should likewise provoke diagnostic review; (iv) impaired smell sense is seen in some patients at 50% risk of parkinsonism; and (v) biopsy of olfactory nasal neurons reveals non-specific changes in IPD and at present will not aid diagnosis.

Poster: Development of the Gustatory System

EGF signaling in patterning fungiform papillae in embryonic rat tongue

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In previous experiments to understand how papillae form in patterns on the embryonic tongue, we demonstrated roles for the morphogen, sonic hedgehog (Shh) in fungiform papilla development. Fungiform papillae form in doubled numbers and atypical posterior locations in tongue cultures when Shh signaling is selectively disrupted with the alkaloid, cyclopamine. To further understand molecular regulation

and interactions in papilla formation, we are studying effects of epidermal growth factor, EGF. A polyclonal antibody to EGF receptor (EGFR) was used to immunolocalize EGFR in rat embryo tongues at gestational days (E) 13–18. Also, in whole tongue cultures begun at E14, we examined effects of exogenous EGF on fungiform papilla development and on the cyclopamine-induced change in papilla patterning. EGFR localized in all layers in the dorsal epithelium of embryonic rat tongue at all ages examined. Immunoproducts were weak and homogeneous in the tongue epithelium at E13–E14. Signals became progressively more intense in the inter-papilla space, but not within the papilla epithelium, in association with papilla development from E15–E18. In E14+2 day cultures, EGF dose-dependently decreased the number of fungiform papillae while stimulating epithelial proliferation. Moreover, pre-incubation with EGF, followed by culture with EGF plus cyclopamine, prevented the cyclopamine-induced change in papilla pattern. The data demonstrate that EGF signaling plays an important role in spacing of fungiform papillae in embryonic tongue, and interacts with Shh signaling in regulating development of papilla pattern.

Supported by NIH NIDCD grant DC000456 to C.M.M.

Poster: Development of the Gustatory System

Taste bud primordia develop in rodent tongue cultures

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Lingual taste buds form within papillae in mammals. We are studying the molecular aspects of taste bud differentiation during development. Keratin 8 is expressed in epithelial cell clusters in distinctive patterns across the developing tongue in mouse embryos before the innervation of tongue epithelium. To learn whether keratin 8 expression proceeds *in vitro* as *in vivo*, we examined the expression patterns of keratin 8 in mouse tongue cultures. Tongue or lower jaw explants were taken from gestational days 11.5 and 12.5 mouse embryos and maintained at liquid-gas interface in conventional organ cultures. Explanted tongues were harvested after 1–6 days in culture and assayed for keratin 8 by immunocytochemistry. In E11.5 and 12.5 mouse tongue explants, keratin 8 is broadly expressed in the lingual epithelium, as *in vivo*. Over the next 24–48 h, keratin 8 becomes restricted to clusters of cells located in the center of epithelial placodes. Surprisingly, keratin 8 continues to be expressed in few clusters after 6 days of culture. These results establish that much of taste bud development is nerve dependent in mammals. I am currently examining the gene repertoire expressed in tongue placodes that may possibly play a role in the determination of taste buds.

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Poster: Development of the Gustatory System

Identification of developmentally regulated genes expressed in taste buds

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During their differentiation and development, taste cells express a variety of molecules in spatially and temporally regulated fashion. Although several markers selectively expressed in mature taste cells have been identified, few markers of developing taste cells are known, making it difficult to study how taste cells develop and mature from stem cells and precursor cells. To search for genes selectively expressed during mouse taste cell development we used Representational Difference Analysis (RDA) and Affymetrix gene chip analysis to compare mRNAs from circumvallate (CV) papillae ('taste') versus surrounding non-sensory lingual epithelial tissue ('non-taste'). Most of the genes cloned by RDA, including small proline rich proteins and mesothelin, were also found by Affymetrix gene chips. Selective expression of these genes was confirmed by semi-quantitative RT-PCR. Those genes confirmed to be differentially expressed in taste versus non-taste were examined by *in situ* hybridization. To date, we have identified >20 genes, including TRPM5 and G gamma 13, that are selectively expressed in adult taste cells. We carried out *in situ* hybridization on a series of CV sections from early through mature stages of taste bud development to examine temporal and spatial patterns of expression of these genes. We have identified several marker genes for taste cells which were expressed in one of two patterns: (i) early through late stages; and (ii) only in the late stage of taste cell development.

Poster: Development of the Gustatory System

Relationship between expression of postsynaptic density protein 95 and the development of taste buds in the circumvallate papillae of rat compared with G-gustducin and protein gene product 9.5

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It is well known that neurotrophic factors are necessary for formation, development and maintenance of mammalian taste buds, and that type III taste cells have synaptic connections with gustatory nerves. However, it is unclear how synapses develop in taste buds. In this study we examined the distribution of postsynaptic density protein 95 (PSD-95) immunohistochemically in circumvallate papillae (CVP) in rats aged 0, 1, 2, 4, 5, 7, 14, 21 and 28 days postnatal and in adults. At adulthood, strong PSD-95-like immunoreactivity (PSD-95-LI) existed around the taste pore and some of them overlapped with neuron and type III taste receptor cell specific gene, protein gene product 9.5 (PGP 9.5). Type II cells are typically viewed as making contacts with nerve fibers, but they do not make classical synapses. However, we found that some cells having G α -gustducin-LI, considered as a marker of type II cells, also had PSD-95-LI. Developmentally, many taste buds existed in the CVP at birth, but PSD-95-LI did not occur until postnatal day 5. Thereafter, the density of PSD-95-LI increased progressively until it was similar to adults between PN 21–28 days. These results suggest that there may be subtypes of receptor cells based on the presence of synapses. Further, functional synaptic contacts in the CVP do not mature until after the third postnatal week.

Poster: Development of the Gustatory System**Neurotrophic factors regulate the sensitivity of geniculate axons to *Sema3a* during innervation**

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Geniculate axons begin to penetrate the rat dorsal lingual epithelium at E17, followed by trigeminal axons (Farbman and Mbiene, *J. Comp. Neurol.* 306: 172–186), despite the presence of epithelial *Sema3A* mRNA. Trigeminal axons are repelled by *Sema3A* *in vitro* through E18 (Dillon *et al.*, *J. Comp. Neurol.* 470: 13–24), suggesting that *Sema3A* repellent activity or sensitivity to *Sema3A* is regulated *in vivo*. The neurotrophic factors that stimulate geniculate axon outgrowth in collagen gels and the response of this outgrowth to *Sema3A* during late embryonic stages are not known. BDNF, NT4, GDNF and NGF (15–25 ng/ml) stimulate axon outgrowth above control media levels, although the NGF effect was small. BDNF stimulated the most rapidly advancing outgrowth, NT4 the slowest. In contrast to our results with the E18 trigeminal ganglion, *Sema3A*-transfected COS7 cells did not repel BDNF- ($n = 14$) and GDNF-stimulated ($n = 9$) outgrowth compared with control transfected COS7 cells ($n = 10$ for each). However, NT4-stimulated outgrowth ($n = 17$) was repelled ($P < 0.05$, ANOVA) compared with control cells ($n = 11$). Geniculate ganglia dissected on E17 and cultured in BDNF were repelled by *Sema3A* transfected COS7 cells ($n = 8$), compared with controls ($n = 7$). Thus, when geniculate axons are penetrating their targets *in vivo*, BDNF supported axons are downregulating their sensitivity to *Sema3A*. We are currently determining if lingual sensory axons are repelled by *Sema3A* *in situ* by using a gene gun to transfect dorsal lingual epithelial cells with *Sema3A* plasmids. As a first step, we have succeeded in transfecting the dorsal lingual cells with GFP.

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Poster: Development of the Gustatory System**Differences in trophic factor receptor and Eph expression may contribute to geniculate nerve divergence**

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The posterior auricular/chorda tympani (PA/CT) and the greater superficial petrosal (GSP) nerves diverge at the geniculate ganglion and innervate the outer ear/tongue and the palate, respectively. Divergence depends on differential responses to guidance cues and may result from differential expression of cue receptors. To determine if E11 mouse geniculate neurons differ in trophic factor receptor or Eph expression, we dissected single neurons labeled by applying dye to the PA/CT or GSP. We used antisense RNA amplification and PCR to assess receptor mRNA expression in 20–30 cells/nerve. TrkB and truncated TrkB were detected in every cell. TrkA and TrkC were expressed in more PA/CT neurons than GSP neurons, and the difference was significant ($P < 0.05$) for TrkC. P75 was not detected. GFR α -1, -2 and -3 were also expressed. Only GFR α -2 expression differed significantly between the GSP

(83%) and PA/CT (30%). We also compared the length and density of regenerating E13 rat PA/CT and GSP axons in the presence of each neurotrophic factor *in vitro*. Only BDNF, NT4, GDNF, and Neurturin stimulated axon growth. Neurturin promoted denser outgrowth from GSP than from PA/CT neurons, commensurate with the GFR α -2 data. The percentage of neurons expressing a receptor was otherwise not proportional to the outgrowth observed with the receptor's ligand. Regarding Eph receptors, A1, A3 and A6 were expressed in significantly more PA/CT than GSP neurons; the converse for EphA4. Thus, guidance cue receptors are differentially expressed in PA/CT and GSP neurons and will help identify divergence relevant cues.

Supported by NIH DC05253-02.

Slide: Development of the Gustatory System**Taste placodes are primary targets of early geniculate but not trigeminal peripheral nerve endings in the developing tongue of mouse embryos**

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The establishment of a reproducible pattern of connectivity is as essential to neuron's function as its physiological properties. Until recently innervation of gustatory papillae was thought to occur after their initial morphogenesis. However, new results from mammals indicate that much of molecular markers of papilla development are expressed prior to their overt formation. Given that markers of taste bud genesis begin to be expressed before papilla morphogenesis or its completion, we hypothesized that lingual epithelial placodes (which later will harbor the embryonic taste buds) are specific targets for taste afferents. To test this idea, we traced chorda tympani and lingual nerves with different lipophilic dyes and compared the patterns of their terminal arbors in tongues of mouse embryos from embryonic day (E) 11.5 to E14.5. The results indicate that chorda tympani, in contrast to lingual nerve fibers, pioneer the tongue at E11.5. Over the next 24 h, at E12.5, the tongue epithelium is still not contacted by nerves but the ramification patterns of both nerves in the tongue are identical. At E13.5 (when the first taste buds are apparent) chorda tympani axons make contacts with the epithelial placodes under which they exhibited distinctive terminal arbors. At E14.5 (when the fungiform papillae first form) chorda tympani enter and reach the apical epithelium of the papilla where the embryonic taste buds form. These results indicate that taste buds form at the same time as they are innervated and will be discussed in the context of the current view of non-neural induction of taste buds.

Grant sponsor: NIDCD; grant number: DC03503.

Poster: Development of the Gustatory System**Roles for Hedgehog proteins in supporting neuron survival and neurite extension in embryonic geniculate and trigeminal ganglia**

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Sonic hedgehog (Shh) and other Hedgehog (Hh) proteins have known roles in regulating proliferation of neuron precursors, neuron differentiation and neurite outgrowth, and Schwann cell—neuron interactions. Because Shh is intensely localized in developing papilla placodes and fungiform papillae in embryonic rat tongue, we hypothesized that Shh, and/or other Hh proteins, also would have roles in regulating neuron number and neurite extension in the geniculate (gg) and trigeminal (tg) ganglia, which innervate these papillae. Ganglia were dissected from gestational day 13 and 16 rat embryos and explanted onto matrix-coated coverslips. Gg and tg cultures were maintained in standard medium (B27), with or without neurotrophins, BDNF or NGF, for 2–5 days. To test roles for Hh proteins in ganglion cultures, we added the alkaloid cyclopamine (CYCL), a specific blocker of hedgehog signaling at the receptor complex, to B27, BDNF or NGF culture media. In all cultures with CYCL, there were decreased numbers of ganglion neurons, and the extent of neurite outgrowth was reduced. CYCL effects were concentration-dependent (from 1 to 10 μ M). With immunohistochemistry we demonstrated that gg and tg neurons contain the hedgehog receptor proteins, patched and smoothened, both in cultures and *in vivo*. Another molecule in the signaling pathway, hedgehog-interacting protein, also was immunolocalized in the ganglia. The data provide evidence that members of the Hedgehog family support survival and neurite extension of embryonic gg and tg neurons.

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Poster: Development of the Gustatory System

Gustatory phenotype in double neurotrophin knockout mice

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BDNF and NT-3, but not NT-4, are expressed in developing gustatory papillae and taste buds and participate in establishing the gustatory and somatosensory innervation of the tongue. BDNF, NT-3 and NT-4 null mutated mice show deficits in their lingual gustatory and somatosensory innervation. We have generated double neurotrophin knockout mice and have analyzed the differences in the gustatory phenotypes of wild type, BDNF, BDNF/NT-3 and BDNF/NT-4 mice. Because BDNF/NT-3 and BDNF/NT-4 mice die at birth, we have focused our study on newborn animals. We have focused the present study on the number, average size and innervation of the gustatory papillae in these mice. Fungiform papillae numbers were decreased significantly in all mutant mice while BDNF/NT-3 mice exhibited the most severe phenotype. Size measurements revealed a slight decrease in the size of BDNF and BDNF/NT-4 fungiform papillae, but the strongest significant size reduction was observed in BDNF/NT-3 mice. Innervation measurements followed the same trend, with BDNF mice having the least severe loss of innervation, followed closely by BDNF/NT4 mice while BDNF/NT-3 mice exhibited the most severe phenotype. Taken together, we propose NT-3 dependent innervation plays crucial roles in maturation and maintenance of fungiform papillae while NT-4 phenotype is mimicked by BDNF in the anterior part of the tongue.

Poster: Development of the Gustatory System

Neuronal death in the rat geniculate ganglion during development

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The geniculate ganglion provides sensory innervation to taste buds on the anterior tongue and the palate, and to the skin of the ear. During vertebrate development other sensory ganglia undergo a period when neuron cell death occurs, often because of insufficient target-derived trophic substance for maintenance of all ganglionic neurons. We examined neuronal death in the geniculate ganglion to try to determine when critical developmental events might occur that would be related to the survival or death of geniculate neurons. Thus far, 10 μ m histological sections of geniculate ganglia from fetal (E17–E22) and newborn (P1–P3) rats have been examined following fixation in 4% paraformaldehyde and standard staining procedures. Earlier stages are currently under investigation. At each age total numbers of neurons and pycnotic neurons were counted in eight ganglia. The average incidence of pycnotic neurons at E17 was 9% of total neurons. The percentage then decreased progressively, leveling off at 0.2% by E22. Findings suggest that critical events related to neuronal survival occur from at least E17 to E21, when neuronal death is elevated. Trophic substances required for survival might be present in limited amounts so that only those neurons that receive sufficient amounts will survive. These trophic factors could originate from several sources, including the neurons themselves, the supporting cells around the neurons in the ganglion, the peripheral targets or the central targets of the neuronal projections, or a combination of sources.

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Poster: Bitter Taste

Blocking glutamate receptors in the parabrachial nucleus reduces aversive oromotor responses to quinine in conscious rats

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Microinjection of glutamate into the classic gustatory region of the parabrachial nucleus (PBN), the 'waist' region (W), in conscious rats elicits ingestive oromotor behaviors, but the exact functional role of glutamate in W is unclear. This pilot study was designed to examine taste reactivity responses during oral infusion of tastants while blocking glutamate neurotransmission in W. Cannula (Plastics One) were placed bilaterally into W in eight male Wistar rats. The cannula were connected to osmotic pumps (Alzet) that were filled with either aCSF ($n = 3$) or kynurenate (KYN, an ionotropic glutamate receptor antagonist) in aCSF [250 ($n = 2$), 10 ($n = 2$) or 2.5 mM ($n = 1$)]. The pumps delivered 0.25 μ l/h for 7 days. Rats also were implanted with intra-oral cannula for the infusion of tastants. After recovery from surgery and habituation to the behavioral arena, rats were videotaped on two successive days during 1 min (0.233 ml) oral infusions of dH₂O, 0.1 M NaCl and 0.03 M HCl

on the first day, and dH₂O, 0.1 M sucrose and 0.003 M quinine hydrochloride on the second day. Ingestive oromotor responses to all chemicals were similar in all groups, however aversive responses to quinine (e.g. gapes) were dramatically reduced in rats receiving infusions of 250 mM KYN (mean of 56 versus 2, $P < 0.01$). There also was a trend for the lower doses of KYN to reduce the number of aversive behaviors to quinine. Though preliminary, these data suggest that the activation of ionotropic glutamate receptors in W is important for the elicitation of gapes in response to oral stimulation with quinine.

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Poster: Bitter Taste

The time-course and specificity of long-term adaptation to a 'bitter' taste stimulus in mice

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Little is known about the extent to which mammals (including humans) can overcome their aversion to 'bitter' taste stimuli. Although several early reports indicated that the aversive response of rodents to sucrose octaacetate could be adapted through several months of dietary exposure, the time-course and specificity of this long-term adaptation phenomenon was not explored. We studied long-term adaptation to a harmless bitter taste stimulus (denatonium benzoate) in C57BL/6J mice. In experiment 1, the 'exposed' mice received water containing 6 mM denatonium as their only source of water for 21 days; the 'control' mice received untreated water. We recorded licking responses of the mice to three concentrations of denatonium (0, 3 and 10 mM) on days 1, 7, 14 and 21 of the experiment. After 7 days, the aversive response to denatonium in the exposed mice was significantly diminished (but not abolished), whereas that in the control mice was unchanged. The magnitude of the aversive response in both groups remained stationary over the next 14 days. In experiment 2, we examined the specificity of the adaptation phenomenon by determining whether it generalized to isoaversive concentrations of three other 'bitter' taste stimuli: quinine, sparteine and caffeine. We found that the aversive response to quinine and sparteine (in the exposed mice) was partially adapted, whereas that to caffeine was completely adapted. This latter finding indicates there are multiple bitter signaling pathways, and that chronic exposure to denatonium adapts some of these bitter signaling pathways more effectively than others.

Poster: Bitter Taste

Covariation in taste responses to multiple bitter stimuli in rats

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It has been proposed that bitterness constitutes a unitary taste quality based on molecular findings that many bitter taste receptors are expressed within the same receptor cells and behavioral evidence

that rats fail to discriminate between certain bitter compounds. Conversely, other studies have shown specificity of taste receptor cell responses to different bitter substances and have suggested that humans and rats can distinguish among certain stimuli within the bitter category. We examined behavioral responding to various bitter tastants [quinine-HCl, denatonium benzoate, cycloheximide, MgCl₂] in short-term lick tests in rats to assess the degree of covariation among responses to these stimuli and infer commonalities in their receptor and neural mechanisms. Rats were tested with a given pair of bitter stimuli during three sessions comprising randomized trial blocks of six concentrations of each stimulus + deionized water. Psychophysical functions were generated for individual rats for respective stimulus pairs and concentrations of each stimulus that produced 50% suppression of licking relative to water were correlated across subjects. Behavioral sensitivity to quinine was highly correlated with that to denatonium and cycloheximide ($r_s > +0.9$) but not with responses to MgCl₂. These results suggest overlap in the coding of bitter taste information for the former three compounds but heterogeneity in the mechanisms for quinine versus bitter salts, consistent with taste discrimination studies in rats. Additional bitter stimuli will be tested.

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Poster: Bitter Taste

PLC2 knockout mice display lick avoidance to high concentrations of quinine and denatonium

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The T1R and T2R families of taste receptors apparently mediate taste receptor cell responsiveness to 'sweet' and 'bitter' compounds, respectively. Yet these two distinct families are believed to share key components of their signaling pathways, namely TRPM5 ion channels and an isoform of phospholipase C (PLCβ2). There is evidence that mutant mice lacking either PLCβ2 or TRPM5 are completely unresponsive to these compounds. We sought to confirm the behavioral response characteristics of PLCβ2 knockout (KO) mice and wild-type (WT) controls by using a brief-access taste test. Unconditioned licking responses of KO and WT mice were measured to sucrose (S), sodium chloride (N), quinine hydrochloride (Q), denatonium benzoate (D) and citric acid (C). KO mice were unresponsive to S, whereas WT mice showed concentration-dependent increases in licking. The concentration-response functions of KO mice were shifted to the right for both Q and D, but these animals nonetheless clearly showed lick suppression at higher concentrations. There were no significant differences between the C concentration-response functions from WT and KO mice. The results for N were somewhat more complex, but differences between WT and KO mice were modest. Thus mice can respond to higher concentrations of Q and D in the absence of PLCβ2, suggesting that these 'bitter' stimuli can activate additional transduction pathways that do not depend on this enzyme.

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Poster: Bitter Taste**Functional characterization of human T2R bitter receptors**

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To characterize the functional properties of members of the human T2R family of bitter receptors, we are using an *in vitro* reconstitution assay. Baculoviral expression vectors were constructed with the human T2R genes and the mouse cycloheximide receptor gene, mT2R5. Western analyses with cellular membranes isolated from insect cells infected with these baculoviruses indicate that the membranes are highly enriched in bitter receptors. To validate our methods, we demonstrated functional reconstitution of the mT2R5 receptor by the addition of cycloheximide and purified G-proteins to mT2R5-enriched membranes, as previously reported. We are currently screening orphan members of the hT2R family with a panel of 60 bitter-tasting compounds to uncover additional ligand-receptor interactions. Thus far, we have identified a bitter receptor that selectively responds to denatonium and another receptor that displays a broader response profile, responding to several of the bitter-tasting compounds tested. The pharmacophore for the seemingly more promiscuous receptor is under investigation. Given that the identities and concentrations of both the ligands and G-proteins added in the assay can be controlled, this approach should allow the quantitative assessments of the ligand binding properties and the G-protein selectivities of these receptors.

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Slide: Bitter Taste**The evolutionary diversity of bitter taste**

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Bitter taste is a complex topic viewed from perspectives of chemical, receptor, behavioral and species diversity. Many chemically diverse compounds are bitter to humans, and multiple G-protein coupled receptors (GPCRs), the T2 candidate bitter receptors, have been identified. We are conducting a series of studies addressing which of those compounds may be 'bitter' to golden hamsters (*Mesocricetus auratus*). Behavioral cross-generalizations indicate that the ionic denatonium and MgSO₄, and non-ionic caffeine and sucrose octaacetate (SOA) have distinct sensory qualities to hamsters. In hamsters only the ionic stimuli activate the chorda tympani nerve (CT) and cross-generalize with quinine, the human bitter prototype. Some inbred strains of golden hamsters, e.g. ACNT, prefer non-ionic SOA and caffeine, which taste bitter to humans. Hamster behavioral preference and CT neural thresholds for denatonium are five orders of magnitude higher than human thresholds. Thus, although denatonium may be 'bitter' to hamsters and humans, the receptors are likely species-specific. Simple salts such as MgSO₄ are also bitter and other mechanisms must be considered; nonetheless, candidate T2 GPCRs likely contribute to the observed bitter

diversity within and across species. By analyzing nucleotide and amino-acid sequences published in the NCBI genetic database, we are working to develop models to recognize orthologous (between species) and paralogous (within species) variation among T2s in order to sort out bitter diversities.

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Poster: Bitter Taste**High resolution mapping of the bitter taste sensitivity locus *Qui***T.M. Nelson¹, S.D. Munger¹ and J.D. Boughter¹*Anatomy & Neurobiology, University Maryland School of Medicine, Baltimore, MD, USA and* ²*Anatomy & Neurobiology, University Tennessee Health Science Center, Memphis, TN, USA*

Although several genes have been implicated in the detection and transduction of taste stimuli, there is still a sizable gap in our understanding of the mechanisms of taste transduction and sensory coding, as well as how taste-related genes combine to underlie behavioral responsiveness. To better understand the genetic basis of bitter taste behavior, we have sought to identify genes that contribute to specific bitter taste sensitivities. Using a brief-access taste test, which minimizes non-gustatory variables, we determined the taste sensitivities of BXDTy recombinant inbred mouse strains [as well as their parental strains, C57BL/6J (B6) and DBA/2J (D2)] to several bitter stimuli. Our initial interval mapping of the BXDTy dataset confirmed a significant quantitative trait locus (QTL), *Qui*, for quinine taste sensitivity (e.g. Lush, 1986) on distal Chr 6. We have defined the physical interval that contains *Qui*. This interval includes a cluster of 24 *Tas2r* genes encoding T2R putative bitter taste receptors. Comparisons of the protein coding sequences across B6, D2 and BXDTy lines revealed distinct B6 and D2 alleles for a number of *Tas2r* genes; these allelic variants correlate with the taster status of individual BXDTy lines. These studies should facilitate the identification of major quantitative trait genes underlying bitter taste.

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Poster: Bitter Taste**Relationship between genotypes of the TAS2R38 gene and bitter perception in**

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The present study aimed to determine how variation in the TAS2R38 gene influences the gustatory experience and preferences of children and adults for bitterness. To this aim, genotype and behavioral analyses were performed on 5- to 10-year-old children of African (43 girls, 44 boys) or European (31 girls, 23 boys) descent and their mothers. Genomic DNA was extracted from cheek cells and alleles of the TAS2R38 gene were genotyped for the variant sites using allele-specific probes and primers. Forced-choice procedures embedded in the context of a game were used to determine sensitivity to the bitter taste of 6-n-propylthiouracil (PROP) during one test session and food habits during another. Regardless of race,

alleles at TAS2R38 were significantly associated with PROP thresholds in children ($P < 0.00001$) and adults ($P < 0.00001$). Other genetic-related differences in food habits and mother-child interaction will be presented. These findings highlight the importance of studying children.

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Poster: Bitter Taste

Bitter taste markers identify sweet and alcohol hedonics and intake

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Human and animal data suggest connections between sweet and alcohol behaviors. We tested the ability of markers of variation in taste to identify preference for and intake of sweet foods and alcoholic drinks. Following the pioneering work of Fischer *et al.* from the 1960s, taste variation was characterized by bitterness of 6-n-propylthiouracil (PROP) and quinine hydrochloride (QHCl). Bitterness varies genetically and across the lifespan (e.g. hormonally, exposure to taste pathology). Adults (74 F, 51 M; 22–58 years) used the general Labeled Magnitude Scale to rate bitterness of 3.2 mM PROP and .32 mM QHCl and preference for sampled and survey sweet foods. Subjects reported alcohol and sweet food intake via frequency interview and energy from added sugar via food records analyzed with the USDA Pyramid Servings Database. Multiple regression revealed that lower preference for or intake of sweets and alcohol was predicted by greater PROP bitterness but lower QHCl bitterness. Although PROP and QHCl showed significant correlation, subjects fell into groups that were discordant in these bitters but nearly matched for sex. Those who tasted QHCl as more bitter relative to PROP ($n = 23$) had significantly greater preference for and intake of sweets and greater alcohol intake than those who taste PROP as more bitter relative to QHCl ($n = 24$). In summary, use of both bitter makers increased the ability to explain sweet and alcohol behaviors. Whether quinine bitterness reflects another genetic taste variant or an environmentally-mediated pattern of sensation is unknown.

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Poster: Olfactory Bulb: Coding

Inhibition of adenylyl cyclase in lobster olfactory receptor neurons enhances central responses to odors

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Olfactory receptor neurons (ORNs) in some animals can be inhibited as well as excited by odorants. The question is whether such bipolar input is functionally significant in olfaction. Pharmacological intervention of the cyclic nucleotide signaling cascade in lobster ORNs selectively alters inhibitory responses to odorants. As a first step to addressing this question, we assessed the effect of

blocking adenylyl cyclase (AC) peripherally on the output of the first olfactory relay, the olfactory lobe (OL), in a perfused lobster nose-brain preparation. We show that pharmacologically blocking AC in the periphery had no effect by itself, but could enhance the response of the OL to odorant mixtures, consistent with the predicted reduction in odorant-evoked inhibitory input that should follow AC blockade. Blocking AC could also enhance the response of the OL to single component odorants. Given earlier evidence that monomolecular odorants excite and inhibit different cells, the latter finding suggests that spontaneous activity in the ORNs may be sufficient to register peripheral inhibition in the OL in the absence of co-activation by other odorants. Our results suggest that inhibitory input to the olfactory periphery helps shape the output of the OL and, therefore, potentially contributes to the olfactory code.

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Poster: Olfactory Bulb: Coding

Inter- and intra-species antennal imaginal disc transplants: behavior, sensory and central olfactory neurophysiology

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Vertebrate and invertebrate organisms share a common feature in having the primary olfactory neuropil organized into discrete knots known as glomeruli. The macroglomerular complex (MGC) is a sexually dimorphic cluster of male-specific, pheromone-receptive glomeruli that is configured differently between moth species. The physiology and arrangement of MGC glomeruli is dictated, in part, by the antennal imaginal disc. We have previously demonstrated that pre-metamorphic inter-species transplantation of antennal imaginal discs resulted in the development of a 'donor' type MGC. Our current study seeks to gain further insight into the factors affecting changes in olfactory physiology and behavior through unilateral transplantation of: (i) a *Heliothis virescens* disc into *Helicoverpa zea* (V-Zr); and (ii) a *H. virescens* disc into a *H. virescens* recipient (V-Vr). To test moth behavioral discrimination, unoperated antennae were removed and the ability of moths to differentiate between each species' pheromone blends was tested in a wind tunnel. Both transplant types had similar, unusual sensory neurons tuned to Z9-16:Ald, an essential component of the normal *H. zea* pheromone blend. However, only V-Zr males responded behaviorally to blends containing this compound. Cobalt-lysine stains of the sensilla housing these unexpected sensory neurons and recordings from central interneurons suggested that this behavioral difference was the result of the specific MGC glomeruli activated by this compound.

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Poster: Olfactory Bulb: Coding

Macroglomeruli in the worker caste of leaf-cutting ants

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Workers of leaf-cutting ants express an extraordinary size polymorphism with a factor of 1000 in body mass. Foraging workers show less variation but still can be discriminated in minor and medium workers. We asked the question whether these workers differ in (i) their morphology of the first olfactory neuropil, the antennal lobe; (ii) their olfactorial behavior to trail pheromones; and (iii) their receptor neuron response to trail pheromones. We found, for the first time in nonsexual individuals, a greatly enlarged glomerulus. The comparison of two closely related species *Atta sexdens* and *Atta vollenweideri* by 3-D reconstructions of the antennal lobes revealed striking similarities as well as very distinct differences in the arrangement of macroglomeruli among the two species. Size polymorphism is found in antennal lobe structures, the glomeruli. While medium workers have a macroglomerulus, the glomeruli of the minor workers are all of similar size. We tested the antenna in EAGs with two common and main components of the trail pheromone of leaf-cutting ants (4-methylpyrrol-2-carboxylate and 2-ethyl-3,5-dimethylpyrazine) and found that the relative response to those two components differs significantly. If a larger glomerulus reflects a larger number of terminating receptor neurons, this result supports the idea that the macroglomerulus is involved in trail detection. In behavioral tests we found that trail following behavior is somewhat lower in minor workers than in medium workers. Surprisingly, in a choice experiment with gland extracts of nestmates and of the comparison species the minor workers outperformed the medium workers.

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Poster: Olfactory Bulb: Coding

The effects of stimulus dynamics on olfactory lobe responses in the crayfish, *Procambarus clarkii* using ensemble recording techniques

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Two major issues facing sensory ecologists are how olfactory information is coded in the nervous system and that information correlates with behavior. Behavioral results from our lab indicate that crayfish are sensitive and respond to temporal components of an odor signal. As such, we expect to find that underlying this sensitivity to stimulus dynamics, are neural correlates evident within the olfactory system. Thus, in this preliminary study, we investigated how the temporal aspects of an odor signal are coded in the olfactory lobe of the crayfish. Neural ensemble recordings were made on an isolated head preparation perfused with oxygenated crayfish saline. Silicon multichannel electrode arrays were inserted into the olfactory lobe of the crayfish brain. The medial antennule was placed into an olfactometer and stimulated with three types of stimuli: glutamate, glycine and shrimp extract. The stimuli were presented at a specific molar concentration (10^{-5} M) and duration (500 ms), varying only the intermittency between odor pulses. Our results suggest that coordinated clusters of units, which collectively produced odor-dependent responses but these responses were further dependent on stimulus intermittency. These results are consistent with our behavioral data demonstrating that crayfish are sensitive to the manner in which odors are experienced.

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Poster: Olfactory Bulb: Coding

The effect of stimulus duration on euclidian response distance measures of odor discrimination across antennal lobe populations in *Manduca sexta*

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Behavioral studies suggest that animals such as the moth *Manduca sexta* perceive subtle differences between odorant based on changes molecular features such a carbon chain length. Recent theories of temporal coding predict that closely related odors are 'decomposed' over time and that longer duration stimulations should provide additional information used by the antennal lobe (AL) or olfactory bulb to enhance discrimination. To test this we measured the degree to which AL ensembles could statistically discriminate closely related odors as a function of increasing stimulus duration. Multiunit recordings from moths ALs were analyzed in response to six alcohols and ketones that were repeatedly presented at odor pulse durations ranging from 50 to 4000 ms. Principle Components Analysis, on binned data (10 ms), extracted orthogonal factors each representing a collection of units with common and coordinated responses. General Linear Modeling identified factors with odor-dependent temporal effects ($P < 0.001$). Odor-dependent factors were treated as independent dimensions and the Euclidian distance between odor responses at bin was calculated in a high dimensional space. We find that population trajectories rapidly peak (animal specific ~120–240 ms) and does so at the same time irrespective of stimulus duration. For longer durations, trajectories do tend to stay separated for the duration of the pulse length. These preliminary results suggest that olfactory systems have the capacity to represent odorant identity of subtly different odors rapidly.

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Poster: Olfactory Bulb: Coding

Characterization of labeled cells in the olfactory bulb of transgenic zebrafish expressing the simian cytomegalovirus promoter

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The cytomegalovirus (CMV) promoter is commonly used for the production of transgenic animals due to its effective and essentially ubiquitous expression. We used a simian CMV (sCMV) promoter to drive the expression of a fluorescent reporter protein, dsRed, in zebrafish. Expression of the sCMV promoter has been confirmed in several regions throughout the nervous system including a specific subset of cells in the olfactory bulb. These olfactory bulb cells were among the first to express the transgene early in development. The

purpose of this study was to examine the morphology, distribution, and identity of the labeled cells in the olfactory bulb using confocal microscopy, immunocytochemistry, and retrograde tract-tracing methods. The labeled cells have teardrop-shaped somata that are 5–10 μm in diameter. The cell bodies are found throughout the glomerular and superficial internal cell layers, and they possess a single prominent process containing tufts in the glomerular layer. Although these cells are found in the same location as mitral cells, they do not appear to be output neurons since retrograde labeling of the olfactory tracts with a fluorescent dextran identifies a different subset of bulbar cells. We are continuing our exploration of the identity of the bulbar neurons that are labeled in these transgenic zebrafish.

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Poster: Olfactory Bulb: Coding

Cadherin and catenin expression in the olfactory nerve

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Olfactory sensory neurons extend axons to the olfactory bulb, where they form the olfactory nerve layer (ONL), which is subdivided into outer (ONLo) and inner (ONLi) sublaminae. In the ONLo axons primarily undergo extension and gross targeting while targeting specific glomeruli occurs within the ONLi. Mechanisms for differential axonal adhesion within the ONLi versus the ONLo are not known. However, the cadherin family of molecules are candidates. Together with their intracellular binding partners, the catenins, they mediate homotypic cell–cell adhesion and have been implicated in many roles during neural development, including axonal extension and specific circuit formation. Using immunohistochemistry, we have localized N-cadherin (CDH2) and several catenins in the mouse olfactory pathway. CDH2 and several catenins are more strongly expressed in the ONLo than the ONLi during perinatal development, a pattern that is absent in adulthood. γ -Catenin, which binds directly to cadherins and links to the cytoskeleton, is expressed strongly in the ONLo, as is δ -catenin, which binds directly to cadherins and modulates their function. In contrast, β -catenin, which competes with γ -catenin for cadherin binding, is expressed uniformly in the ONLo and ONLi, as is p120-catenin, which competes with δ -catenin. These findings suggest the presence of two different cadherin-based adhesion systems in the ONL, one of which contains CDH2, γ -catenin and δ -catenin, and helps restrict axons to the ONLo during extension. Preliminary evidence further suggests that intermediate filaments may be involved in the function of this adhesion system.

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Poster: Olfactory Bulb: Coding

Action potential backpropagation and modular processing of vomeronasal receptor input in rat accessory olfactory bulb

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In main olfactory bulb, receptor cells expressing one OR gene project to unique paired glomeruli. Mitral cells link to single glomeruli via simple primary dendrites which reliably propagate action potentials. Accessory olfactory bulb (AOB) exhibits more complex wiring: sensory neurons (VSNs) expressing one VR gene make divergent projections to many glomeruli, and mitral cells link to multiple glomeruli via branched primary dendrites. We applied Ca-imaging to track backpropagating action potentials (BPAPs) in rat AOB primary dendrites. Under whole-cell dialysis with 100 μM Ca-orange, somatic spike-evoked fluorescent signals were detected over the entire dendritic tree; $\Delta F/F$ was nearly constant on all branches from soma to glomeruli. Backpropagation relied on Na^+ channels: in 1 μM TTX, somatic AP voltage-clamp commands evoked dendritic Ca-transients that declined significantly with distance compared with current-clamp controls. Dual soma–dendrite recording confirmed that BPAPs were unattenuated, while sub-threshold voltage transients declined markedly. Ca-transients were not significantly altered by 100 μM APV or 50 μM bicuculline, suggesting BPAPs are unaffected by local synaptic input. Genetic tracing in AOB has suggested homotypic connectivity—individual dendritic arbors project only to glomeruli targeted by VSNs expressing the same VR gene. Non-decremental, non-dichotomous backpropagation in AOB primary dendrites ensures fast, reliable communication between mitral cells and their homotypic glomeruli, binding them into coherent modules in accordance with their VR-coded inputs.

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Poster: Olfactory Bulb: Coding

Mitral/tufted and granule cell response specificity in the mouse olfactory bulb

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Odor representations passed from the olfactory bulb to higher brain centers are contained in the firing patterns of olfactory bulb mitral/tufted (M/T) neurons. The degree of stimulus specificity of M/T neurons has important implications for distinguishing between competing hypotheses of olfactory coding. Assessing stimulus specificity has been difficult due to the huge size of 'odor space': the range of potentially active volatile chemicals that could activate a neuron. To examine M/T response specificity, we use a computer-controlled, robotic odor delivery system that allows fast and simple application of hundreds of odorants with precisely controlled dilution and timing. Extracellular single unit recordings from the mouse OB *in vivo*, using odorant concentrations within a behaviorally relevant range, revealed that M/T responses are extremely specific. When presented with a large panel of structurally diverse and closely related categories, M/T neurons typically respond to <5% of stimuli, and usually to just a single odorant. This implies that M/T cells are not broadly tuned. In contrast to M/T neurons, granule cells (GCs) responded to a much broader range of odorants. At stimulus concentrations producing sparse M/T responses, a large percentage (30–50%) of odors produce responses in GCs. This is consistent with the idea that GCs integrate inputs

from many M/T cells and provide widespread local inhibition within the OB.

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Poster: Olfactory Bulb: Coding

Responses of olfactory interneurons in the behaving odor-conditioned mouse

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Recording neural activity in a sensory system in a behaving animal has three important aspects: (i) in an awake animal feedback from higher brain areas is fully active, which can change sensory information processing even at early processing stages; (ii) the changes in the neural representation of an odor due to learning and plasticity can be recorded in real time; and (iii) the animal's behavior can report the result of sensory information processing. A microdrive with three motors moving three electrodes was implanted to record from the olfactory bulb of a mouse previously trained in two-odor discrimination task. We record nose pokes into the odor sampling port, the water reinforcement port, and stimulus delivery timing along with the single unit responses from olfactory bulb cells. After initial discrimination training, the mouse was trained to make an association between a new odor and water in one training session lasting 2 h. Temporal patterns of presumed mitral/tufted and granule cells were analyzed relative to the timing of nose pokes signaling onset of odor sampling or results of olfactory computations (responses at the water port). As an example of unexpected behavioral modulation of units in olfactory bulb, cells were found that reduced their firing prior to execution of a nose-poke into the odor sampling port. As more units are recorded during odor-guided behavior, our analysis will be directed to understand the neural code for odor, the way it is read by the animal, and the way in which odor learning may modify it.

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Poster: Olfactory Bulb: Coding

Ontogeny of odor discrimination

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Individual olfactory bulb mitral/tufted cells respond preferentially to groups of molecularly similar odorants. Bulbar interneurons are thought to influence mitral/tufted odorant receptive fields (RFs) through mechanisms such as lateral inhibition. Infant rats, however, lack a majority of these inhibitory interneurons until the second week of life. It is unclear if these developmental differences affect mitral/tufted RFs or affect behavioral odor discrimination. The following experiments aimed at better understanding olfactory bulb mitral/tufted cell receptive fields, odor coding and behavioral odor discrimination in the developing olfactory system. Single-unit and local field potential recordings were made from mitral/tufted cells of freely breathing urethane-anesthetized rats [postnatal day (P) 4–adult]. RFs to a homologous series of esters of different carbon chain lengths were mapped for each age group. Odorants were equated for concen-

tration (150 p.p.m.) using a flow dilution olfactometer. Preliminary results suggest minimal differences between infant and adult single-unit mitral/tufted RFs. However, odor-evoked local field potential oscillations showed a strong age-dependent change in dominant frequency over the age range tested. Behavioral odor discrimination to the same set of odorants is currently being examined using our cardiac orienting response paradigm, which is effective even in very young (P4) animals.

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Poster: Olfactory Bulb: Coding

Effects of functional group position on glomerular activation patterns evoked by ester and alcohol odorants

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Studies of the relationship between odorant structure and bulbar activity patterns have identified glomerular modules that recognize discrete odorant molecular features such as functional groups. In the present experiments, we asked if the position of functional groups within odorants influences spatial activity patterns. By mapping uptake of [¹⁴C]2-deoxyglucose across the entire glomerular layer, we compared the influences of functional group position and carbon number using systematically differing series of 21 alcohols and 16 esters. Along every carbon number and positional series, representations were chemotopic, with the most similar chemicals evoking the most similar patterns. However, the relative impact of the position of the functional group differed greatly for esters and alcohols. Changing the position of the ester functional group determined whether or not particular glomerular modules were activated, but caused only small differences in the overall glomerular activity pattern. In contrast, changing the position of the alcohol functional group evoked very distinct patterns involving entirely different groups of glomerular modules. Thus, the position of functional groups is an important feature determining patterns of glomerular activity, although the nature of the difference in the evoked response depends on the functional group.

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Poster: Olfactory Bulb: Coding

Responses to ketones are not organized chemotopically within a ketone-responsive glomerular module

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We have shown previously that every odorant activates a unique combination of glomerular modules in the rat olfactory bulb. Some of the modules appear to respond to a range of odorant molecules sharing a chemical feature such as a functional group. Within modules responding to aldehydes, alcohols and acids, responses are arranged chemotopically with respect to molecular length. Ketones activate a module located dorsomedially in the bulb and in the present study,

we investigated the possibility of a chemotopic organization within this module. We mapped uptake of radiolabeled 2-deoxyglucose in the olfactory bulbs of rats during stimulation by straight-chain ketone odorants that differed systematically in their carbon numbers (2-pentanone to 2-undecanone) or in the position of their carbonyl group (2- and 3-hexanone and 2-, 3- and 4-heptanone). As the carbon number increased, the pattern of activity in the posteriolateral, and medial parts of the bulb shifted toward ventral positions. However, unlike the responses of other functional groups, there was no change in the location of the responses within the ketone-responsive module as carbon number increased. Changing the position of other functional groups has been shown to affect odorant response patterns, but such changes were not seen within the ketone-responsive module. These data indicate that the organization of responses within the olfactory system differs across odorant functional groups.

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Poster: Olfactory Bulb: Coding

Informatics tools for global mapping of odor-induced neural activity in the glomerular layer of the rodent olfactory bulb

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Aims: we are developing computer software tools, OdorMapBuilder and OdorMapComparer, to generate and analyze odor maps in the rodent olfactory system. **Methods:** The software programs are written in the Java programming language. OdorMapBuilder allows users to trace the glomerular layer in a MRI slab, which then serves as a template for the glomerular fMRI density slab. The patterns from all slabs are combined into a 2-D map image. Each position in the odor map represents a unique site in the OB glomerular layer, with its optical density representing the neural activity. To aid in analyzing the maps, OdorMapComparer allows users to import two maps onto a framed canvas, perform warping, and carry out simple addition, subtraction and statistical analysis between the two images. **Results:** the programs provide a user-friendly interface with a rich set of menus. OdorMapBuilder generates odor maps in different perspectives: dorsal, lateral, ventral and medial. The map images can be saved in JPEG and GIF format or exported as raw pixel data. OdorMapComparer enables quantitative comparisons of two images and calculations of the difference or additive effects of any two odor maps. **Conclusions:** the present study illustrates the critical role of informatics tools in analyzing the neural basis of the olfactory processing. In addition to fMRI data, these tools also apply to 3-D data obtained using other methods.

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Poster: Olfactory Bulb: Coding

Lateral inhibition: it makes scents as a neuronal coding strategy in olfaction

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There is good evidence that the primary representation of an olfactory stimulus is shaped by inhibitory interactions between glomeruli, but there are few olfactory systems in which the odor-tuning properties of identifiable and accessible glomeruli are known. The moth antennal lobe contains a small number of sexually dimorphic glomeruli: the macroglomerular complex (MGC) in males and the large female glomeruli (LFGs) in females, and evidence is increasing that the MGC, LFGs and the remaining glomeruli share similar principles of synaptic organization. To test this idea further, we used intracellular recording and staining to examine the odor-evoked responses of projection neurons (PNs) innervating spatially identifiable glomeruli in both sexes. In males, our earlier results showed that the synchronous firing of PNs innervating one MGC glomerulus is modulated by local inhibition from the neighboring glomerulus. Similarly, in females, we found that PNs innervating one identifiable glomerulus (G40) were depolarized selectively by *cis*-3-hexenyl acetate, while PNs innervating the lateral LFG (which neighbors G40) were activated selectively by (+)linalool. In contrast, (±)linalool evoked a rapid inhibitory potential in PNs innervating the adjacent G40 glomerulus. Similarly, (+) but not (−) linalool also evoked an IPSP in medial LFG-PNs. These results provide direct evidence for odor-mediated, lateral inhibitory interactions between glomeruli in both the pheromonal and non-pheromonal olfactory subsystems in this insect. Pharmacological tests that selectively influence GABAergic transmission are expected to shed light on the neurochemical basis for these inhibitory synaptic interactions.

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Poster: Olfactory Bulb: Coding

Configurational and elemental odor mixture perception can arise from local inhibition

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Contrast enhancement via lateral inhibitory circuits is a common mechanism in sensory systems. We here employ a computational model to show that, in addition to shaping experimentally observed molecular receptive fields in the olfactory bulb, functionally lateral inhibitory circuits can also mediate the elemental and configurational properties of odor mixture perception. To the extent that odor perception can be predicted by slow-timescale neural activation patterns in the olfactory bulb, and to the extent that interglomerular inhibitory projections map onto a space of odorant similarity, the model shows that these inhibitory processes in the olfactory bulb suffice to generate the behaviorally observed inverse relationship between two odorants' perceptual similarities and the perceptual similarities between either of these same odorants and their binary mixture.

Slide: Olfactory Bulb: Coding

High-dimensional contrast enhancement in odor space

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The topographical mapping of external stimulus spaces is a common principle of neural organization, and contributes to sensory input processing by facilitating contrast enhancement mechanisms such as center-surround lateral inhibition. This process requires that the intrinsic topology of the contrast enhancement mechanism match the underlying topology of the sensory space upon which it acts. For example, the spatial contrast of retinal images is enhanced by lateral inhibitory projections along the two dimensions of the retinal field, and auditory frequency tuning in the inferior colliculus is similarly sharpened by lateral inhibition along the single dimension of frequency space. Contrast enhancement in the olfactory system is believed to sharpen odor quality representations, which map onto an indeterminate but certainly high-dimensional sensory space. This high-dimensional odor space must map onto functionally two-dimensional neural cortices while retaining unambiguous high-dimensional similarity relationships that are common among conspecifics; hence, physical proximity cannot reliably connote stimulus similarity. For a 'lateral-inhibitory' mechanism of contrast enhancement to function, bulbar activation by a given odorant feature must specifically inhibit numerous other bulbar regions activated by chemically similar odorant features and sparsely distributed in space; current models have not addressed the scope of this fundamentally high-dimensional problem. We here propose and demonstrate a novel bulbar mechanism for such hyperlateral inhibition in odor similarity space, and challenge this model with evidence from the experimental literature.

Poster: Olfactory Bulb: Coding

Glomerular on-off model of olfactory coding

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We present a model for olfactory coding based on spatial representation of glomerular responses. In this model distinct odorants activate specific subsets of glomeruli, dependent upon the odorant's concentration. The glomerular response specificities are understood statistically, based on experimentally measured distributions of odor detection thresholds. A simple version of the model, in which glomerular responses are binary (the on-off model), allows us to quantitatively account for the following results of human/rodent psychophysics: (i) just noticeable differences in perceived concentration of a single odor (Weber ratios) are $dC/C \sim 0.1$; (ii) the number of simultaneously perceived odors can be as high as 12 (Jinks and Laing, 1999); (iii) extensive lesions of the olfactory bulb do not lead to significant changes in detection or discrimination thresholds. A more detailed model allows us to reproduce closely the conditional probabilities obtained in human psychophysical experiments on perception of complex odors.

Poster: Social Odors and Behavior

Understanding chemical communication under lotic and lentic conditions in the laboratory with crayfish

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The physics of environments can structure how animals send and receive signals. In fact, habitat specific physics may constrain signal transmission and provide a mechanism for evolutionary sensory biases. This experiment investigates how the use of chemical (olfactory) signals to convey social signals is influenced by environmental physics. If environments can place constraints upon chemical communication, then we would predict that crayfish found in lotic (flowing water) systems should be adapted for more effective communication within this environment and crayfish in lentic (low or no flow) systems should be adapted for effective communication in this environment. This hypothesis extends the 'Matched Filter' idea of Wehner to the level of behavior. We hypothesize that crayfish are influenced by the environment, thus dominant crayfish collected from lotic systems will take residence upstream in the flow, whereas under lentic (no flow) conditions the position of dominant individuals will be random. Lotic conditions consisted of two flow regimes (5 and 10 cm/s) and lentic conditions had no flow (0 cm/s). A second experiment demonstrated when urine was released under these conditions. The crayfish received an injection of 0.1% sodium fluorescein at a dose of 2–6 $\mu\text{g/g}$ body mass. The fluorescein injection results in the visualization of urine released during the course of an agonistic bout. This section of the study examined the ability to project urine in the different flow regimes. This study is a model for elucidating how environments structure communication systems and also whether crayfish release urine in manner that suggests that the signal is deliberately released to communicate status.

Poster: Social Odors and Behavior

HPLC analysis of the chemical composition of urine in the crayfish, *Orconectes rusticus*

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Social communication is important for the formation of social hierarchies in crayfish. Communication uses various sensory modalities that play a role in establishing these relationships. In crayfish, chemoreception has been found to play an important role in the development and maintenance of social hierarchies. During an agonistic bout, urine is released from the nephropores and is propelled in the anterior direction toward a conspecific by use of the fan organs (maxillae and maxillipeds). Conspecifics detect chemical signals with two pairs of antennules. Previous work has been done on the frequency and duration of urine releases during agonistic bouts between crayfish. The results show that dominant crayfish have a longer duration and more frequent release of urine than subordinates. However, there may also be differences in the chemical composition of urine in dominant and subordinate animals, perhaps due to intrinsic variability. This project used HPLC to examine the different chemical components present within the urine of dominant and subordinate individuals. The social status and previous social experience of crayfish altered the presence or absence of chemical cues within the urine. In addition, the quantity of urine released differed between dominant and subordinate crayfish. Therefore, social status alters the composition of chemicals within urine. Consequently, it appears as if crayfish can alter the behavior of conspecifics by releasing urine during a fight, and that this urine may be a true indicator of social status and fighting ability.

Poster: Social Odors and Behavior**The utilization of the major chelae by male crayfish (*Orconectes rusticus*) for detecting female pheromones**

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The abundance and spatial distribution of the sensory hairs of the major chelae of the crayfish (*Orconectes rusticus*) are dependent upon the sex and reproductive form of the crayfish. It has been previously demonstrated that the major chelae may have chemosensory abilities that function to detect pheromones or mating signals. Given these previous results, we determined if form I (reproductive) male crayfish use their major chelae to detect potential female pheromones in order to locate potential mates. This study provides a link between the previous morphological studies and reproductive behavior. We videotaped and analyzed the behavioral reactions of form I males ($n = 20$) to female conditioned water ($n = 6$), male conditioned water ($n = 6$) and control water. Following this, we used males that had their chelae sensory deprived (lesioned) by coating them with super glue. We found that unlesioned form I males responded significantly more ($P = 0.02$) to female odor than males with lesioned chelae ($P = 0.61$). These results clearly show that the major chelae serve a chemosensory function that is related to distinguishing sex in crayfish. It may be possible that the chelae also play a chemosensory role in mate localization and courtship behavior.

Poster: Social Odors and Behavior**Individual recognition in the lobster, *Homarus americanus*: the loser remembers**

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Lobsters can distinguish conspecifics individually by odor, and use this information, carried in the urine, to establish their social structure through a series of agonistic encounters. In the first encounter, unfamiliar lobsters learn the individual odortype of their opponents. In second and subsequent encounters, they do not engage in highly aggressive interactions. In this study, we questioned which of an agonistic pair makes the decision not to fight a second time. We paired male lobsters in two successive boxing matches. Before the second fight, we disabled the critical antennular chemoreceptors of either the winner or the loser of fight one. The effects of the lesion on the behavior of both animals as well as the overall characteristics of the fight were recorded. Results show that when the subordinate's chemoreceptors are disabled and the dominant remains intact, all behaviors and fight characteristics remain largely the same in fight two as in fight one. In two cases, the lesioned loser of fight one beat his dominant in fight two, thus overturning their dominance relationship. When the winner's nose is disabled and the loser remains intact, however, the duration of the fight and all other measures of aggression decrease significantly for both winner and loser. These findings confirm that the loser of a fight determines the intensity of subsequent fights, fleeing significantly sooner and more often, thereby eliciting less aggression from the winner.

Poster: Social Odors and Behavior**Chemical signals and chemosensory pathways involved in spiny lobster sheltering behavior**

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The chemosensory system of the Caribbean spiny lobster (*Panulirus argus*) is organized into two parallel pathways that originate in different populations of antennular sensilla and project to specific neuropils in the brain. Although unique roles for each pathway in olfactory mediated behaviors have not been described in lobsters, work on other crustaceans suggests that the pathways may have different roles in the detection of intraspecific signals. Caribbean spiny lobsters are gregarious animals that often shelter together in communal dens. Several previous studies have demonstrated that this aggregation behavior is mediated by chemical signals released from sheltering conspecifics. However, the nature of the aggregation signal and the chemosensory pathways involved in its detection are currently unknown. We developed a shelter choice assay in a large seawater flume and examined the sheltering behavior of lobsters in response to a range of odorants including diluted conspecific urine (pheromone) and its chemical fractions, shrimp extract (food), and octopus odor (predator). Lobsters sheltered preferentially with diluted conspecific urine, showed no preference for the shrimp odor and avoided shelters with octopus odor. Thus sheltering behavior is specific to conspecific odors, and the aggregation signal is contained within urine. At present we are attempting to chemically characterize the aggregation pheromone, and we are also examining the importance of each of the chemosensory pathways in this behavior through selective ablation of different populations of antennular sensilla.

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Poster: Social Odors and Behavior**In search of sex pheromones in blue crabs**M. Kamio¹, J. Kubanek² and C.D. Derby¹¹*Biology, Georgia State University, Atlanta, GA, USA and* ²*Biology, Georgia Institute of Technology, Atlanta, GA, USA*

Blue crab *Callinectes sapidus* premolt females release a sex pheromone in their urine. Males detect this pheromone using antennular sensors, resulting in mating behaviors that include precopulatory display (rhythmic waving of swimming legs) and grabbing and guarding the female. Male crab also releases a sex pheromone that attracts premolt females (Gleeson, *Crustacean Sexual Biology*, Columbia University Press, 1991). 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. We collected female and male urine and separated them by ultrafiltration into three molecular weight fractions: <500 Da (small), 500–1000 Da (middle) and >1000 Da (large). Small and middle fractions of female urine induced male precopulatory display as well as standing high on legs, spreading chelae and grasping. These same size fractions of male urine induced males to perform all of these behaviors except precopulatory display. These results indicate that female

urine contains a sex-specific pheromone, and male crab urine contains other chemicals such as species- or male-specific odors that stimulate agonistic behavior in other males, or even non-specific odors. Our results also show that the sex pheromone is either a single molecule of ~500 Da or mixture of molecules including some of <500 Da and others of 500–1000 Da. NMR spectroscopy identified differences in the chemical composition of male and female urine.

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Poster: Social Odors and Behavior

Possible involvement of phosphatidylcholines as a signal substance mediating the recognition of school in the catfish, *Plotosus lineatus*

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Group living is integrated in the communication between group members; chemical signals are widely used in many species. The catfish, *Plotosus lineatus*, forms a dense ball-shaped school soon after hatching as a clutch; interestingly, the original schools are reformed even if two schools are mixed. It is known that *P. lineatus* prefers their own schoolmates' seawater to seawater, as well as to other school's seawater, indicating the implication of chemical substance mediating the recognition of school [school recognition substance (SRS)]. We attempted to clarify the chemical nature of SRS. We first established a reliable bioassay to detect the activity of SRS, which is based on 'turn behavior' to the agar block containing skin mucus collected from schoolmates. The bioassay guided-fractionation of skin mucus yielded a single active peak in HPLC. The substance in the active fraction was analyzed by NMR and mass spectrometry, which led to an identification of phosphatidylcholine (PC); actually it was a mixture of PC molecular species as analyzed by LC/ESI-MS. From these results, we assumed that a mixture of PC molecular species is involved in the school recognition of *P. lineatus*.

Poster: Social Odors and Behavior

Preen gland secretions of a scented and unscented seabird

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The crested auklet (*Aethia cristatella*) exhibits a tangerine-like scent that has been implicated in 'nape-sniffing' behavior during courtship (Hagelin *et al.*, 2003). In contrast, its nearest relative, the least auklet, *Aethia pusilla*, is unscented (to human noses), but exhibits a head-wagging behavior that could be involved in chemical signaling. We examined both the volatile and non-volatile components of auklet preen gland secretions in order to: (i) determine whether least auklet secretions contained any key compounds found in crested

auklet odor; and (ii) characterize the compounds secreted by the preen gland of each species. Our analyses indicated distinct differences between secretions of crested and least auklets. Only crested auklets exhibited *Z*-2-decenal and octanal, two compounds related to the characteristic tangerine-like scent. Volatile compounds unique to least auklets included a series of C₁₄–C₁₈ alcohols. With regard to larger, less volatile compounds, crested auklets appeared to secrete a less complicated mixture of molecules than least auklets. We discuss our findings in light of recent work by Reneerkens *et al.* (2002), who suggest that the chemistry of preen glands may have consequences for nest predation in some groups of birds.

Poster: Social Odors and Behavior

New insights on the social structure and odor function of a tangerine-scented seabird

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The crested auklet (*Aethia cristatella*) is an arctic seabird that emits a tangerine-scented odor during breeding (Hagelin *et al.*, 2003). Birds rub their beaks in the scented nape of a display partner, providing a possible mechanism for odor assessment. The exact social function of crested auklet odor is, as yet, unknown. Two possible hypotheses include: (i) odor correlates with social rank, such as dominance status; and (ii) odor acts as a sexual ornament that attracts members of the opposite sex. We analyzed patterns of aggression and courtship within a captive population of 14 birds. We also collected chemical samples from both the oil gland and freshly clipped feathers. Behavioral analysis revealed a strong linear hierarchy within our population. Among females, social (dominance) rank was positively related to the degree to which a female associated with a mate. Birds also mated assortatively with respect to body size. Chemical analyses revealed that both oil glands and feathers contained compounds characteristic of the auklet's seasonal scent (e.g. *cis*-4-decenal and octanal; Hagelin *et al.*, 2003). From the samples of top ranking females we identified >25 volatile compounds in oil gland secretions, including a variety of C₈–C₁₀ acids, aldehydes and alcohols. An analysis of the relative concentration of specific compounds versus social rank is currently underway. Such a comparison promises to reveal patterns related to odor function, given the surprisingly linear social hierarchy of our captive population.

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Poster: Social Odors and Behavior

Behavioral and physiological responses to a putative alarm odor in European starlings (*Sturnus vulgaris*)

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Wild European starlings (*Sturnus vulgaris*) produce a pungent odor that is detectable to humans when birds are held in captivity. Despite growing evidence for chemosignals in birds, alarm odors have not been investigated. We tested whether fecal odor of stressed European starlings produced a behavioral or physiological alarm response in non-stressed individuals. We videotaped focal birds ($n = 18$) while exposed to one of three chemical stimuli: (i) fecal odor from a stressed conspecific; (ii) fecal odor from an unstressed conspecific; and (iii) plain air. Fecal samples of focal birds were also collected before and after exposure to each treatment, and assayed for a common stress hormone [corticosterone (CORT)]. Analyses of gaping and breathing rate, common distress behaviors in birds, are currently underway. We detected no significant difference between the three treatments in the frequency of other behaviors such as hopping ($P = 0.58$), preening ($P = 0.93$) or feather fluffing ($P = 0.51$). With regard to physiology, starlings tended to exhibit less of a net increase in fecal CORT levels after exposure to stressed conspecific odor, compared with other chemical treatments ($P = 0.097$). Such a result suggests that starlings might experience a physiological change after exposure to the scent of stressed conspecifics. Though our preliminary study failed to meet statistical significance, it is an intriguing first step that highlights the need for additional, careful study of avian chemical signals.

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Poster: Social Odors and Behavior

The influence of context on female MHC-based mate choice

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The primary function of the molecules of the major histocompatibility complex, the MHC, is to enable antigen presentation to T-cells, thereby initiating an immune response. The MHC also influences mating behavior such that individuals choose mates who express MHC alleles that are different from their own. MHC-based disassortative mate choice has been shown in mice, fish, and humans. Females more so than males may mate disassortatively with respect to the MHC because of potential immune and fitness benefits that this genetic diversity provides offspring. However, reports from some MHC-based odor studies, wherein females were presented with the odors from males possessing many different MHC alleles, did not indicate preferences for the most MHC differences. Rather, females preferred the odors of males with whom they shared a few of the same alleles, suggesting that the preferred MHC difference is relative to the female's own MHC diversity. We previously tested this hypothesis and presented data consistent with preference for differences—the greater the MHC difference, the greater the preference. However, we also showed that the direction of preference depended on the type of choice being made—whether females were choosing between a male identical in MHC and one different or between two males that were both different in MHC, in number of alleles. To further investigate the influence of context, we have expanded the study with more subjects and another MHC

haplotype. We present data that female rats (*Rattus norvegicus*) consider the context of choice, as indicated by male MHC differences, when making mating decisions.

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Poster: Social Odors and Behavior

The scent of friendship: high school students research the mysteries of human odor recognition

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Though several studies have examined the effect of human odor on kin recognition and mate choice, few have focused on the impact of familiarity on recognition of non-relatives. As part of a program designed to engage students in scientific research, 55 10th grade and Advanced Placement biology students researched, planned and implemented a project to analyze the effect of odor on human recognition of, and preference for, close friends, gender and self. Each student and friend of their choosing wore a T-shirt for three consecutive nights. During that time, subjects were controlled for exposure to extraneous perfumes, household odors and other humans. The students were then asked to smell a series of shirts and evaluate them with respect to pleasantness. Students were also asked to identify the two shirts belonging to themselves and their friend, and determine the gender of the person who had been wearing each shirt. Results of this testing will be presented along with a discussion of its implications for human social behavior.

This research is supported by the NSF Graduate Teaching Fellows in K-12 Education Program and Cornell University through the Cornell Science Inquiry Partnerships Program.

Slide: Olfactory Behavior & Psychophysics

Foraging in a complex chemical landscape: DOM from elevated CO₂ detritus and its impact on crayfish orientation to a food source

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Crayfish must locate food resources in a chemically complex environment where chemicals from various sources interact and mix with one another. We tested whether an additional chemical food source of leachate from detritus (dissolved organic matter, DOM) produced at ambient (AMB) or elevated (ELEV) atmospheric CO₂ and presented at two different concentrations would affect crayfish orientation behavior. Crayfish (*Orconectes virilis*) orientation was observed in a recirculating flume where a fish odor source was placed downstream of a DOM odor source. Preliminary analysis of the chemical signal demonstrated that the fine-scale spatiotemporal structure of the odor plume was different upon introduction of a background DOM odor. Behavioral treatments were as follows: (i) CON (control; no DOM present); (ii) AMB-Low (AMB DOM present at 3 mg/l); (iii) AMB-High (AMB DOM present

at 6 mg/l); (iv) ELEV-Low (ELEV DOM present at 3 mg/l); and (v) ELEV-High (ELEV DOM present at 6 mg/l). Crayfish in the AMB-High treatment were more successful in locating the source. In contrast, animals in the ELEV-High treatment had higher turning angles and heading angles than all other treatments. No differences were found for temporal parameters of orientation. Overall, these results indicate that crayfish orientation to a fish odor source is affected by the presence of DOM from detritus when it is present at the high end of a natural range of DOM concentration, perhaps resulting in a chemical ‘cocktail party’ effect.

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Slide: Olfactory Behavior & Psychophysics

Chemically induced antennular grooming in the spiny lobster, *Panulirus argus*, is mediated by non-olfactory sensilla

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In decapod crustaceans, the first antennae, bearing olfactory and other chemo-mechanosensory sensilla, are groomed by mouthpart appendages in a stereotyped behavioral pattern. In the spiny lobster, *Panulirus argus*, antennular grooming behavior (AGB) is elicited by L-glutamate, and ablation experiments led to the conclusion that AGB is mediated by olfactory sensilla (aesthetascs) located on the lateral flagella of the first antennae (Wroblewska *et al.*, 2002, *Chem. Senses* 27: 769–778). The aim of our study was to examine this conclusion since the aesthetascs are closely associated with other sensilla, the asymmetric setae, which had also been eliminated. In two independent sets of experiments, we first showed that intact animals responded with AGB upon stimulation with 0.5 mM L-glutamate, then removed either all asymmetric setae ($n = 8/n = 7$) or all aesthetascs ($n = 8/n = 6$). Aesthetasc ablation did not reduce AGB, whereas ablation of asymmetric setae almost completely eliminated it. A third set of experiments showed that aesthetasc ablation also has no positive effect on AGB. Electron and confocal microscopy suggest that asymmetric setae are bimodal chemo-mechanosensory sensilla, defined by the presence of a terminal pore and a scolopale. We conclude that AGB is elicited by chemosensory input provided by asymmetric setae and that it is not mediated by the olfactory pathway but by a parallel chemo- and mechanosensory pathway constituted by the lateral antennular neuropils (Schmidt and Ache, 1996, *J. Comp. Physiol. A* 178: 579–604).

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Slide: Olfactory Behavior & Psychophysics

Discrimination between enantiomers of carvone and terpinen-4-ol odorants in normal rats and those with lesions of the olfactory bulbs

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Rats trained on an operant conditioning task were used to assess whether the enantiomers of terpinen-4-ol, odorants that activate very similar areas of the olfactory bulb are more difficult to discriminate than those of carvone, odorants that activate different areas of the olfactory bulb, and to determine the effects of disrupting identified bulbar sites activated by these odorants. In psychophysical tests in which odor concentration was gradually reduced to near threshold levels, normal rats discriminated between the enantiomers of terpinen-4-ol and of carvone equally well. Olfactory bulb lesions that removed the majority of bulbar glomeruli activated by these odorants (as demonstrated in prior olfactory bulb studies using optical imaging and 2-deoxyglucose) resulted in increased detection thresholds but little or no deficits in discriminating between supra threshold concentrations of the enantiomers. These results fail to confirm predictions based on maps of bulbar activity that enantiomers of terpinen-4-ol should be more difficult to discriminate than those of carvone and that the ability to discriminate between enantiomers of an odorant are based on differences in patterns of bulbar activation produced by the odorants.

Slide: Olfactory Behavior & Psychophysics

Size and numbers don't matter (that much)—relative size of olfactory brain structures and number of functional olfactory receptor genes are poor predictors of olfactory performance

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Primates are typically regarded as ‘microsmatic’ animals. This view, however, is only based on an interpretation of neuroanatomical and recent genetic findings and not on physiological evidence. We determined olfactory detection thresholds in squirrel monkeys and pigtail macaques for >40 odorants from different chemical classes and compared their performance to that of human subjects and nonprimate mammals. We found that—contrary to the traditional view—human subjects do not generally perform more poorly than monkeys, and Old World primates do not generally show higher threshold values than New World primates. Furthermore, human and nonhuman primates show an olfactory sensitivity which for several substances matches or even is markedly better than that of species believed to be ‘macrosmatic’, such as dogs, rats or mice. Similarly, human and nonhuman primates do not generally perform poorer in discriminating between structurally related odorants compared with nonprimate mammals and insects. We conclude that between-species comparisons of neuroanatomical features or of the number of functional olfactory receptor genes are poor predictors of olfactory performance. Differences in olfactory sensitivity and discrimination abilities within and between species seem to reflect evolutionary adaptations to ecological niches.

Slide: Olfactory Behavior & Psychophysics

A psychophysical test of the vibration theory of olfaction

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At present no satisfactory theory exists to explain why a given molecule has a particular smell. A recent book about the physiologist Luca Turin has generated new interest in the theory that the smell of a molecule is determined by its intramolecular vibrations rather than by its shape. We present the first psychophysical experiments in humans that test key predictions of this theory. The results suggest that molecular vibrations alone cannot explain the perceived smell of a chemical. Specifically, we have found that: (i) in a component identification task no vanilla odor character was detected in the mixture of benzaldehyde and guaiacol; (ii) odor similarity ratings did not reveal that even and odd numbered aldehydes form two odor classes; and (iii) naive subjects who could easily discriminate the smell of two molecules that differ in shape but not in molecular vibration failed to discriminate two molecules with similar shape but different molecular vibrations in three different experimental paradigms (similarity rating, duo-trio test, triangle test). Taken together our findings are consistent with the idea that the smell of a molecule is determined by its shape but we found no evidence that the smell of a molecule is influenced by its vibrational properties.

Slide: Olfactory Behavior & Psychophysics

Functional connectivity of the hippocampus during an olfactory task: differences observed between young and elderly

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Interest in the role of the hippocampus (HIP) for olfactory function has increased since the discovery that initial neuropathological changes indicative of Alzheimer's disease (AD) occur in mesial temporal olfactory regions. The present study investigated differences in the functional relationship between the HIP and classic olfactory regions in young and elderly adults in response to odor stimulation. Activation in reference voxels located in left and right hippocampi were separately averaged and correlation coefficients between these and all other brain voxels were calculated. Group analyses were also performed in order to detect brain areas with significant differences in their strength of functional association to the hippocampi. For the young, both left and right hippocampi demonstrated strong functional associations with classic olfactory areas while the elderly exhibited a reduced number of functional associations. In addition, while both right and left hippocampi demonstrated functional connectivity with ipsilateral orbital frontal cortices in the young, functional connectivity was not observed between the left HIP and left orbital frontal cortex in the elderly. However, a notably larger area of the right orbital frontal cortex displayed connectivity with the right HIP, suggesting the possibility of a compensatory process in the elderly. Group analyses further demonstrated age differences in strength of functional associations among regions. The present study illustrates the effect of aging on hippocampal function and provides a foundation for further understanding of its dysfunction when affected by a neurodegenerative disease such as AD.

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Slide: Olfactory Behavior & Psychophysics

Impact of the chemical senses on augmenting memory, attention, reaction time, problem solving, and response variability: the differential role of retronasal versus orthonasal odorant administration

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Past research has consistently noted a significant interplay between odors and human behavior. Multiple studies have shown that the administration of particular odorants can enhance athletic performance, sleep, pain tolerance, mood and cognitive processing. In addition, odorants have a differential effect on human behavior, dependent upon route of administration (retronasal versus orthonasal). The present study examined the differential effects of odorants administered retronasally and orthonasally on cognitive performance. During Phase I, 31 participants completed cognitive tasks on a computer-based program (Impact®) under five 'chewing gum' conditions (no gum, flavorless gum, peppermint gum, cinnamon gum, and cherry gum). During Phase II, 39 participants completed the same cognitive tasks under four odorant conditions (no odor, peppermint odor, jasmine odor and cinnamon odor). Participants also completed pre- and post-test assessments of mood, and rated their perception of the required workload. Results revealed a task-dependent relationship between odors and the enhancement of cognitive processing. Cinnamon, administered retronasally or orthonasally, improved participants' scores on tasks related to attentional processes, virtual recognition memory, working memory, and visual-motor response speed. Implications of the present study are most promising in providing a non-pharmacological adjunct to enhancing cognition in the elderly, individuals with test-anxiety, and perhaps even patients with diseases that lead to cognitive decline.

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Slide: Olfactory Behavior & Psychophysics

The magic number 3 applies to components identified in complex odor-taste mixtures

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Humans can identify up to three components in taste or odour mixtures (Laing and Francis, 1989; Laing *et al.*, 2002), but their capacity to analyse multi-component odor-taste mixtures is unknown. With binary mixtures both components are usually perceived, however, only tastants were identified in three-component mixtures containing one odorant. (Laing *et al.*, 2002), suggesting taste may dominate smell in odor-taste mixtures. Here, the aim was to determine the number of identifiable components in complex odor-taste mixtures and investigate the report of dominance of taste over smell. Forty-three subjects were trained to identify 'equi' intense aqueous solutions of the tastants sucrose, sodium chloride and citric acid, and the odorants cinnamaldehyde (cinnamon), *cis*-3-hexenol (grass-like) and 2-pentanone (like nail polish remover). Over two

test sessions they were asked to identify the components of 36 mixtures containing from one to six components presented in random order. Stimuli were sampled by mouth using a straw sited in a hole in the lid of a 30 ml plastic cup. Subjects readily identified components in one- and two-component stimuli, but with 3–6 component mixtures the mean number identified was 3. Clearly, increasing the number of modalities in a mixture did not increase the number of components identified. As reported earlier, tastes were more readily identified than odors in mixtures. The limit of three components strongly suggests the limiting factor is working memory, the memory that is used to rapidly identify a stimulus and initiate a response within a second or two.

Symposium: Non-neuronal Cells of the Olfactory System in Development

Sustentacular cells—more active than we ever imagined

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Sustentacular cells are morphologically polarized and have structural features that allude to functions of secretion, absorption, phagocytosis, maintenance of extracellular ionic gradients, metabolism of noxious chemicals, and regulation of cell turnover. Here, we review the well-characterized morphology and ultrastructure of mammalian sustentacular cells and present data investigating their dynamic activity. We show, using a mouse olfactory epithelium slice model, that sustentacular cells have voltage-gated Na⁺ and K⁺ channels and are capable of evoking rapid, robust increases in intracellular calcium in response to P2Y purinergic receptor activation. In addition, sustentacular cells propagate waves of calcium oscillations initiated by either endogenous release or exogenous application of ATP or other putative neurotransmitters. Oscillations in intracellular calcium may govern secretion, proliferation and development in sustentacular cells, and, via calcium dependent exocytosis, may provide chemical signals to basal cells, neuronal precursors, or neurons. Recent discoveries of voltage-gated channels, receptors and transmitter release in both peripheral and central glial cells suggest direct communication between neurons and glia. The identification of similar components in sustentacular cells suggests that, like their glial counterparts, they are capable of rapid communication between themselves and the neural elements of the olfactory epithelium.

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Symposium: Non-neuronal Cells of the Olfactory System in Development

A glia–axon *pas de deux* underlies olfactory receptor axon sorting

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The problem: olfactory receptor neurons expressing the same olfactory specificity are not topographically ordered within the receptor epithelium, but their axons extend to just one or a pair of glomeruli

in the olfactory bulb. Axon sorting is thus a critical process that must occur somewhere along the olfactory pathway. In the moth *Manduca sexta*, sorting takes place not in the nerve layer that circumscribes the olfactory neuropil, but rather in a discrete region of the nerve that we have called the axon sorting zone. This sorting zone has three important features: it is densely populated by glial cells, the glial cells can be killed without killing neurons, and it can easily be surgically isolated for various *in vivo* and *in vitro* experimental manipulations. Recent experiments have provided clear evidence for reciprocal interactions between the ingrowing receptor axons and the glial cells that affect glial cell proliferation, axon fasciculation, and growth cone behavior, and also are beginning to reveal the underlying molecular players.

Symposium: Non-neuronal Cells of the Olfactory System in Development

Sorting and glial–neuronal interactions in the olfactory nerve layer

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Olfactory sensory neurons (OSNs) are generated throughout life and extend axons that form fascicles within the olfactory nerve (ON). As axons enter the olfactory nerve layer (ONL) of the olfactory bulb (OB), they reorganize before synapsing in glomeruli. Each OSN expresses one odorant receptor (OR) from a family of ~1000 ORs. Neurons expressing the same OR target specific glomeruli. Thus, OSN axons face unique challenges when sorting. First, axons from neurons located widely within the OE must identify common targets, taking varied trajectories and presumably using multiple guidance cues. Second, as neurons are continually regenerated throughout life, axons must navigate a complex meshwork of pre-existing axons to identify target glomeruli. Throughout this process the unmyelinated OSN axons maintain intimate contact with a unique population of glia, the olfactory ensheathing cells (OECs). The morphological and molecular properties of OECs vary along the length of the axonal projection, suggesting that subsets of OECs subserve different functions. In the ON and peripheral regions of the ONL, OECs tightly bundle OSN axons and express p75. However, as axons approach glomeruli and radically change their trajectories, the population of glia they interact with changes. The OECs found in this region express NPY but not p75. OSN axons also encounter astrocytic processes within this region which emanate radially into the ONL from glomeruli. It is likely that a spatiotemporal combination of cell surface axon-axon and axon-glia interactions underlie glomerular targeting in the OB.

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Symposium: Non-neuronal Cells of the Olfactory System in Development

Losing the path; cell migration in a changing forebrain

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The presence of a germinal layer and the capacity to generate neurons, once thought restricted to the embryonic brain, persists in the forebrain of postnatal and adult mammals. The olfactory bulb (OB), unlike the cortex and other regions, continues to receive new neurons throughout life. Despite dramatic changes in the structure and cellular organization of the forebrain from embryo to adult, these newly generated cells still migrate from germinal regions to specific targets. In early embryos, the OB has a cortical-like organization with radial glia spanning the ventricle to pia. Radial glia are likely to provide a migratory scaffold for cells exiting the ventricular germinal layer and migrating radially. As development progresses numerous cells are generated from a stem cell pool located in the lateral ventricle subventricular zone (SVZ). SVZ-derived progenitor cells migrate into the OB following the rostral migratory stream (RMS) and into other postnatal forebrain structures along different migratory pathways. After birth radial glia disappear and reorganize into a series of glial tubes along the RMS. However, once radial glia in the bulb disappear there is no longer a clear radial scaffold for cells to follow when migrating radially. Such modifications of the non-neuronal migratory environment are paralleled by changes in lineage characteristics of SVZ progenitors. Namely, progenitors that leave the SVZ at different developmental times generate different types of mature OB neurons. Thus, cell migration in the bulb is a developmentally dynamic process between immature cells and different non-neuronal cellular elements at different times.

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Poster: Chemical Ecology

Orientation to temporally and spatially complex odor signals in the crayfish, *Orconectes rusticus*

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Organisms in natural environments encounter complex information and must be able to decipher this information in order to respond behaviorally. This complex information can be a result of various aspects of the natural environment, such as hydrodynamics, changing habitats, and conflicting or multiple odor cues. In many habitats, multiple odor plumes in various spatial configurations often mix and animals must extract relevant spatial information in order to make appropriate orientation decisions. In this study, we show how the spatial configuration of multiple odor plumes influences the orientation abilities of crayfish. By altering the spatial configuration of odor sources without altering concentration or hydrodynamics it is possible to understand the underlying mechanisms of orientation. In this study, we have quantified the hydrodynamics, chemical dynamics and animal behavior within the artificial stream. Our results show that the different spatial configuration influences the distribution of odors within our stream and that these odors influence the subsequent orientation behavior. Our work indicates that it is the

fine-scale distribution of odors that is regulating the orientation behavior of the crayfish.

Poster: Chemical Ecology

Fluid dynamics and chemical signals in the crayfish walking legs

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In aquatic environments, chemical cues are important in many species for finding resources and locating mates. Two processes disperse these cues: fluid flow and molecular diffusion. Fluid flow is dominant in spatial scales larger than 10 mm, while molecular diffusion is dominant at smaller spatial scales. For animals with chemoreceptive capabilities, sampling the environment is critical to obtain information. Receptor structure morphology is responsible for changing the flow surrounding receptor cells by creating a boundary layer. Fluid that is in direct contact with the receptor surface does not flow, which is known as the no-slip condition. The boundary layer acts as a filter, changing the temporal and spatial structure of the chemical signal arriving to receptor cells. In this study, we will employ the technique used by Moore and Atema (1991) to study the fluid dynamics in the chemosensory chelae and walking legs of the crayfish, *Orconectes rusticus*. Our goal is to determine the function of the boundary layer before and after chemical information has been filtered (i.e. during flicking) to the receptor cells. The experiment will be conducted in a recirculating flow tank in which the stimulus will be delivered with a plastic pipette. High-speed electrochemical recordings will be made with BAS epsilon system. It is clear that the morphology of these appendages influence the structure of chemical signals arriving at receptor cells. These results provide insight into how crayfish perceive chemical signals and extract information from turbulent odor plumes.

Poster: Chemical Ecology

The role that boundary layers around crayfish sensory appendages act as temporal filters for odor plumes

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Sensory systems have a complex task of extracting relevant information from the environment. The interaction between fluid flow and the morphology of sensory appendages acts as a physical filter and plays an important role in extracting information. This interaction determines the boundary layer structure which alters the spatial and temporal distribution of chemical signals arriving at the receptors. This study was designed to examine the microscale fluid and chemical dynamics around the sensory appendages of the crayfish, *Orconectes rusticus*. Previous research has determined the biomechanics of sampling behaviors of the lateral antennule of the crayfish. In this study, we have used these biomechanical results with an electrochemical technique to quantify the role that boundary layers play around various sensory appendages in filtering the temporal

dynamics of odor plumes. We measured the boundary layer structure and chemical dynamics of sensory appendages that were mounted in a flow tank under known flow conditions. We utilized the BAS epsilon system with electrochemical microelectrodes to record and quantify chemical dynamics with multiple electrodes around the appendage. The preliminary findings indicate the majority of the chemical signal flows around the appendage rather than through it. Boundary layer structure greatly affects the spatial and temporal nature of chemical signals by acting as a smoothing filter for incoming signals. The exact nature of this physical filter depends upon the ambient flow speed and the angle of the sensory appendage relative to flow. This study illustrates the importance of form and function in chemoreception and in an organism's ability to detect environmental cues.

Slide: Chemical Ecology

From odor plume to antennule: do crayfish antennules vary with flow habitat as predicted to maximize odor molecule capture?

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Many aquatic crustaceans use water-borne chemical cues in ecologically critical activities such as finding food, mates, habitat and avoiding predators. These chemical cues are present as odor plumes consisting of fine filaments of highly concentrated odor molecules interspersed with the surrounding fluid. The structure of the odor plume, and thus how the plume is encountered by navigating animals, is affected by factors such as the size-scale of the bottom substrate and flow conditions such as the mean velocity, turbulence level, and the gradient of flow speed above the substratum. Several species of Ohio crayfish (*Cambarus* spp. and *Orconectes* spp.) were collected from a variety of flow habitats including still ponds and turbulent streams. Since odor plume structure varies according to flow habitat, crayfish antennules from species living in different flow environments should have different patterns of aesthetasc arrangements on their filaments, to best encounter odors in that habitat. I used Image J to measure structural parameters such as aesthetasc number, length and diameter, and the ratio of the gap between aesthetasc rows to the aesthetasc diameter (an indication of odor penetration into the sensor array) from SEMs on three individuals per species. As predicted from odor plume structure, species from high flow habitats have longer aesthetascs ($P = 0.009$; $rsPc = 0.684$) and a smaller ratio of the gap between aesthetasc bundles to the aesthetasc diameter ($P = 0.092$; $rsPc = 0.908$) than species from low flow habitats).

Funding: Denison University Research Foundation.

Poster: Chemical Ecology

Do movements of honeybee antennae enhance capture of odorants?

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Honeybees have numerous olfactory sensory hairs that make up their sensory epithelium, which is distributed along the length of

the antennae. The antennae are shaped as tubular structures that can be placed in different orientations and moved with low frequency through air. In general, antennal sensilla increase in number from proximal to distal. Because of this uneven distribution of sensors and the way in which the shape of the antennae interacts physically with the environment, antennal movements have important consequences for odorant molecule capture and, therefore, perception. Antennal movements were videotaped during presentation of two odorants (geraniol and 1-hexanol) and a control blank. Bee responses were recorded both before and after being trained to associate a food reward with an odorant, as demonstrated by a proboscis extension response. The movements of the antennae were analyzed in three-dimensional space by digitizing the base, elbow, and distal tip of each antenna. Antennal movements increased in response to odorants. Antennal movements were not simple oscillations in a plane but were complicated excursions in three-dimensional space. Antennae were oriented in an anterior direction more noticeably in response to geraniol. Left and right antennae are moved independently of each other. These increased movements will increase the volume of air sampled by the antennae and a potential increase in odorant capture rate from the moving air.

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Poster: Chemical Ecology

Olfactory-mediated search behaviors of migratory sea lampreys seeking pheromone-laden spawning streams in the Great Lakes

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The sea lamprey (*Petromyzon marinus*) spends its larval life in streams, enters large lakes or oceans as a parasitic juvenile, and eventually returns to streams as an adult to reproduce. Laboratory studies strongly suggest that lampreys locate suitable spawning streams using a bile acid-related migratory pheromone released by larval conspecifics (see Fine and Sorensen, this symposium). This study examined olfactory-mediated search behaviors of lampreys as they searched for pheromone-laden streams emptying into Lake Huron. Both olfactory-occluded and untreated lampreys implanted with acoustic telemetry tags were tracked from a GPS-equipped boat and their movements related to stream water concentration (as measured by conductivity). Outside the apparent influence of stream water, lampreys ($n = 12$) swam rapidly (1.5 km/h) and continuously on relatively straight bearings (straightness index = 0.78) while performing repeated vertical excursions through the water column. Occluded and intact animals swam with different compass bearings, perhaps due to differing responses to lake currents/odors. Upon encountering river plumes ($n = 10$), lampreys with an intact olfactory sense commenced circling (straightness index = 0.42). The speed and success of locating the stream mouth was correlated with the thoroughness of mixing of the stream and lake waters. These behaviors, together with responses to stream waters previously documented in laboratory mazes, suggest the use of klinotactic chemo-orientation in stream finding.

Funded by the Great Lakes Fisheries Commission.

Poster: Chemical Ecology**Chemical fractionation demonstrates that the sea lamprey migratory pheromone is comprised of several bile acid-like compounds**

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The sea lamprey (*Petromyzon marinus*) starts its life in freshwater streams, which it then leaves to parasitize lake/oceanic fishes before eventually re-entering streams to spawn. Laboratory and field studies have shown that adult lampreys locate spawning streams using a pheromone released by stream-resident larval lampreys (see Vrieze and Sorensen, this symposium). Initial biochemical characterization of this cue found it to contain the sulfated bile acid petromyzonol sulfate (PS) which adult lampreys detect at 10^{-12} M. Using a combination of HPLC fractionation, olfactory recording, behavioral assays, and mass spectrometry we have recently isolated two additional compounds from larval holding water that have pheromonal activity. The most important has a molecular weight of 704, co-elutes with PS by HPLC, and is behaviorally attractive at concentrations below 10^{-14} M, a record for fish. The second has a molecular weight of 590 and is less potent. In a two-choice preference maze, adult lampreys did not distinguish between larval water and a mixture comprised of PS and these two compounds, demonstrating that this mixture constitutes the majority of the pheromone. It is as yet unclear whether the lamprey has evolved to respond to multiple compounds released by larvae to increase their sensitivity to larval odor or to discern it more specifically. Efforts are presently underway to elucidate the structures of the unknown compounds.

Funded by the Great Lakes Fishery Commission.

Slide: Chemical Ecology**Larval reef fish discriminate between reef odors and may use this in recruitment**J. Atema¹, G. Gerlach² and M. Kingsford³¹Marine Program, Boston University, Woods Hole, MA, USA,²Marine Resources, Marine Biological Laboratory, Woods Hole, MA, USA and ³James Cook University, Townsville, Queensland, Australia

Larval reef fishes typically have an early pelagic phase from which they must return back to the reef environment to survive. The discrepancy between passive dispersal models and actual recruitment data suggests that these small animals participate actively in the recruitment process, but the behavioral mechanisms are not clear. The ontogeny of swimming efficiency of several species is now known; sensory capabilities remain poorly understood. We have been pursuing the olfactory hypothesis that early imprinting on the home reef odor allows them to remain near the (natal) reef and perhaps may guide their return. We have now demonstrated for different species that at the stage that they return to the reef they can discriminate between the odor of different reefs and among five choices prefer the odor of the reef to which they returned. We are now using genetic markers to see if this sensory capability has led to

strong homing that is reflected in population genetic substructuring at the scale of single reefs separated by kilometer distances with and without current barriers.

Poster: Chemical Ecology**Fruit odor discrimination and host race formation in *Rhagoletis* fruit flies**

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Rhagoletis pomonella is a model for incipient sympatric speciation (divergence without geographic isolation) via host plant shifts. We are testing the hypothesis that *R. pomonella* flies originating from hawthorn, apple, and flowering dogwood fruit use fruit volatiles to distinguish among their respective host plants. Solid phase micro-extraction combined with gas chromatography and electroantennogram detection were used to identify unique blends of volatiles from each fruit type. In flight tunnel assays flies preferentially flew upwind (>70%) to the volatile blend from their natal host. Field tests also showed that over the fruiting season significantly more flies were captured with natal fruit volatiles. Because *R. pomonella* rendezvous on or near the unabsorbed fruit of their hosts to mate, the behavioral preference for natal fruit odor translates directly into premating reproductive isolation between the fly races. To explore the genetic basis of the trait crosses were produced between the different fly populations. Flight tunnel tests showed that hybrid flies have significantly reduced response levels to natal or combined volatile blends, with only 40% of the flies exhibiting upwind flight, and only with a concentration ten times that used with parent flies. Flies generated from F2 crosses exhibited response profiles similar to F1 hybrids (60%) or to the parent populations (40%). The behavioral data lay the foundation for QTL analysis.

Supported by NSF DEB-9977011.

Poster: Chemical Ecology**CO₂ is involved in the oviposition behavior of *Manduca* moths**P.G. Guerenstein¹, L. Abrell², W.L. Mechaber¹, G. Stange³ and J.G. Hildebrand¹¹ARL Division of Neurobiology, University of Arizona, Tucson, AZ, USA, ²Biosphere 2 Chemistry Unit, Columbia University, Oracle, AZ, USA and ³Biological Sciences, Australian National University, Canberra, Australia

Moths detect CO₂ in the environment, but the role of CO₂ in the biology of these insects is not well understood. It has been suggested that CO₂ plays a role in the oviposition of the moth *Cactoblastis cactorum* and in the nectar foraging of *Manduca sexta*. We asked if CO₂ also plays a role in oviposition in *Manduca*. Two groups of plants, each surrounded by a ring of ductwork, were placed inside a flight cage. Fans pumped ambient air into the ducts in which small holes provided for gas outflow. CO₂ was injected at the inlet for one group of plants, thus generating an artificial plume of high CO₂ around that group. Female *Manduca* were released into the cage singly. We measured number of eggs oviposited. We found that,

as in *Cactoblastis*, females preferred the control plants (no CO₂ added), possibly because natural CO₂ fluctuations generated by the test plants were masked by an artificially high CO₂ plume. Thus, CO₂ affects the oviposition behavior of *Manduca*. We did not find a dose effect, suggesting that the moths' response was saturated at the CO₂ levels used. The response of *Manduca* was lower than that reported for *Cactoblastis*. Because *Cactoblastis* oviposits on (CAM) plants that generate sinks of CO₂ on their surface while *Manduca* oviposits on plants that are sources of CO₂, we suggest that the expected rise in global ambient CO₂ levels will affect more strongly moths that rely on CO₂ sinks than those that rely on CO₂ sources.

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Poster: Chemical Ecology

Developmental expression and tissue distribution of an odorant-binding protein in the male yellow fever mosquito *Aedes aegypti*

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Major concerns of an adult male mosquito are finding mates and food sources. While most studies concentrate on female specific olfactory processes such as seeking a host for a suitable blood meal, little is known regarding other olfactory based behaviors, including those of the male. Even less is known at the molecular level. We have cloned and characterized an odorant-binding protein (OBP) from a male antennal cDNA library. The Aaeg-OBP sequence is most similar to members of the *Drosophila* and *Anopheles* OBP family. Due to the limited amount of material, we used the quantitative real-time PCR method to measure expression levels among different tissues and at different developmental times in both males and females. Aaeg-OBP expression is adult and male specific. Moreover, its expression is restricted to appendages known to carry chemosensory organs, including antennae and wings. The expression of this gene in adult but not larval males suggests the protein functions in a male specific adult context, mediating chemosensory signals important to behaviors that are more relevant to the males than to the females. The identification of such neural pathways significantly expands our opportunity for developing strategies which disrupt chemosensory mosquito behavior and thus mitigate human-mosquito interactions.

Poster: Chemical Ecology

Mechanisms of action of defensive secretions of the sea hare *Aplysia californica* against the spiny lobster *Panulirus interruptus*

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Active chemical defenses of *Aplysia*, which include secretions from the opaline and ink glands, deter attacks by potential predators. The mechanism of action of these secretions differs among predators. Against the sea anemone *Anthopleura sola*, ink from *Aplysia californica* contains a protein that is aversive and cytolytic (Kicklighter *et al.*, 2004, AChemS poster). Against the spiny lobster *Panulirus interruptus*,

sea hares that release secretions upon attack are more likely to be dropped, allowing sea hares to escape. Ink and opaline appear to protect sea hares through a combination of phagomimicry, chemical confusion and aversion. Phagomimicry, in which predators are deceived into attending to a false food stimulus from the secretions, and chemical confusion occur because the defensive secretions contain millimolar concentrations of amino acids and coat the sensory organs of lobsters. These levels of amino acids in ink and opaline are enormously stimulatory to the lobster's chemosensory cells, and the stickiness likely causes a persistent stimulation. The aversive effect against lobsters is mediated by components in opaline. In addition, ink and opaline are highly acidic (4.9 and 5.8 respectively), suggesting that pH may have either a direct or indirect effect on the secretions' efficacy. We are currently examining these pH effects in behavioral and electrophysiological assays.

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Poster: Chemical Ecology

Protein-mediated defense in *Aplysia californica* against the predatory anemone *Anthopleura sola*

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The sea hare *Aplysia californica* defends itself from predators with two secretions, ink and opaline, which affect predators differently. Against the spiny lobster *Panulirus interruptus* ink is attractive while opaline is aversive (Shabani *et al.*, 2004, AChemS poster). Conversely, against the predatory sea anemone *Anthopleura sola*, ink is aversive, causing tentacles to shrivel and the gastrovascular cavity to evert. Opaline appears to stimulate feeding, as it elicits tentacle movement to the mouth, which opens. Our investigations indicate that ink contains one dominant protein, 'escapin', which occurs at a concentration of 0.025 mg/ml of ink. When applied to anemone tentacles, escapin elicits cell lysis, suggesting that escapin may be responsible for ink's aversiveness to sea anemones. To further pursue escapin's defensive role for *Aplysia*, we are using the technique RNA interference to knock down production of escapin. Ink produced by *Aplysia* injected with double-stranded RNA elicits less tentacle shriveling than ink produced by control *Aplysia*. We are currently using this technique to assess the survival of sea hares that can and cannot produce ink containing escapin against sea anemones.

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Poster: Chemical Ecology

Predator odors and reproduction in house mouse under laboratory and semi-natural conditions

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We examined the influence of predator odors (*Felis catus*) on reproductive output of house mouse (*Mus musculus*) under laboratory and semi-natural conditions. Laboratory naive animals responded to predator chemical cues either with block of pregnancy or reduced litter size and skewed sex ratio. Under laboratory conditions block of

pregnancy in experimental group was observed twice higher than in both of the control groups ($P < 0.001$). In enclosures 30% of females were not pregnant (seasons of May–September 2002–2003) when cat urine was applied onto the bedding each other day. At the same time in control enclosure 92% ($n = 38$) of females were pregnant. The total number of offspring in control enclosure also was significantly higher ($P < 0.001$) than in experimental one. We observed seasonal changes in sensitivity of mice to predator chemical cues. It is noteworthy that suppression of rodent reproduction also occurred when rodents were exposed to urine of conspecifics housed under high population densities. The fact that mice respond to certain chemical signals in predator urine in similar fashion may be fortuitous, and may have more to do with the coincidence that the urine contain similar chemical cues resulting from protein digestion in carnivores and protein catabolism in nutritionally deprived rodents rather than specific predator-prey adaptations.

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Slide: Chemical Ecology

Manufacture and testing of chemical-signal-enhanced devices for deterring crop-raiding elephants

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Human–elephant conflict is a significant economic and ecological problem in southeast Asia, escalating as human populations continue to expand and the forest habitat for elephants is destroyed. In their native society Asian elephants utilize two pheromones that elicit well-defined behavioral responses and a variety of chemical signals that influence other behaviors, including movement and choices. Utilizing this information combined with several years of testing various anti-feedants and deterrents, and detailed first-hand knowledge of the walking behavior of elephants, Riddle and Rasmussen invented a mechanical device, enhanced with chemical signal dispersion units. Recently we built twelve such devices at a village site in southern India and tested their efficacy during harvest season, the period of maximum crop-raiding by elephants. As crop-raiding in this region occurs primarily nocturnally, observations were conducted using night vision recording equipment. Results demonstrate that (i) the manufacturing process was cost effective; (ii) the devices were efficacious as deterrents; and (iii) new ambulatory capabilities and high risk behaviors by wild male elephants were revealed. We present this study in the context of the relevance of basic behavioral and chemical signal research toward practical economic use.

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Poster: Accessory Olfactory System

Modification of odor investigation by female opossums (*Monodelphis domestica*) after accessory olfactory bulb ablation

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Monodelphis domestica are South American marsupials that commonly scent-mark their environment. We have previously shown that female opossums discriminate between conspecific odors from different sources. The purpose of the present study was to determine if the vomeronasal system is important for females to discriminate these odors. Investigation of conspecific odors and distilled water was tested in 12 female opossums in a two-choice paradigm and the time spent in snout contact with each stimulus was analyzed from video recordings. Females were tested before treatments and after control treatment [anaesthesia and partial electrolytic accessory olfactory bulb lesions (AOBP, $n = 6$), or anaesthesia followed by complete AOB ablation (AOBX, $n = 6$)]. Neither anaesthesia nor AOBP had an effect on odor investigation. AOBX resulted in a significant reduction of investigation of certain odors, but only when those odors were paired with certain other odors. For example, when male suprasternal gland (SG) odor was paired with urine of the same stimulus male, there was a significant decrease in the time spent investigating SG odors following AOBX. However, AOBX did not modify the investigation of male SG when it was paired with the same-male submandibular odors. Our data suggest that the accessory olfactory system may be important for the investigation of conspecific odors by female opossums, but it does not appear to be essential for such odor discrimination.

Supported by NIH grant DC02745.

Poster: Accessory Olfactory System

Two populations of granule cells in the accessory olfactory bulb of the opossum, *Monodelphis domestica*

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In the accessory olfactory bulb (AOB) the principal neurons, mitral/tufted cells, receive synaptic inputs from vomeronasal receptor cell axons. Mitral/tufted cell activity is modulated by feedback control of interneurons. Granule cells are the major type of inhibitory interneurons. In this study, we used immunocytochemical technique to analyze the expression of the calcium binding proteins calretinin and calbindin D28k in the granule cell layer of the opossum AOB. We identified two separate populations of granule cells in the granule cell layer. One population expresses only calretinin, and the other expresses only calbindin D28k. Their somatic distribution in the granule cell layer was intermingled and their dendritic projection into the external plexiform layer was also similar. No such differential expression of the calcium binding proteins was detected in the mouse or rat AOB, or in the main olfactory bulbs of the rat, mouse and opossum using the same antibodies. This observation suggests that in the opossum AOB the granule cell population is not homogenous.

Supported by NIDCD grant DC02745.

Poster: Accessory Olfactory System

Vomeronasal and olfactory convergence in medial amygdala

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Conspecific chemosensory signals communicate social information in hamsters, and are distinguished from heterospecific, socially non-relevant, stimuli by Fos responses within medial amygdala (Westberry and Meredith, AChemS 2003). Signals stimulate the vomeronasal organ (VNO) or main olfactory system, activating medial amygdala via accessory and main olfactory bulbs (AOB, MOB). We used electrical stimulation of the VNO and MOB in anesthetized hamsters to show that both systems have input to the medial amygdala. MOB electrodes were in the rostral-lateral bulb to avoid driving VNO nerves. Simultaneous recordings were made with four electrodes inserted into a 3 × 8 grid pattern (200 μm spacing) covering most of the anterior (MeA) and posterior (MeP) medial amygdala. Robust field potentials appeared throughout with moderate to high stimulus intensity (400–1000 μA, 500 μS) and a few single units were driven by both systems. Full mapping of MeA and MeP was obtained in five hamsters and partial mapping of MeP in two others, as verified by histology. Olfactory input clearly has access to medial amygdala even though it was not necessary, in the previous Fos studies, for stimulus categorization by MeA/MeP. Electrical VNO stimulation in awake hamsters activated Fos expression in MeA but not MeP (as for heterospecific chemosensory stimulation). Here we found approximately equal efficiency for short-latency VNO driven activation of MeA and MeP, possibly due to higher stimulus levels than used in awake animals (150 μA), or to the cumulative effect of activation on Fos expression over 15 min. These hypotheses will be tested in future experiments. Supported by NIDCD grant DC005813.

Poster: Accessory Olfactory System

Categorization of chemosensory input in medial amygdala requires vomeronasal input in both sexually naive and experienced male hamsters

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In male hamsters, medial amygdala responds categorically to chemosensory input based on the species and social relevance of a stimulus. Using immediate early gene (IEG) expression, we have demonstrated that the anterior medial amygdala (MeA) responds to both conspecific and heterospecific stimuli, but posterior medial amygdala (MeP) responds only to conspecific (socially relevant) stimuli. Another part of the amygdala, the largely GABAergic intercalated nucleus (ICN) was activated when MeP was not activated with heterospecific stimuli, suggesting inhibition of MeP by ICN. This categorization appears to be hard-wired. In sexually naive males, lesions of the main olfactory epithelium (OLFX) did not change the pattern of IEG activation in the MeA or MeP elicited by conspecific and heterospecific stimuli, while removal of the VNO (VNX) eliminated categorization and most of the responses in the MeA and MeP. Sexually experienced males can use main olfactory input to compensate for lack of VNO input. Here we investigated activation and categorization in MeA and MeP in experienced VNX males. Preliminary data indicates that experienced VNX males show activation of MeA and MeP with both conspecific and heterospecific stimuli, indicating a lack of categorization of these stimuli. Without VNO input, there was never activation of

ICN with either class of stimuli. There were no significant differences in attention to the stimuli (on swabs) that could account for differences in amygdala FRAs expression between groups.

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Poster: Accessory Olfactory System

Cortical response to androstadienone with or without functional occlusion of the vomeronasal duct—a functional magnetic resonance imaging study

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There has been controversy as to whether the vomeronasal duct (VND) in humans is mediating any sensory information or is merely a nonfunctional vestige. This study assessed the cortical responses following stimulation with the putative pheromone Androstadienone (AND) in women, with and without coverage of the VND and using AND in concentrations beyond olfactory threshold. We hypothesized that activation is independent of the VND's status. Following detailed examination including tests for olfactory functions, 16 women (age range 21–27 years), fertile and all presenting with a VND, underwent functional magnetic resonance imaging. The odour stimuli were AND (3 mol/l) and phenylethyl alcohol (PEA) (pseudo-randomized block design, 30 s on, 30 s off; 1 s stimulus duration, 3 s interstimulus interval). Subjects did not know about the status of the VND's coverage. Imaging data were analysed with SPM2. In a group random effects analysis, coverage of the VND did not show significant influence on cortical activation. On first sight, AND produced an activation pattern similar to that of the control odour PEA. When further assessing the differences (AND versus PEA, group random effects analysis), significant subcallosal activation extending to the septal region was detected. In summary, access to the VND does not seem to play a major role in the perception of AND odor. The subcallosal and septal activation specific to AND is in line with previous findings with PET.

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Poster: Accessory Olfactory System

Characteristics of general and specific chemosensory responses in the snake accessory olfactory bulb

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Converging evidence indicates that the main olfactory system detects general odors using a combinatorial coding strategy. Currently, the coding strategy in the vomeronasal (VN) system is unknown, but this system detects highly selective chemosignals, and

probably depends on the activation of highly tuned VN receptor neurons. In snakes, the VN system is critical for detecting chemosignals emitted by conspecifics and prey, but also responds to general odors and amino acids when they are delivered as liquid stimuli. Using voltage sensitive dyes and selective chemoattractants, we studied the distribution of neural responses evoked by electrical, general and specific chemosensory stimuli in the snake accessory olfactory bulb (AOB). All stimulus types evoke non-homogeneously distributed patterns within and throughout the layers, and seemed to be organized in clusters. The spatial distribution of responses to prey chemicals suggest the presence of a zone of preferential activation located in the posterior AOB. Changes in the spatial distribution of activity occurred over time, suggesting a stimulus-specific dynamic organization of responses. Foci of activity also exhibited dissimilar thresholds. These results suggest that sensory discrimination in the snake AOB is based on a combinatorial coding scheme.

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Slide: Olfactory Sensory Neuron Physiology

Chloride homeostasis in mouse ORNs

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Olfactory transduction was seen as a system that worked analogously to vertebrate phototransduction: stimulation of G-protein-coupled receptors triggers an electrical response by activating CNG channels. This view was altered when Kleene and Gesteland reported the presence of a Ca^{2+} -activated Cl channel on olfactory cilia. Odor stimulation increases the ciliary Ca^{2+} concentration by opening CNG channels, generating a secondary Cl current. This current is inward, therefore excitatory, implying a relatively high internal chloride concentration. Eighty percent of the odor-induced current in rodents and 36–65% in amphibians is carried by chloride. Two functions for this unusual Cl current have been proposed. First, it provides a nonlinear, low-noise amplification of the CNG current. Second, it reduces the dependency of the receptor current on external mucosal Na^+ , which might vary depending on the environment, a situation more relevant for amphibians and fish than for mammals. Many of the characteristics of the odor-induced response therefore depend on the interplay between the CNG channel (the Ca^{2+} source) and the Cl channel, and their regulation. Furthermore, since spike frequency is a function of the overall receptor current, the interaction of these two channels will affect action-potential generation. We present molecular and electrophysiological evidence for a mechanism by which internal Cl-concentration is maintained such that its reversal potential is positive relative to the resting membrane potential in mouse ORNs.

Poster: Olfactory Sensory Neuron Physiology

Chloride homeostasis in mouse olfactory neurons

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Intracellular Cl, $[\text{Cl}]_i$, is important in determining the ultimate response of olfactory sensory neurons (OSNs) exposed to odor. Although secondary to the non-selective cation current, the Ca-dependent Cl current is primarily responsible for the generator potential. Thus, Cl homeostasis is important to odor responses. We used MEQ, a chloride sensitive fluorescent dye, to measure $[\text{Cl}]_i$ across a population of isolated OSNs. Since MEQ is a non-ratiometric dye, a set of Cl bath standards was used to establish the initial level of internal Cl. Our results indicated a wide range of $[\text{Cl}]_i$ exist in isolated OSNs (range: 20–145 mM; mean: 62 mM). As a control, $[\text{Cl}]_i$ measured in erythrocytes had a mean of 55 ± 5 mM, similar to other reports for RBCs. Although the population of isolated OSNs displayed a wide range of $[\text{Cl}]_i$, in any individual OSN $[\text{Cl}]_i$ was stable during the recording period (up to 2 h). The only changes observed in $[\text{Cl}]_i$ were due to stimulation or altering bath conditions to affect Cl cotransporters. $[\text{Cl}]_i$ of OSNs responded in a dynamic fashion, changing in response to odor stimulation or by increasing $[\text{cAMP}]_i$. With $[\text{Cl}]_i$ unusually high in some olfactory neurons, there must be an active transporter or exchanger(s) that maintains this gradient. Several different transporters have been reported to carry Cl in neurons. We focused on NKCC1 and KCC2 since these Cl cotransporters have been shown to be critical to $[\text{Cl}]_i$ in developing neurons. Drugs were used to selectively inhibit either NKCC1 or KCC2. Data from these experiments, imaging and perforated patch clamp, suggest these transporters are functional in OSNs and work to set $[\text{Cl}]_i$.

Poster: Olfactory Sensory Neuron Physiology

Expression of Cl⁻ cotransporters in mouse olfactory neurons

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Chloride fluxes and intracellular Cl, $[\text{Cl}]_i$, are important in determining the ultimate response of olfactory sensory neurons (OSN) exposed to odor. In some OSNs, Cl can contribute most of the odor-elicited generator current, giving it a vital role in olfactory signaling. Populations of isolated mouse OSNs have been shown to express a wide range $[\text{Cl}]_i$. To determine what Cl transporters might be involved in this variance we characterized the expression of two chloride cotransporters in mouse OSNs: isoform 1 of the Na/K/2Cl cotransporter (NKCC1) and isoform 2 of the K/Cl cotransporter (KCC2). NKCC1 moves one Na ion, one K ion and two Cl ions into the cell. Conversely, KCC2 moves one K and one Cl ion out of the cell. Since these are cotransporters, if any one of ions being moved across the membrane is not present, no movement occurs. Western blots of nasal epithelium and immunohistochemistry of sectioned tissues, showed that both KCC2 and NKCC1 were present in mouse olfactory neurons and both transporters appeared to be located in the apical region of OSNs. To further characterize the expression patterns of KCC2 and NKCC1 we examined isolated cells using deconvolution microscopy. OSNs showed variable expression of KCC2 and NKCC1. Some OSNs displayed intense labeling for NKCC1 and KCC2 in their dendritic region, while others express these cotransporters at lower levels throughout the entire cell. A few OSNs showed only faint expression of either cotransporter. We have shown that NKCC1 and

KCC2 are present in mouse OSNs, although expression is not uniform across the population of OSNs.

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Poster: Olfactory Sensory Neuron Physiology

Pendrin, a chloride transporter, is expressed in olfactory receptor neurons

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During odorant transduction, a calcium-activated chloride current contributes to the depolarization of olfactory receptor neurons (ORNs). The purpose of this study is to identify anion exchangers that regulate internal chloride concentration in ORNs. Toward this end, reverse transcriptase-polymerase chain reaction was performed on RNA isolated from murine nasal mucosa using primers specific for members of the SLC26 family, a highly conserved group of anion exchangers that transport chloride. Our results identified the expression of pendrin in murine nasal mucosa. Pendrin is an ion transporter that exchanges chloride for bicarbonate, hydroxide, iodide or formate, but not sulfate. It also exchanges chloride for fructose and mannose. Pendrin is abundantly expressed in a distinct subpopulation of cells in the thyroid, inner ear and kidney. Mutations in pendrin cause Pendred's syndrome, a hereditary disorder characterized by deafness and goiter. Using Northern blotting, we confirmed that pendrin mRNA is expressed in nasal mucosa. Immunohistochemical staining with specific antibodies indicated that pendrin is expressed in ORNs and sustentacular cells in the epithelium, as well as in mitral cells in the olfactory bulb. In ORNs, sustentacular cells and mitral cells, pendrin is present in spherical, vesicle-like structures. The immunolabeling was specific since it was blocked with the pendrin peptide to which the antibody had been raised. Future studies are aimed at identifying the vesicles that express pendrin and in ascertaining the role of pendrin in regulating chloride concentration in the ORNs.

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Poster: Olfactory Sensory Neuron Physiology

Plasma membrane calcium pumps in the mouse olfactory and VNO receptor cells

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Calcium plays important roles in olfactory signaling and, therefore, it must be important to control intracellular Ca levels in olfactory and VNO sensory neurons by binding proteins, intracellular pumps, and extrusion from the cell by the Na⁺/Ca²⁺ transporter and plasma membrane Ca pumps (PMCAs). Mammals have four PMCA isoforms, each with different kinetic properties to serve different physiological tasks. We are studying the distribution and roles of these pumps in olfactory and VNO receptor neurons using immunocytochemistry of epithelia and dissociated cells and calcium imaging. The pan-PMCA antibodies show distinct staining patterns

in the olfactory and VNO epithelia: intense in the apical region of the olfactory epithelium but not in the respiratory epithelium; none apparent in the cilia; faint in the region of the neuron cell bodies. Use of specific antibodies shows: intense staining for isoforms 1 and 2 in both VNO and olfactory epithelium; little or no staining for isoforms 3 and 4 in either epithelium; weak to no staining of any isoform in the respiratory epithelium. The most intense staining is in VNO for isoforms 1 and 2 and in olfactory epithelium. Dissociated olfactory sensory neurons (OSNs) show staining for isoform 1 along the entire cell with intense staining where the dendrite meets the cell body. Staining for isoform 2 is prominent at the dendritic knob, cilia and cell body with less in the dendrite. Calcium imaging of isolated OSNs shows that calcium levels can return to basal levels after KCl or odorant stimulation even in the absence of transporter and SERCA pump function, which suggests a role for PMCAs in Ca removal in response to odor stimulation.

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Poster: Olfactory Sensory Neuron Physiology

Olfactory epithelial localization and dendritic morphology of golf negative olfactory sensory neurons projecting to medial olfactory bulb glomeruli in the larval sea lamprey (*Petromyzon marinus* L.)

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The objective of this study is to examine the dendritic morphology and olfactory epithelial distribution olfactory sensory neurons (OSNs) that project to spatially conserved medial glomeruli in the sea lamprey, an ancestral vertebrate with migratory and reproductive behavior mediated through pheromones (Li *et al.*, 1995, *J. Gen. Physiol.* 105: 567; Li *et al.*, 2002, *Science* 296: 138–141). These medial glomeruli lack Golf expression; yet this GTP binding protein is localized in the remaining six glomerular territories (Frontini *et al.*, *J. Comp. Neurol.* 465: 27–37). Following micro-injection of fluorescent dextran amines into the medial glomeruli, dextran labeled OSNs were observed in the ventral hemisphere of the olfactory mucosa. The dendrite of these OSNs was long and slender, and the cell body was located in the basal half of the olfactory epithelium. In comparison, G_{olf}-immunoreactive OSNs were widely distributed in the olfactory epithelium. The morphology of the G_{olf}-immunoreactive OSNs included short, thick dendrites with cell bodies in the apical portion of the olfactory epithelium, and OSNs with slender dendrites and cell bodies in the basal half of the olfactory epithelium. This study shows that OSNs in the sea lamprey are dimorphic, and supports the idea that sub-populations of OSN terminals are distributed according to functional parameters in this species.

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Poster: Olfactory Sensory Neuron Physiology

Electrophysiology of sustentacular cells in mouse olfactory epithelium

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Sustentacular cells (SCs) are exquisitely sensitive to P2Y receptor stimulation and generate robust increases in $[Ca^{2+}]_i$, implicating ATP as an important paracrine regulator in the olfactory epithelium (OE) (Hegg *et al.*, *J. Neurosci.* 23: 8291–8301). Here we determined the electrical properties and responses to ATP of SCs in 250 μm OE slices from mice (P1–P4) using whole-cell techniques. Capacitances (C_m) of SCs averaged 17.9 ± 0.1 pF and input resistance (R_{in}) was 173.1 ± 15.6 M Ω ($n = 134$). SCs generated a fast inward Na^+ current that was half-maximally activated and inactivated at -52 mV and -86 mV respectively. Blocking gap junctions with 1 mM 1-octanol did not significantly alter C_m (20.9 ± 1.4 pF before versus 20.7 ± 2.2 pF during 1-octanol, $n = 11$), while R_{in} increased significantly ($P < 0.05$, paired *t*-test) from 140 ± 48 to 187 ± 58 M Ω . The poor electrical connectivity between SCs was confirmed by an absence of dye coupling in 8 of 10 cells filled with Lucifer yellow (0.2%). In the presence of 1-octanol, ATP (30 s, 20–50 μM) transiently decreased R_{in} by 10%, which then increased by 20% during the 5 min wash. In eight SCs, a 30 s application of ATP increased C_m by $\sim 2.5\%$ over 10 min. Our results indicate that SCs generate inward Na^+ currents which, due to the low R_{in} of SCs, cannot support action potentials. The low R_{in} is apparently not due to coupling to neighboring cells. In addition, ATP elicits a biphasic response in the R_{in} of SCs and may trigger exocytosis, as indicated by an ~ 400 fF increase in C_m . Further experiments will identify the resting conductances of SCs and those elicited by ATP, as well as the Ca^{2+} -dependence of the ATP-mediated exocytosis.

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Poster: Olfactory Sensory Neuron Physiology

Transcripts enriched in sensory neurons and supporting cells of the olfactory epithelium

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Using a fluorescent LacZ substrate and flow cytometry, we collected LacZ+ and LacZ– cells from the olfactory epithelium of OMP-LacZ3 mice (Walters *et al.*, 1996, *J. Neurosci. Res.* 43: 146). Olfactory marker protein was 100-fold enriched in the LacZ+ RNA, indicating successful purification of mature olfactory sensory neurons (OSNs). Representational difference analysis confirmed by quantitative RT-PCR identified 54 differentially distributed transcripts. The majority of these transcripts encode proteins that have no known function. *In situ* hybridization identified 11 transcripts expressed only by OSNs and vomeronasal sensory neurons (VSNs). Some were restricted to mature OSNs. Twelve additional transcripts were enriched in OSNs and VSNs, but were also expressed in other cells in the epithelium. One novel transcript was expressed in the OSNs, sustentacular cells and Bowman's glands only within a restricted region of the epithelium. The six transcripts restricted to sustentacular cells and Bowman's glands include several enzymes that metabolize odorants. We also identified a transcript, pancreatitis-associated protein, restricted to cells of the respiratory epithelium. As a group, these olfactory enriched transcripts provide useful markers of certain cell types within the epithelium and potential insights into the function of several of these cell types.

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Poster: Olfactory Sensory Neuron Physiology

Expression profiling of phenotypically identified olfactory sensory neurons

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GFP+ and GFP– cells were collected using flow cytometry from the olfactory epithelium of OMP-GFP mice (Potter *et al.*, 2001, *J. Neurosci.* 21: 24). RNA from these two populations of cells, and from the brain of OMP-GFP mice, was hybridized against Affymetrix MOE430 A and B GeneChip microarrays. Adenylyl cyclase type III and $G_{\alpha olf}$ were present in the GFP+ RNA but not detected in the GFP– RNA, indicating that olfactory sensory neurons (OSNs) were enriched in the GFP+ population. We identified 730 probe sets that were present only in the GFP+ population. Of these, 490 represent expressed sequence tags (ESTs), genes encoding proteins of unknown function, and unannotated genes. A wide range of functions were represented by the other 240 sets. Transcripts were detected with roles in odorant detection (22), cell adhesion (10), metabolism and biosynthesis (21), transcription regulation (11), cell signaling (16), proliferation and differentiation (19), ion permeation and small molecule transport (24), and intracellular transport (9). Comparisons with previous experiments profiling transcripts expressed in the olfactory epithelium or in OSNs revealed overlaps with $<5\%$ of our list. Our data therefore appear to greatly expand the number of transcripts known to be enriched in OSNs.

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Poster: Olfactory Sensory Neuron Physiology

How sensitive can a 'broadly tuned' olfactory receptor be?

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There is an apparent paradox in the current literature of vertebrate olfaction. On the one hand, many kinds of evidence show that receptors are 'broadly tuned'; each receptor responds to numerous compounds. On the other hand, mammals can detect very low concentrations of odorants and single receptor neurons may detect single odorant molecules. Broad tuning appears to require low-affinity binding of odorants; high sensitivity is most easily explained by high-affinity binding. Thus, there appears to be an inverse relation between sensitivity and 'broad tuning'. We have used a simple model to explore this tradeoff. To obtain sensitivity near the theoretical limit of one molecule, one bound ligand—or one active receptor—must produce spikes in the olfactory neuron. Amplification mechanisms within the cell can accomplish this; however, in a receptor with low affinity and binding energy thermal fluctuations may produce a significant probability of the active state in the absence of a ligand. Unless the total spontaneous activity of all the receptors in the cell is well below threshold, these thermal fluctuations will produce spikes. This requirement determines a minimum energy difference between active and inactive states of the receptor

and, hence, a minimum binding energy and affinity of the ligand (odorant). Calculations using plausible estimates of the number of olfactory receptors in a cell show that at a moderate binding energy, the probability of spontaneous receptor activity is low enough to permit a cellular response to a single active receptor.

Poster: Olfactory Sensory Neuron Physiology

Olfactory 'interferometry'—non-contiguous distributions of olfactory receptor neurons expressing one olfactory receptor

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Following elucidation of olfactory receptor (OR) candidates (Buck and Axel, 1991), it was shown that olfactory receptor neurons (ORNs) expressing one OR (designated here ORNxs) are positioned in a non-contiguous fashion within olfactory epithelial (OE) 'zones' (Ressler *et al.*, 1993; Vassar *et al.*, 1993). We have recently shown similar, non-contiguously positioned ORNxs in salamander OE (Marchand *et al.*, 2004, in revision). From a developmental perspective (how stem cells generate one ORNx population) and from a targeting perspective (how ORNxs find glomerular targets in the bulb), it would seem more efficient that ORNxs be in contiguous groups. The question thus arises as to what advantages might non-contiguous ORN placement afford. In the present study, we hypothesize that non-contiguous ORNx placement provides the opportunity for odor-generated activity in each ORNx neuron to be compared by the receiving circuitry of the target glomerulus with respect to similarity. Responses from ORNxs placed in different regions of the incoming air flow, should be similar because they arise in ORNs expressing the same OR. The degree to which such responses are seen as similar by glomerular circuits is a measure of signal robustness. Such comparisons, especially for near threshold signals, performed in appropriate time windows (e.g. during sniffs), can provide for a kind of 'interferometric' process that could increase signal/noise ratio and improve the sensitivity (by averaging) of the ensemble, over individual ORN, responses.

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Poster: Olfactory Sensory Neuron Physiology

Assessing airflow parameters in rat EOGs

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The chromatographic model of olfactory response by Mozell and colleagues predicts that response size should vary with the interaction of flow rate and the physicochemical properties of odorants. We investigated this question in the rat nose by EOG recordings in the dorso-lateral recesses of the intact nasal cavity. Nasal flow rates of 50, 100, 200 and 500 ml/min were used to characterize the response size and latency. We found that latency measurements in the lateral recess had to guard against complex waveforms arising from stimulation at other sites. These waveforms are artifactual because they do not appear if odorants are directly applied to the opened epithelium and they disappear if the dorsal recess is made unresponsive by pro-

longed exposure to low molecular weight esters. With isoamyl acetate stimulation, the response in the lateral recess grows more rapidly with increase in nasal flow rate than the response in the dorsal recess. The latency in the lateral recess also decreases as flow rate increases. This effect was present over at least a 3 log unit range of concentration, down to $\sim 10^{-3}$ of vapor saturation. We suggest that the greater response latency in the lateral sites is indicative of slower airflow in the lateral recesses, which would favor odorant sorption before it reaches the recording site. As airflow increases, that loss of odorant due to sorption is decreased and the response increases markedly. This effect is smaller with odorants that normally evoke large lateral responses (hexane, α -terpinene and limonene). We interpret these findings to mean that the sniff velocity may dynamically control both the timing and size of olfactory responses in different parts of the epithelium.

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Poster: Olfactory Sensory Neuron Physiology

Altered olfactory sensory neuron phenotype in mucopolysaccharidoses I and VI

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Mucopolysaccharides (MPS) are thought to play a role in neural development and axon guidance. To assess their importance in the olfactory system, we studied the effects of two feline genetic models lacking different enzymes involved in MPS processing; one (MPSI) is associated with mental retardation, while the other (MPSVI) is not. We used tissue obtained at autopsy from unaffected control and affected cats. Stimulus-induced changes in intracellular calcium were studied using fluorescence imaging of live olfactory sensory neurons (OSNs). There were significantly fewer odorant responsive cells in the tissue from MPSI affected cats ($n = 7$; 1/57 ORNs), while $\sim 25\%$ of 149 cells isolated from controls ($n = 18$) responded to a standard battery of 13 odorants. In contrast, the frequency of responses to elevation of cAMP or membrane depolarization were similar in OSNs from control and affected cats. Fewer OSNs were obtained from animals with MPSVI ($n = 4$; 11 OSNs), but they responded normally to odors, depolarization and cAMP elevation. These results indicate that the defect in alpha-L-iduronidase activity (MPSI), but not arylsulfatase B activity (MPSVI) alters the normal development or maintenance of the olfactory epithelium. These data suggest that abnormal MPS processing interferes with OSN function and that certain MPSs play a role in development or maintenance of the olfactory system.

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Poster: Olfactory Sensory Neuron Physiology

Biophysical model of olfactory receptor neuron pairs reveals mechanism for gap junction mediated synchronized firing at threshold odor concentrations

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Recent studies in our laboratory have shown that multiple connexins are expressed in mature olfactory receptor neurons (ORNs) in mice, suggesting that gap junctional coupling might modify ORN function. A recent study in *Necturus maculosus* (Delay and Dionne, 2003, *Chem. Senses* 28: 807) found only a small fraction of coupled ORNs arguing against a general functional role for gap junctions. However, sparse coupling does not imply lack of a functional role of gap junctions in sub-groups of neurons expressing the same olfactory receptor. If connexins are only expressed in ORNs expressing certain olfactory receptors, the function of those ORNs could be modulated by gap junctional coupling. We formulated a Hodgkin and Huxley compartmental model of two gap junction coupled ORNs in order to create the theoretical framework necessary to test the hypothesis that gap junctions modulate function of specific subgroups of ORNs. We asked how the strength of the coupling would change the odor-induced activity of the coupled neurons. We report that at intermediate coupling strength, ORNs of different odor specificity display synchronized firing at threshold odor concentrations. At higher concentrations, the firing is not synchronized and differs substantially between the cell that is stimulated with the cognate odor and the adjacent cell. This mechanism would allow for increased odor sensitivity of certain odors without compromising odor quality discrimination.

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Poster: Sweet Taste

Reduction of sweet-suppressing effects of gurmarin by kallikreins increased in the submandibular saliva of rats fed gymnema-containing diet

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A tropical plant, *Gymnema sylvestre* (gymnema), contains gurmarin that selectively inhibits responses to sweet substances in rodents. The present study investigated possible interaction between gurmarin and the submandibular saliva in rats fed diet containing gymnema. At 1–2 days after the start of the gymnema diet, preference for saccharin and D-phenylalanine decreased and subsequently returned closely to the control level within several days. Electrophoretic analyses demonstrated that relative amounts of two proteins in the saliva clearly increased in rats fed the gymnema diet. Rats previously given section of the bilateral glossopharyngeal nerve, however, showed no such salivary protein induction, suggesting importance of sensory information for the protein induction. Analyses of amino acid sequence indicate that two proteins are rat kallikrein 2 (rK2) and rat kallikrein 9 (rK9). rK2 and rK9, a family of serine proteases, have resemble cleavage sites in the protein substrates of which comparable residue is also contained in sequence of gurmarin. Finally, kallikreins purified from saliva clearly inhibited the immunoreaction between gurmarin and antigurmarin antiserum. These results suggest that rK2 and rK9 increased by the gymnema diet via oral sensory system cleave gurmarin and reduce its sweet suppressing effect in rats.

Poster: Sweet Taste

Behavioral testing of the interaction of sweet taste and solution temperature in the rat

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Electrophysiological recordings have shown that there is an interaction between taste and temperature in the rat, but there is scant behavioral evidence to support this finding. The present study was conducted to test the licking behavior of non-deprived rats in a short-term test using different concentrations of nutritive and non-nutritive sweeteners across temperatures ranging from 10 to 40°C. A testing apparatus was developed to control the temperature of a solution with a resolution of 1°C. An infrared beam was passed across the opening to the sipper tube, so when the rat's tongue broke the beam, licks could be counted. Shutters could be opened, allowing access to the sipper tubes. During daily testing sessions, eight 30 s taste-temperature trials were given by opening the shutter, allowing the rat access to a sucrose or saccharin solution. The inter-trial interval was 90 s, allowing time for the temperature of the solution to be raised 5°C for the subsequent trial. On a given day, the concentration of the sucrose or saccharin was held constant and the temperature was varied from 10 to 40°C in an ascending manner across the eight trials. Concentrations used for sucrose were 0.00075–0.25 M and for saccharin 0.001–0.066 M. The software analysis program allowed for a microanalysis of the licking behavior during the 30 s presentations. As has been shown, licking increases as a function of concentration for sucrose and is expressed as an inverted U-function for saccharin. In all cases, licking decreases as the temperature of the solution is increased to >22°C.

Poster: Sweet Taste

Molecular studies of sweet taste receptor function

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Heterologous expression of T1R2 plus T1R3 yields a functional 'sweet receptor' that is responsive to a diverse range of sweet tasting ligands. Sweet tastants include sugars (e.g. glucose, fructose, sucrose), sugar alcohols, small molecule artificial sweeteners (e.g. saccharin and acesulfame K) and certain proteins (e.g. brazzein, monellin and thaumatin). There is no common structure that unites all of these diverse compounds, although several attempts have been made to infer a universal sweet motif. Brazzein, like monellin and thaumatin, is a naturally occurring protein that humans, apes and old world monkeys perceive as intensely sweet, but which is not preferred by, and is apparently tasteless to, other species such as new world monkeys, rats and mice. Using mouse-human T1R chimeras, site-directed mutagenesis and calcium imaging of

heterologously-expressed T1R2 + T1R3 we have determined the molecular basis for this species-specific sensitivity: we have identified a site within human T1R3 that is required for brazzein to stimulate T1R2 + T1R3. Other mutations in this same region of human T1R3 had effects on receptor activity toward monellin, and in some cases, overall receptor responsiveness toward most or all sweet compounds. This suggests that this region of T1R3 may play a role in both ligand binding (especially for the protein sweeteners) and in the transition between inactive and active states of the receptor.

Poster: Sweet Taste

Allelic variation of the TAS1R3 taste receptor gene selectively affects behavioral and neural taste responses to sweeteners in the F2 hybrids between C57BL/6ByJ and 129P3/J mice

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Recent studies have shown that the T1R3 receptor protein encoded by the *Tas1r3* gene is involved in transduction of sweet taste. To assess ligand specificity of the T1R3 receptor, we analyzed the association of *Tas1r3* allelic variants with taste responses in mice. In the F2 hybrids between the C57BL/6ByJ (B6) and 129P3/J (129) inbred mouse strains, we determined genotypes of markers on chromosome 4, where *Tas1r3* resides, measured consumption of taste solutions presented in the two-bottle preference tests, and recorded integrated responses of the chorda tympani gustatory nerve to lingual application of taste stimuli. For intakes and preferences, significant linkages to *Tas1r3* were found for the sweeteners sucrose, saccharin and D-phenylalanine, but not glycine. For chorda tympani responses, significant linkages to *Tas1r3* were found for the sweeteners sucrose, saccharin, D-phenylalanine, D-tryptophan and SC-45647, but not glycine, L-proline, L-alanine or L-glutamine. No linkages to distal chromosome 4 were detected for behavioral or neural responses to non-sweet quinine, citric acid, HCl, NaCl, KCl, monosodium glutamate (MSG), inosine 5'-monophosphate (IMP) or ammonium glutamate. These results demonstrate that allelic variation of the *Tas1r3* gene affects gustatory neural and behavioral responses to some but not all sweeteners. This study describes the range of ligand sensitivity of the T1R3 receptor using an *in vivo* approach.

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Poster: Taste Psychophysics

Perceptual variance: how discrimination methods become less discriminating

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Discrimination tests are incorporated into threshold measurement and are used as difference tests, in the sensory evaluation of food.

Yet, the various methods are not equivalent. This can be understood in terms of perceptual distributions (signal and noise) and d' . For a given perceptual difference, the greater the variance of these distributions, the smaller will be the value of d' . There are several sources of perceptual variance that can affect discrimination: adaptation and the sequence of tasting, memory and forgetting, and criterion variation. The goal of this study was to investigate these effects, using discrimination between purified water and 'threshold' concentrations of NaCl. Firstly, the 2-AFC, triangle, duo-trio and same-different methods were compared. Values of d' were computed and used for statistical analysis. After warm-up, which had the effect of stabilizing its criterion, memory effects rendered the same-different test as sensitive as the 2-AFC, despite its less advantageous sequence effects. Both were significantly more sensitive than the duo-trio or triangle methods. However, without warm-up criterion variation reduced the sensitivity of the same-different method to that of the triangle. Secondly, the effects of adaptation were further demonstrated using the two possible 3-AFC tests (NaCl-odd versus water-odd) and varying their relative sensitivity by manipulating interstimulus rinses. Either 3-AFC elicited significantly higher d' values when the interstimulus rinses were different from the stimuli than when they were the same.

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Poster: Taste Psychophysics

Bimodal distribution of sucrose octaacetate bitter taste sensitivity, and heritability of this trait among twins

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Sucrose octaacetate (SOA) tastes bitter to most tasters but the degree of perceived bitterness varies considerably. SOA is one of the most extensively investigated bitter taste traits in mice and has led to the identification of the *Soa* locus on mouse chromosome 6. To determine whether human sensitivity to the bitterness of SOA is also a genetic trait, we screened 130 mono- and dizygotic twins for their bitterness recognition threshold using a modified Harris-Kalmus sort test. In addition, these same subjects tasted and rated the bitterness intensity of six concentrations (including water) of SOA twice on the general Labeled Magnitude Scale. Monozygotic twins share 100% of their alleles in common whereas dizygotic twins share 50% of their genes on average. Thus, if a perceptual/behavioral trait has a significant genetic component, then monozygotic twins should be more similar to each other for this trait than are dizygotic twins. We found that the perceived bitterness of SOA was more similar among monozygotic twins than dizygotic twins for both the Harris-Kalmus test and the bitterness ratings. We further found that bitterness ratings were reliable and, at low concentrations, formed a bimodally distributed frequency histogram due to a subset of the population that cannot taste SOA until higher concentrations. At higher SOA intensities, the distribution appears more unimodally distributed. We also determined that the intensity ratings at low concentrations and the Harris-Kalmus bitterness recognition threshold levels were correlated, which validates these observations. Human Chr 12 syntenic to the *Soa* locus on mChr 6 should yield candidate genes.

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Poster: Taste Psychophysics**Gustatory response times to intensity and hedonic judgments**

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Choice response times of intensity and hedonic judgments were compared. Subjects made forced choice paired comparisons of orange lemonades with various concentrations of added quinine sulfate. Subjects were instructed to focus either on intensity or on pleasantness. Computerized administration of the stimuli and registration of the responses enabled response times measurements. Gustatory response times to intensity and hedonic judgments were compared in a within-subjects ANOVA. Preliminary results of 24 subjects indicate that a focus on intensity or hedonic aspects had no effect on response times [$F(1,23) = 2.451$, $P = 0.131$], whereas there was an effect of concentration on response times [$F(3,69) = 6.164$, $P = 0.001$]. The similarity of the response times for intensity and pleasantness may be due to the simultaneous availability of hedonic and intensity information to cognitive processing. Such a conclusion may be more compatible with a model of parallel processing of hedonic and perceptual information than with serial models of hedonic and perceptual processing.

Funding by Helmholtz Institute, University of Utrecht.

Poster: Taste Psychophysics**Thermal taste is associated with generally higher taste responsiveness**B. Green¹ and P. George²

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It was recently found that for some people, temperature alone can stimulate taste sensations (thermal taste). To investigate a possible source of individual differences in thermal taste we tested whether sucrose sweetness, which can be modulated by temperature, would be rated higher by thermal tasters ($n = 14$) than by thermal non-tasters ($n = 14$). Subjects used the gLMS to rate taste intensity on the tongue tip for three concentrations of sucrose, with saccharin and NaCl included as controls. Contrary to the hypothesis, thermal tasters gave higher ratings to all three stimuli [$F(1,26) = 21.0$, $P < 0.001$], rating the sweetness of sucrose and the saltiness of NaCl 2.1 times stronger, and the sweetness of saccharin 3.7 times stronger than did nontasters. To see if the group difference were the result of a general response bias (e.g. different scale use), thermal tasters and nontasters used the gLMS to rate temperature sensations on the lower lip and hand. There was no difference in temperature ratings between groups. We then measured taste on the back of the tongue (glossopharyngeal nerve) and soft palate (superficial petrosal nerve) with additional taste stimuli (PROP, quinine, citric acid) and new subjects. The differences between groups were even larger than on the tongue tip (chorda tympani nerve). Surprisingly, correlations among taste ratings for the various stimuli within and be-

tween sites were generally lowest for PROP. The consistency of the thermal taster advantage across stimuli and taste nerves suggests that in addition to peripheral factors such as receptor expression and innervation density, individual differences may arise in part from variations in central neural processes that influence taste intensity.

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Poster: Taste Psychophysics**Electric stimulation and metallic taste**H.T. Lawless¹ and D.A. Stevens²

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Metallic tastes can arise from various stimuli, including a retronasal smell sensation after rinses with ferrous sulfate, and a more gustatory or tactile sensation from exposure to metals such as copper or zinc. This study examined whether electrical stimulation produces a sensation similar in intensity, quality and location/sensitivity to stimulation with metals. Ten subjects (five male, age 18–52 years) volunteered. Three stimuli were affixed to handles: a 1.5 V watch battery (anode side exposed), a 2 cm copper disk or a 2 cm disk, half zinc and half copper. Four sites were stimulated on the right and left sides for 2 s: the anterior tongue near the edge, the medial tongue ~2 cm posterior to the tip, the inside of the upper lip and the buccal surface. Magnitude estimates were made relative to 0.10 M NaCl. A series of salt and citric acid stimuli were also rated. Analysis of variance showed an interaction of stimulus by site, main effects for those factors and no lateral differences. The battery and the zinc/copper disk were about equally effective stimuli, the copper-only disk less so. Sites were responsive in the following rank order: anterior tongue, medial tongue, cheek, lip. The rated intensity on the anterior tongue was about equal to 0.1 M NaCl. Individual differences were seen in responsiveness, although most subjects rank ordered the stimuli and sites similarly. The most frequent descriptor on the anterior tongue was 'metallic.' One mechanism for the generation of metallic taste may be electrolysis (generation of ion currents).

Poster: Taste Psychophysics**Sucrose and sodium chloride self-adaptation using 'taste'**A. Ashkenazi¹, J.F. Gent², L.E. Marks¹ and M.E. Frank³

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Recently we developed a computer-controlled, automated, open flow system for gustatory research (Ashkenazi, Fritz, Buckley and Marks, in press). Using pressurized air to control delivery of the solutions, this system provides precise temporal control over as many as 16 possible stimuli. In the current study we tested whether a common finding in the taste domain, that of self-adaptation, can be replicated using TASTE (Temporal Automated System for Taste Experiments). Subjects rated the intensity of either

0.5 M sucrose or 0.5 M sodium chloride on a computerized version of the Labeled Magnitude Scale (Bartoshuk, Duffy, Fast, Green, Prutkin, and Snyder, 2003). Intensity ratings were greater for both test stimuli when preceded by a 30 s water rinse compared with a 30 s self-adaptation rinse. Further, the pattern of results was similar when effects of order were controlled. These findings, similar to findings obtained with traditional methods of stimulus delivery, support the operational validity of TASTE for studies of adaptation.

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Poster: Taste Psychophysics

Perceived intensity functions generated under simulated fMRI scanning conditions

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Currently, research on the psychophysics of taste has become increasingly important, e.g. for interpretation of functional MRI data. However, an important question is how slopes of functions relating perceived intensity to concentration of taste stimuli measured under conditions outside of the scanning environment will be related to slopes of intensity functions for stimuli presented under the severe constraints on stimulus delivery that apply in fMRI experiments. The current study investigated perceived intensity across a number of taste stimuli at various concentrations, in healthy young adults, with a stimulation protocol adapted for fMRI scanning. Stimuli were presented at regular intervals in small boluses (0.03 ml) to minimize swallowing when the subject is lying on the back inside the scanner. Intensity was measured using Green's Labeled Magnitude Scale. Slopes of intensity functions for stimuli presented in the simulated scanning conditions of the present study were compared with slopes from previous studies using the dorsal flow and 'sip and spit' techniques. Results indicate that the slopes of intensity functions for sucrose, NaCl, caffeine, saccharin and MSG in the simulated scanning conditions were similar to the values for slopes from dorsal flow studies and generally lower than in studies done with the sip and spit method. Previous psychophysical taste research using the dorsal flow technique may be particularly useful for interpretation of future fMRI experiments.

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Poster: Taste Psychophysics

Supertasting is not explained by the PTC/PROP gene

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An important gene (TAS2R38) contributes to PTC/PROP perception (Kim *et al.*, 2003). Two common forms of this putative taste

receptor are defined by single nucleotide polymorphisms that result in three amino acid substitutions: Pro49Ala, Ala262Val, and Val296Ile, leading to haplotypes proline-alanine-valine (PAV) and AVI (I = isoleucine). The ancestral human haplotype is PAV, which was associated with lower PTC thresholds (tasters); AVI was associated with higher PTC thresholds (nontasters). Subjects provided PROP thresholds ($n = 54$), rated the bitterness of PROP (0.032–3.2 mM) with the general Labeled Magnitude Scale and were genotyped for the PTC/PROP gene ($n = 91$). Threshold data corroborated those presented by Kim *et al.*; PAV/PAV thresholds were lowest, AVI/AVI thresholds highest and PAV/AVI thresholds intermediate. However, suprathreshold functions showed that PAV/PAV tasters perceived significantly but only slightly more bitterness than did PAV/AVI tasters; AVI/AVI nontasters perceived the least bitterness. Of critical note, PAV/PAV tasters are not identical with supertasters defined by psychophysical criteria. Some of the factors (in addition to the PTC/PROP gene) likely to contribute to the perceived bitterness of PROP are density of fungiform papillae, other bitter genes and pathology. The distinction between medium and supertasters plays an important role in health outcomes related to oral perception.

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Poster: Taste Psychophysics

Childhood tobacco exposure increases obesity risk in adult men

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Maternal smoking during pregnancy promotes childhood obesity, but its impact on adult body mass remains unclear. Notably, these findings fail to consider the role of tobacco use in the home, where chronic exposure throughout childhood may confer long-term health risk. Early tobacco exposure promotes childhood ear infections, which alter oral sensation by damaging the chorda tympani; for male supertasters of PROP (6-n-propylthiouracil), such changes may encourage fat intake and adult-onset obesity. Consistent with this model, we show that postnatal tobacco exposure correlates with increased body mass index (BMI) in adult men. Participants in a smoking cessation program ($n = 288$) provided height, weight, family smoking history, and patterns of tobacco and alcohol use and abstinence. Tobacco exposure produced significant differences in men only; women were unaffected. Adult men raised in homes with 2+ smokers during the ages 1–10 years had higher BMIs than did men raised among fewer smokers; they were also more likely to be overweight. Although BMI often rises with smoking cessation, these men were willing to tolerate extreme gains leading to clinical obesity. Childhood exposure to maternal smoking contributed to higher rates of quit-related BMI gain, but prenatal exposure was unrelated to any adult BMI measure. These data suggest that postnatal tobacco exposure advances obesity risk in adult men by supporting pathologic changes in oral sensation.

Supported by the Transdisciplinary Tobacco Use Research Center at Yale and grants from NIDA/NCI (S.S.O.) and NIDCD (L.M.B.).

Poster: Taste Psychophysics**The influence of head trauma, otitis media, and tonsillectomy on oral sensation, fat acceptance, and body mass index (BMI)**A. Chappo¹, J. Alex², D. Coelho¹, V.B. Duffy³, D. Snyder¹ and L. Bartoshuk¹¹*Surgery, Yale University, New Haven, CT, USA,* ²*Otolaryngology, Lahey Clinic Medical Center, Burlington, MA, USA and* ³*Dietetics, University of Connecticut, Storrs, CT, USA*

Due to the close proximity of cranial nerve IX to the tonsillar bed, taste could be damaged by tonsillectomy. The present study explores oral sensation, food preferences, and body mass index (BMI) related to tonsillectomy and other taste-related pathology. Lecture attendees ($n = 674$) reported weight, height, and pathology [otitis media (OM), head trauma (HT) and tonsillectomy] and rated remembered sensations, butterscotch candy sweetness and acceptance of 26 foods on the general Labeled Magnitude Scale (gLMS). A statistically reliable group of nine high-fat foods was positively associated with BMI. Females aged 25–65 years who demonstrated proper use of the gLMS (most extreme light and sound greater than/equal to strong) were included and classified as follows: controls reported no pathology, ‘OM/HT’ reported OM and/or HT (indicating VII loss), ‘TONs’ reported tonsillectomies (indicating IX loss), and ‘COMBOs’ reported OM and/or HT with tonsillectomy (indicating VII and IX loss). Compared with controls and TONs, COMBOs perceived less candy sweetness, suggesting taste loss; in those aged 40+ years, COMBOs liked fat foods more and had greater BMIs. OM/HTs perceived less sweetness than controls. In those aged 40+ years, OM/HTs tended to like fat foods more than controls but the two groups showed no differences in BMI. Summary: damage to VII and IX may release inhibition on oral somatosensation; subsequent changes in fat acceptance may modulate obesity risk. Damage to VII alone may produce intermediate effects.

Supported by grant DC00283.

Slide: Taste Psychophysics**Gustatory responses to unilateral glossopharyngeal nerve damage**

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At our Wake Forest University Smell and Taste Center we have been referred several patients (in a 6 month period) with the complaint of taste distortion following unilateral tonsillectomy. We report a patient that complains of taste distortion following a right tonsillectomy for unilateral tonsillar hypertrophy. After a complete clinical evaluation and taste testing it was found that the patient suffered an injury to the right lingual branch of the glossopharyngeal nerve. The close anatomical relationship between the palatine tonsil and lingual branch of the glossopharyngeal nerve makes the nerve vulnerable during tonsillectomy. This injury has caused the patient to suffer ageusia to the right posterior one-third of

the tongue compensated by a contralateral phantogeusia (phantom taste) with clinical dysgeusia. The phantogeusia was abolished by application of anesthetic to the area where the phantom was perceived. We propose that the phantogeusia is the result of release-of-inhibition in the contralateral glossopharyngeal nerve. Taste distortion (including, phantogeusia and dysgeusia) after tonsillectomy is rarely reported as complication, but has a significant impact on quality of life. This preliminary study examines the taste distortion presence as a complication following glossopharyngeal nerve damage during tonsillectomy.

Slide: Cortical Signal Processing**Flexibility, not content, of cue representations in ABL depends on input from OFC**G. Schoenbaum¹, M.P. Sadoris² and M. Gallagher²¹*Department of Anatomy and Neurobiology, University of Maryland at Baltimore, Baltimore, MD, USA and* ²*Department of Psychological and Brain Sciences, Johns Hopkins University, Baltimore, MD, USA*

Interactions between orbitofrontal cortex (OFC) and basolateral amygdala (ABL) are critical to the encoding and use of information regarding incentive value. We have reported that neurons in ABL develop differential firing to odor cues paired with appetitive and aversive tastant outcomes. These differential responses reflect the acquired value of the cue and the predicted outcome. To assess the influence of input from OFC, we recorded from ABL neurons in rats with ipsilateral neurotoxic lesions of OFC as the rats were learning and reversing novel two-odor discrimination problems. Correlates of these neurons were compared with those of ABL neurons recorded in rats with sham lesions. We compared activity on S+ and S- trials during odor sampling, responding and outcome presentation. We found that OFC lesions had little effect on the proportions of cue-selective neurons observed or on how selectivity emerged in these neurons with learning. Nor did the OFC lesions affect the proportion of the cue-selective neurons that also exhibited differential activity before and during outcome presentation. OFC lesions did, however cause a dramatic reduction in the flexibility of these representations after reversal. While 53% of the cue-selective neurons reversed odor preference after reversal in intact rats, only 15% did so in rats with OFC lesions. These results suggest that although input from OFC may not be fundamental to the ability of ABL to represent cue value, this input does appear to be critical for the rapid flexibility of those representations, consistent with the deficit in reversal learning that is the hallmark of OFC lesions in rats and primates.

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Slide: Cortical Signal Processing**Brain activation pattern in response to olfactory recognition memory**B. Cerf-Ducastel¹, M. Chen², E. Abou³, L. Haas³ and C. Murphy¹¹*Psychology, San Diego State University, San Diego, CA, USA,*²*University of California, San Diego, San Diego, CA, USA and*³*San Diego State University, San Diego, CA, USA*

Ten young subjects (aged 20–25 years) participated in a functional Magnetic Resonance Imaging (fMRI) study with a 3 T MR scanner. Before entering the scanner subjects were presented with 16 familiar odors. They then were scanned for three runs, during which they were presented with words on a screen every 4 s which were either names of odors previously presented (targets) or names of new odors (foils). Subjects responded by pressing either button 1 if they recognized the odor or button 2 if not. Each run alternated four ‘ON’ periods containing seven targets and two foils (36 s) and four ‘OFF’ periods with seven foils and two targets (36 s). Data were processed with AFNI (Cox 1996) and compared ON and OFF periods, extracting regions that responded to cross-modal olfactory recognition memory. Group analysis showed that during the first run activated regions included right hippocampus, piriform/amygdalar area, superior temporal gyrus, anterior cingulate gyrus, inferior frontal/orbitofrontal gyrus, superior/medial frontal gyrus, bilateral parahippocampal gyrus, inferior parietal lobule, supramarginal gyrus, cerebellum, lingual/fusiform area and middle/posterior cingulate gyrus. Region of interest analysis showed that degree of activation significantly decreased from run 1 to run 3 in the right hippocampus, fusiform and lingual gyrus, parahippocampal gyrus and middle frontal gyrus but not in other regions, suggesting that these regions sustain a specific function in olfactory recognition memory that attenuates as foils become more familiar with repeated presentation.

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Slide: Cortical Signal Processing

Context dependent activity in primary olfactory cortex of humans

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Attentional modulation of neural activity patterns has been demonstrated in primary visual and auditory cortex. Similarly, activity in the rat olfactory bulb reflects non-odor cues that are merely associated with odor content, and activity in rat olfactory cortex reflects the motivational significance of an odor. To ask whether contextual modulation is evident in activity patterns of human primary olfactory cortex we used functional magnetic resonance imaging to measure sniff-induced neural activity in and out-of an olfactory context. Each trial of an event related design began with an auditory primer for ‘task detection’ or ‘task inhalation’. In ‘task detection’ subjects took one sniff and determined whether an odorant was present or not. Odor was present on half of these trials. In ‘task inhalation’ subjects also took one sniff, but knew in advance that an odor would never be present. Thus, the only difference between sniffs in ‘task inhalation’, and the no-odor sniffs of ‘task detection’ was that in the latter condition subjects were exploring for the presence of odor. Real-time measurement of nasal respiration assured sniffs were equal across conditions. Twelve subjects were scanned at 4 T (two-shot T2* sensitive EPI, $T_R = 1000$ ms, $T_E = 30$ ms, flip angle = 20° , 64×64 voxel matrix, 192×192 mm FOV, in-plane resolution = 3.5 mm, through-plane resolution = 3.5 mm). Activity in primary olfactory cortex was significantly higher when sniffing clean air in ‘task detection’ than when sniffing

the same content in ‘task inhalation’ ($P < 0.04$). In other words, activity in human primary olfactory cortex was strongly dependant on task context.

Supported by NSF.

Slide: Cortical Signal Processing

Information coding in the olfactory system

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Odorants are detected in mice by 1000 different odorant receptors (ORs) that are expressed by olfactory sensory neurons in the nose. The ORs are used combinatorially to detect different odorants and encode their unique identities. To explore the mechanisms underlying odor perception, we asked how inputs derived from different mouse ORs are organized as signals travel from the nose to the olfactory bulb and then the olfactory cortex. In the nose, each sensory neuron expresses a single OR gene. Neurons with the same OR are dispersed in the nose, but their axons converge in a few glomeruli at two fixed locations in the bulb. The result is a stereotyped sensory map in which inputs from different ORs are segregated in different glomeruli and relay neurons. In the olfactory cortex, inputs from one OR are targeted to clusters of neurons at specific sites, creating a stereotyped map unrelated to that in the bulb. In contrast to the segregation of different OR inputs seen in both the nose and bulb, it appears that different OR inputs overlap extensively in the cortex and single neurons may receive combinatorial inputs from many different ORs. Using c-Fos as an indicator of neuronal activator, we found that different odorants elicit different, but partially overlapping, activation patterns in the cortex. The representation of each odorant is composed of a small subset of sparsely distributed neurons. Quantitative analysis of the odor representations suggests that cortical neurons may function as coincidence detectors that are activated only by correlated inputs from different ORs.

Symposium: Chemical Communication in Mammals: From Pheromones to Individual Recognition

The mammary pheromone of the rabbit: identity, source, and some functions

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Mammalian females have evolved odor cues to guide their newborns to their mammae, whereas newborns have coevolved reliable means to respond to them efficiently. The domestic rabbit is a particularly suitable model to understand these cues because of the extremely sparing nature of maternal care enforcing nose-lead pups to instantaneously seize a nipple. Using a GC-olfaction assay on the volatiles of rabbit milk, an active compound was identified as 2-methylbut-2-enal. It releases stereotypical searching and oral grasping in pups as effectively as milk itself. Its activity is concentration-dependent and qualitatively selective as 40 other odorants from milk, from rabbit secretions or chosen arbitrarily revealed

ineffective. The compound qualifies as a pheromone in the sense defined by Beauchamp *et al.* (1976) and Johnston (2000) for mammals: it triggers invariant responses of clear functional significance, generalizes across pups of various genetic and dietary backgrounds, and acts instantly as an unconditional stimulus in pups devoid of prior exposure with it. As it seems to be emitted *de novo* in the mammary tract, we named it ‘mammary pheromone’ (MP). It is one of the key compounds of ejected milk as its concentration in milk correlates with behavioural activity. In more functional terms, the MP has a powerful activating impact on pups resting in the nest, and directs searching as efficiently as odor cues emitted on the abdominal surface by lactating does. This predisposed odour–behavior coupling will be discussed in the context of mother–young communication and of adaptive development of offspring in mammals.

Symposium: Chemical Communication in Mammals: From Pheromones to Individual Recognition

Making ‘scents’ of ownership

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In order that a receiver can match information in a scent mark to an individual owner, the signal must provide stable and persistent information about the owner’s individual identity. In mice (*Mus domesticus*), two polymorphic gene complexes contribute to the scents that differ between individuals: the major histocompatibility complex (MHC) and the major urinary proteins (MUPs). These polymorphic and polygenic proteins are ‘hard-wired’ in the genome and could provide stable ownership signals, while volatiles determined by developmental and environmental as well as genetic factors provide information on the current status of the owner. MHC-associated odours are important in promoting MHC-disassortative mate selection and kin recognition, but their contribution to individual identity signatures is unclear. Recent experiments reveal that individual MUP patterns are the basis of ownership signals in male competitive scent marks. Our current hypothesis, of an associative mechanism, attempts to bring together the different contributions made by MHC and MUPs. Volatile components perceived at a distance induce investigation through contact. The receiver then learns an association between the variable volatile signature detected through the main olfactory system and the involatile MUP signature detected via the vomeronasal system. Plasticity in this association means that changes in volatile signatures can be reassociated with stable involatile signatures during contact investigation. This permits rapid and distant identification when volatile signatures are familiar, together with a reliable signature of identity even when status or environment changes.

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Symposium: Chemical Communication in Mammals: From Pheromones to Individual Recognition

Why musth (and other) elephants use pheromones

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In the wild *Elephas maximus* and *Loxodonta africana* societies are generally stable and often resilient, two attributes reinforced by longevity, multigenerational matriarchal families, and multimodal signaling between individuals. In Asian elephants successful life strategies and complex behaviors are interlocked with an extensive chemical communication system. Initially, well-defined behaviors of wild and captive elephants formed the foundation for our investigation of olfactory communication. Two chemically identified pheromones, (*Z*)-7-dodecenyl acetate, released by preovulatory females, and frontalin, released by older adult males in musth, elicit distinctive, quantitatively measurable and statistically valid sex- and sexual-state-specific behaviors. The vast size and clearly spatially and functionally differentiated anatomical interrelationships of the main olfactory and vomeronasal organ systems not only allow the investigation of systems interplay, but effectively slow down individual biochemical events as temporally discrete steps, allowing such biochemical olfactory events to be correlated in real time with behavioral actions. The partially elucidated pathways for pheromone transport from trunk tip to sensory epithelia involving specific binding proteins will be demonstrated using concurrent biochemical and behavioral experiments, and will include data on protein–pheromone interactions and kinetic analyses, demonstrating that our Asian elephant model of vertebrate olfaction is an attractive experimental system with which to study distinctive steps, especially perceptive events, in discrete time.

Symposium: Chemical Communication in Mammals: From Pheromones to Individual Recognition

Individual recognition: signals, behavior and neural mechanisms

R.E. Johnston

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Recognition of individuals is essential for many types of social behavior and reproductive fitness in vertebrates. The cues for such recognition are complex and multi-dimensional; odor cues for individual recognition consist of complex mixtures whose proportions vary across individuals (mosaic signals). Since other individuals can have great emotional significance, individually distinctive signals can have powerful influences on behavior (e.g. fear specific to a familiar opponent). Hamsters (*Mesocricetus auratus*) use at least four sources of body odor for recognition of individuals and they have integrated, multi-component memories of such individuals. They also have extraordinary abilities to analyze scent over-marks and to determine which individual’s scent is on top of the other; they use this information to evaluate the vigor and quality of potential mates. At the neural level of analysis, both the main olfactory and vomeronasal systems contribute to discrimination of individuals by odors. Brain imaging studies using immediate early genes as markers for cell activity indicate circuits that are probably involved in recognition, memory, and appropriate emotional responses to familiar individuals (specifically, winners of fights with the subjects). These areas include parts of the hippocampus (dorsal CA1 area and subiculum), other parahippocampal areas and the basolateral amygdala. This model system provides insight into the functions of higher-order olfactory processing and areas of the brain involved in social behavior.

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Poster: Taste: Animal Behavior**Microstructural analysis of licking in the formation and extinction of a conditioned taste aversion**J.P. Baird¹, S.J. St. John² and E.A. Nguyen¹¹Psychology, Amherst College, Amherst, MA, USA and ²Reed College, Portland, OR, USA

LiCl (i.p.) paired with intraoral sucrose causes sucrose taste reactivity to shift to a profile that resembles aversive intraoral QHCl. We used lick microstructure analysis to assess changes in oromotor activity under conditions that may better approximate natural toxin exposure. Water-deprived rats were trained to access dH₂O 15 min/day from a bottle connected to a lickometer. On days 1, 3 and 5, 0.12 M LiCl (eight rats) or 0.12 M NaCl (eight rats) solution replaced dH₂O. On days 7, 9 and 11, only 0.12 M NaCl replaced dH₂O. A second study used 0.24 M NaCl instead of 0.12 M NaCl. On the first LiCl day rats avidly drank LiCl for 3 min; first minute lick rates and mean burst durations were comparable to NaCl controls. As LiCl licking progressed, ingestion rate slowed significantly by the fourth to sixth minute versus controls. On subsequent LiCl tests, intake, initial rate, burst duration, ingestion rate and lick volume were significantly reduced. However, burst count was significantly increased. In rats ingesting NaCl after LiCl, intake, initial lick rate (except 0.24 M NaCl), ingestion rate, burst duration and lick volume remained significantly reduced relative to controls, and burst count remained increased by at least 72%. Over remaining NaCl tests effects reversed and almost all differences were extinguished by the last NaCl day. These effects of LiCl parallel microstructural responses to 0.2 mM QHCl, and shifts in taste reactivity after CTA formation. The generality of these results across two paradigms supports the notion that CTA's can affect hedonic evaluation of tastants. The construct validity for both techniques as useful tools in the measurement of hedonic gustatory processes is also supported.

Supported by a Mellon-8 grant to J.P.B.

Poster: Taste: Animal Behavior**D-Cycloserine potentiates short-delay, but not long-delay, conditioned taste aversion**

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D-Cycloserine (DCS) is a partial glycine agonist at the NMDA glutamate receptor site, and has been shown to facilitate both declarative and non-declarative memory tasks. In order to determine if DCS enhances conditioned taste aversion (CTA) learning, we administered DCS before the pairing of saccharin intake and LiCl injection and measured expression with two-bottle tests. Water-deprived rats were injected with DCS (15 mg/kg i.p.) 15 min prior to 10 min access to 0.125% saccharin. Rats were injected with LiCl (19 mg/kg i.p.) 25 or 60 min after DCS. Controls were injected with saline in place of DCS, or saline in place of LiCl. One day after conditioning, rats were given 24 h, two-bottle preference tests for 14 days. Daily saccharin preference was calculated as saccharin intake over total intake. Control rats showed no CTA when saccharin was paired with saline (pref: 0.9 ± 0.04), and only a moderate CTA after LiCl injection in the absence

of DCS (pref: 0.4 ± 0.1, $P < 0.01$ versus saline). In the presence of DCS, rats showed a stronger aversion after LiCl at 25 min (pref: 0.06 ± 0.02, $P < 0.05$ versus LiCl alone), but the same aversion after LiCl at 60 min (pref: 0.32 ± 0.1). We conclude that DCS enhances a CTA but is dependent on the timing of both the gustatory stimulus and the toxin, such that DCS potentiated short-delay but not long-delay pairing of saccharin and LiCl. This difference may be due to the short-half life of DCS, or it may reflect different roles for NMDA neurotransmission in gustatory and toxin processing. Current studies are probing different time points with DCS to distinguish these possibilities.

Supported by an FSU Neuroscience Fellowship and NIDCD 03198.

Poster: Taste: Animal Behavior**Differences in gustatory behavior between C57BL/6J and DBA/2J inbred mice**S. Raghov¹, J.D. Boughter¹, T.M. Nelson², S.J. St. John³ and S.D. Munger²¹Anatomy & Neurobiology, University of Tennessee, Memphis, TN, USA, ²Anatomy and Neurobiology, University of Maryland at Baltimore, Baltimore, MD, USA and ³Reed College, Portland, OR, USA

The commonly used C57BL/6J (B6) and DBA/2J (D2) strains of inbred mice are among the strains included in the public and private genome sequencing projects and, given the availability of BXD recombinant inbred (RI) strains, provide an extremely desirable model for genetic studies. B6 and D2 mice have been shown to differ in terms of intake behavior to taste stimuli of different qualities, illustrating the potential of the BXD RI set for mapping QTLs influencing taste sensitivity. However, 24 or 48 h intake tests are generally confounded by non-gustatory or post-ingestive factors such as olfactory cues, caloric load and toxicity. We examined gustatory behavior, especially to an array of stimuli characterized as bitter or aversive, in B6 and D2 mice using both intake and brief-access tests. Robust strain differences in sensitivity for bitter stimuli QHCl, PROP and RUA were found using both assays. However, the strains did not differ for cycloheximide, strychnine or MgCl₂ in the brief-access test. We also failed to detect a strain difference for salts using either assay. We also developed a 4 day, high-throughput screen for bitter taste sensitivity. B6, D2 and BXD F1 and F2 intercross mice were tested with two concentrations each of denatonium benzoate, PROP, and QHCl. Mice were also assessed for additional non-gustatory phenotypes, including water lick rate, water consumption, latency to first lick and overall performance in the task. B6 and D2 mice significantly differed in taste sensitivity for PROP and QHCl. However, sensitivity to PROP and QHCL were not correlated ($r = 0.08$) in 126 F2 mice, demonstrating independence of these phenotypes.

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Poster: Taste: Animal Behavior**Gaping to quinine in glossopharyngeal nerve-transected rats after postsurgical taste aversion conditioning**

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Glossopharyngeal nerve transection (GLX) in rats has been shown to significantly reduce gaping, a stereotypical oromotor rejection behavior, in response to intraoral quinine hydrochloride (Q) infusion. We examined whether gaping to Q could be increased by a conditioned taste aversion (CTA) to this stimulus in GLX rats. Rats had intraoral cannulae implanted and either received sham surgery (SHAM) or GLX. After habituation to the test chamber, animals were infused with 0.3 M sucrose for 3 min (1 ml/min) on three consecutive days, followed by 0.6 mM Q infusion, the latter of which preceded an injection (inj) of LiCl or NaCl (2.0 mEq/kg). The rats were videorecorded and this 4 day cycle was repeated three more times with no injections given after the last Q infusion (test day). The first 30 s of the test-day Q infusion were scored for gapes adjusted for unscorable periods. The LiCl-inj SHAM ($n = 7$) and GLX ($n = 9$) groups gaped significantly more to Q compared with the NaCl-inj GLX ($n = 7$) rats and did not differ from each other. Gaping in the NaCl-inj SHAM ($n = 8$) rats fell between these two extremes and only significantly differed from that in the LiCl-inj GLX group. The lack of an expected significant difference in gapes between the two NaCl-inj groups could be due to repeated stimulus exposure effects. We are scoring the first Q infusion to evaluate this and to allow us to conduct within-subjects analyses. At present, these findings suggest that a CTA can increase gaping to Q in GLX rats as has been previously shown for sucrose.

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Poster: Taste: Animal Behavior

Taste preference and taste buds maintenance after unilateral lingual denervation in rats

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Intact gustatory nerve supply appears to be required for the maintenance of taste buds, and unilateral transection of gustatory nerve results in a reduced number of taste papillae, with the degeneration and disappearance of taste buds ipsilateral to the surgical side. We examined the effects of unilateral lingual nerve transection on taste function as well as on the maintenance of taste buds. Male Sprague–Dawley rats weighing 250–300 g underwent a unilateral transection of the lingual nerve and were subjected to preference tests for various taste solutions (0.1 M NaCl, 0.1 M sucrose, 0.01 M QHCl or 0.01 M HCl) with a two-bottle test paradigm at 2, 4, 6, or 8 weeks after the operation. Water and each test solution bottle were supplied for 48 h at each test, with one change of the bottle position at 24 h. The preference score for salty, sweet or sour taste, but not for bitter taste, tended to be higher in the operated rats (though without statistical significance) compared with the sham rats. The overall pattern of the preference scores was not changed by time after operation. These results suggest that unilateral damage of the lingual nerve may not affect taste function of the subject significantly. In order to examine the morphologic changes of tongue, the number of fungi form papillae on the denervated side was counted in comparison with the intact side. The time course of apoptotic death of taste cells in the foliate and vallate papillae was examined as well.

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Poster: Taste: Peripheral Connectivity

Ultrastructure of morphologically identified chorda tympani axons in the nucleus of the solitary tract in developmentally sodium-restricted and control rats

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Dietary sodium restriction during a critical period in gestation results in specific, yet profound morphological changes in the dorsal zone of the chorda tympani nerve terminal field in the adult rat nucleus of the solitary tract. Specifically, there is an approximate two-fold increase in volume of the dorsal zone of the terminal field in sodium-restricted rats compared with controls. In order to better characterize this plasticity, we examined the ultrastructure of terminals identified by way of bulk-labeling the chorda tympani nerve with biotinylated dextran and visualizing with DAB. Quantitative measurements in the dorsal zone of the chorda tympani field in both sodium-restricted and control rats included volumetric density of labeled synapses and synapsing frequency of axons. Preliminary evidence indicates that there is over a fourfold increase in the density of chorda tympani terminals in the dorsal region in restricted rats compared with controls. However, there was no significant increase in synapse density along any given axon, suggesting that elaboration of chorda tympani input to this region occurs by addition of new axonal arbors. Furthermore, there is a conspicuous qualitative difference in the morphology of synaptic terminals in sodium-restricted rats in that their profiles are interrupted by finger-like protrusions of dendritic spines. These results indicate a process of synaptic remodeling as well as an elaboration of connectivity occurs as a result of developmental sodium restriction.

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Poster: Taste: Peripheral Connectivity

Gustatory nerve terminal fields in rats recovered from early developmental sodium restriction

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A critical period for chorda tympani (CT) terminal field development is from embryonic day 3 (E3) to E12. When pregnant rats are fed a low-sodium diet during this period, the CT terminal field in their offspring (E3–E12 restricted) is enlarged compared with controls. Similarly, rats raised on a sodium-restricted diet from E3 to postnatal day 28 and then fed a sodium-replete diet for at least a month (P28 recovered) also have CT terminal fields that are large. To determine the interactions among the CT, GSP and IX terminal fields in controls, E3–E12 restricted and P28 recovered rats, we used a triple fluorescent labeling procedure. In control rats, the IXth field is the most dorsal of the three fields and extends ventrally into the dorsal-most zone of the GSP. The CT field overlaps much of the GSP field, but has no overlap with the IXth field. In E3E12 sodium restricted rats, the IXth field is ~3 times greater than

that in controls and extends medio-laterally and ventrally, well into the CT field. Both the GSP and CT fields are significantly larger than controls. P28 recovered rats had similar alterations of all three fields; however, they were not as dramatic as in E3–E12 restricted rats. Therefore, a very early period of dietary sodium restriction (E3–E12) that precedes the development of peripheral gustatory structures produces the most widespread effects on the development of gustatory afferents in the NTS. These results also suggest a greater functional convergence in the gustatory brainstem and behavioral consequences for sodium restriction during development.

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Poster: Taste: Peripheral Connectivity

Isoforms of the synaptic vesicle protein SV2 have different locations in the rat circumvallate gustatory tissue

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SV2, a 12-transmembrane protein which may function as a calcium regulator in neurotransmission and a component of the intravesicular matrix, has three identified isoforms, A, B and C. Presynaptic size vesicles are present in the gustatory system in primary afferent nerve fibers receiving synapses from taste cells, peptidergic intragemmal nerve fibers and taste cells. Two-color fluorescent microscopy of fixed rat tongue tissue sections is labeled with various SV2 antibodies. A general SV2 antibody labels most nerve fibers, including intragemmal, some perigemmal, peri-apical pore, basal plexus and the sub-epithelial network of nerve fibers. SV2B has a very restricted pattern suggestive of location in taste cells, especially in taste buds in the lower circumvallate crypt. SV2C labels a smaller group of intragemmal, perigemmal and basal plexus fibers than the general SV2 antibody. The SV2 general positive fibers are included in the population of fibers which label with PGP 9.5. Most SV2B and C positive structures colocalize with PGP-positive structures. In contrast, a few intragemmal fibers labeled with either the general SV2 or SV2B antibody appear to colocalize or oppose gustducin-positive taste cells. These results indicate that the antibodies to various SV2 isoforms may be a tool to differentiate various pools of small clear vesicles within the gustatory system. SV2-positive nerve fibers are a subset of the PGP-positive fibers and may identify synaptic sites between some nerve fibers and taste cells.

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Poster: Taste: Peripheral Connectivity

Synaptophysin-like immunoreactivity in circumvallate papillae of the rat and mouse

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Synaptophysin is an abundant protein in the CNS that is found in the membranes of synaptic vesicles. Synaptophysin is thought to

play a role in the regulation of SNARE formation during synaptic transmission through its interactions with other proteins of the synaptic vesicle cycle, namely binding with VAMP/synaptobrevin. We postulate that synaptophysin is associated with the synaptic vesicles at taste cell synapses. Our preliminary results indicate that subsets of taste cells of the circumvallate papillae of both the rat and mouse display synaptophysin-like immunoreactivity (LIR). The immunoreactive cells are slender and span the entire length of the taste bud from the basal lamina to the taste pore. In the rat, nerve processes exiting the taste buds are also immunoreactive. Synaptophysin-LIR does not appear to colocalize with IP₃R3-LIR, but does appear to colocalize with a subset of PGP 9.5 (protein gene product 9.5) immunoreactive cells. The elucidation of proteins such as synaptophysin involved in the synaptic vesicle cycle in taste cells may prove useful in determining how taste cells transmit information.

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Poster: Taste: Peripheral Connectivity

Immunocytochemical analysis of syntaxin in rat circumvallate taste buds

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The SNARE proteins syntaxin, SNAP-25 and synaptobrevin all play key roles during Ca²⁺ dependent exocytosis in the CNS. We hypothesize that taste cell synapses utilize the same protein machinery as used by synapses in the CNS. Our previous studies have shown that taste cells with synapses display SNAP-25- and synaptobrevin-like immunoreactivity (LIR) (Yang *et al.*, 2000; Yang *et al.*, in press). At present, we are studying the presynaptic membrane protein, syntaxin, in rat circumvallate taste buds. Our current results indicate that syntaxin is present in a subset of taste cells and nerve processes in taste buds. Approximately 15% of the taste cells in a taste bud display cytoplasmic syntaxin-LIR and a smaller subset of taste cells also show punctate staining in the cytoplasm. Syntaxin-LIR colocalizes with SNAP-25- and synaptobrevin-LIR in a small subset of taste cells and most nerve processes. Approximately 11% of the syntaxin-LIR cells colocalize with PLCβ2-LIR cells. However, only ~4% of the PLCβ2-LIR cells also display syntaxin-LIR. DAB immunoelectron microscopy reveals that syntaxin-LIR is present in both type II and III taste cells. Type II taste cells display punctate syntaxin-LIR at Golgi bodies, while type III taste cells show cytoplasmic syntaxin-LIR. All of the synapses associated with syntaxin-LIR taste cells are from type III cells onto nerve processes. These results support the notion that taste cell synapses are similar to synapses of the CNS.

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Poster: Taste: Peripheral Connectivity

Taste buds and surrounding fibers are immunoreactive for the ionotropic ATP receptor P2X7

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Processing of taste information probably involves intercellular communication within the taste bud as well as communication between

individual taste cells and innervating nerve fibers. Immunocytochemical studies of transmitter proteins and receptors suggest that several neurotransmitter pathways are used by taste buds. Recent evidence suggests a role for ATP and its receptors in taste bud function (Bo *et al.*, 1999; Kim *et al.*, 2000; Baryshnikov *et al.*, 2003). Further, physiological studies suggest taste cell responses to ATP involve metabotropic (P2Y-like) receptors. The current study was done to determine if ionotropic ATP receptors also are present on taste cells. Tongues from transgenic mice expressing GFP under the control of the α -gustducin promoter were examined for the presence of P2X7 immunoreactivity. We found that P2X7 immunoreactivity includes taste cells as well as nerve fibers in and around taste buds. P2X7 occurs both in cells that express α -gustducin and in taste cells that lack the G-protein. In conclusion, taste cells may signal to one another by both metabotropic and ionotropic ATP receptors.

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Poster: Taste: Peripheral Connectivity

Recovery of gurmarin-sensitive neural responses and expression of T1R3 and gustducin in fungiform papillae after crush of the mouse chorda tympani

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Gurmarin is known to be a peptide that selectively inhibits responses to sweet compounds in the chorda tympani nerve (CT) in C57BL mice. The peptide, however, suppresses only about half of the response to sucrose, indicating existence of both gurmarin-sensitive (GS) and -insensitive (GI) response components in the CT. In the present study, recovery of taste responses and reappearance of taste receptor cells were studied by examining GS of the CT responses and expression of T1R3 and gustducin in fungiform papillae. It was reported that T1R3 contributes responses to sucrose, saccharin and other artificial sweeteners in the CT. At ~2 weeks after the nerve crush, although no significant responses to taste stimuli were observed in the CT, *in situ* hybridization analysis demonstrated that T1R3 and gustducin expressed in subsets of taste bud cells. At ~3 weeks after the CT crush, responses to sweet compounds reappeared and the threshold of sucrose was higher than that shown by intact CT. After more than a month, the CT showed GS comparable with those shown by intact animals. These data suggest that the molecules related to taste transduction might express in taste receptor cells prior to their contact with regenerated taste axons.

Poster: Taste: Peripheral Connectivity

The neural isoform of tryptophan hydroxylase is localized to taste bud cells

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Taste receptor cells are epithelial in origin and form synaptic contact with innervating sensory nerves. The identification of the neurotransmitter(s) in these cells has not, however, been absolutely determined. While a number of studies implicate serotonin as a candidate neurotransmitter, evidence of its synthetic pathway in taste receptor cells is

incomplete. In the current study, RT-PCR identified the neural isoform of tryptophan hydroxylase (TPH2) in rat foliate and vallate isolated taste buds and single taste bud cells (TBC). Sequencing of 666 bp near the 3'-end of the taste TPH2 showed this product to have 99% identity with rat brain TPH2. The peripheral isoform, TPH1, could not be detected by RT-PCR in taste tissue, but was detected in brain cDNA, used as a positive control. Of 12 TBC wherein the synaptic marker syntaxin was detected by RT-PCR, TPH2 but not TPH1 was detected in 10 of these cells, and T1R3 was detected in six. Immunocytochemistry localized TPH2 to a subset of taste bud cells located within the main body of the bud. No staining was seen in the basal region nor in the perigemmal cells. The results support a role for serotonin as a neurotransmitter in taste receptor cells.

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Poster: Taste: Peripheral Connectivity

Taste buds release 5-HT when depolarized

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Serotonin (5-HT) is implicated as a neurotransmitter or neuromodulator in taste buds but to date there has been no direct evidence for its release from taste cells. Using a CHO/biosensor cell, we tested whether isolated mouse vallate taste buds release 5-HT in response to stimulation. Pilot studies confirmed that CHO cells, stably transfected with 5-HT_{2c} receptors (Berg *et al.*, 1994, *Mol. Pharmacol.*) and loaded with the Ca²⁺-sensitive dye, Fura2, to assay responses, were exquisitely sensitive to 5-HT (threshold ~3 nM). These 'CHO/biosensor cells' did not respond to bath-applied KCl (50–100 mM) nor to cycloheximide (100 μ M), a bitter taste stimulus. We confirmed that 5-HT responses in CHO/biosensor cells were reversibly inhibited by mianserin (1–10 nM), a 5-HT_{2c} antagonist. We isolated taste buds from mouse vallate papillae and loaded them with Fura2 to verify that taste cells responded to KCl depolarization (50–100 mM). Finally, to test whether stimulated taste buds released 5-HT, we isolated individual taste buds and plated them on a glass coverslip in a shallow recording chamber. Taste buds were approached with a Fura2-loaded CHO/biosensor cell held onto a fire-polished micropipette by gentle suction. Bath-applied KCl elicited responses in the CHO/biosensor cell when it was positioned against a taste bud. Applying buffer did not elicit responses. Furthermore, moving the CHO/biosensor away from the taste bud abolished responses. These data suggest that when they are depolarized, isolated taste buds release 5-HT.

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Poster: Taste: Peripheral Connectivity

Analysis of a human fungiform papillae cDNA library and identification of taste-related genes

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Various genes related to early events in human gustation have recently been discovered, yet a thorough understanding of taste transduction is hampered by gaps in our knowledge of the signaling chain. As a first step toward gaining additional insight, the expression specificity of genes in human taste tissue needs to be determined. To this end, a fungiform papillae cDNA library has been generated and analyzed. For validation of the library, taste-related gene probes were used to detect known molecules. Subsequently, DNA sequence analysis was performed to identify further candidates. Of 987 clones sequenced, clustering results in 288 contigs. Comparison of these contigs with genomic databases reveals that 207 contigs (71.9%) match known genes, 16 (5.6%) match hypothetical genes, 8 (2.8%) match repetitive sequences and 57 (19.8%) have no or low similarity to annotated genes. The results indicate that despite a high level of redundancy, this human fungiform cDNA library contains specific taste markers and is valuable for investigation of both known and novel taste-related genes.

Poster: Human Olfaction: Pathology

Olfactory functions and volumes of orbitofrontal and limbic regions in schizophrenia

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Olfactory deficits in schizophrenia have been widely reported. These deficits have been hypothesized to be associated with abnormalities in prefrontal (orbitofrontal) and/or mesiotemporal brain regions. No study investigated this structure–function relationship using magnetic resonance imaging. We examined the relationship between olfactory functions and volumes of limbic (hippocampus/amygdala) and orbitofrontal brain regions in young men with schizophrenia and healthy subjects, matched for sex and age. Unirhinal assessment of olfactory measures included main functions (threshold, discrimination, identification) and odor judgements (intensity, familiarity, edibility, pleasantness). Patients performed bilaterally more poorly than controls in the threshold, discrimination and identification tasks, as well as on several odor judgement measures. Compared with controls, patients showed bilateral smaller hippocampus and amygdala volumes. In patients, smaller volumes of the hippocampus were significantly correlated with poorer discrimination performance. Our results corroborate and extend previous findings of olfactory deficits as well as limbic structure volume reduction in schizophrenia, and suggest that olfactory deficits, namely impairments in discrimination, are associated with morphometric abnormalities in the hippocampus.

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Slide: Human Olfaction: Pathology

Diminished posterior nasal volumes in male patients with schizophrenia

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Background: gross midline abnormalities, such as cleft palate and cavum septum pelucidum, impairments in olfactory ability and, to a lesser degree, subtle craniofacial dysmorphogenesis, are considered characteristics of schizophrenia. Due to concordant development of the oral cavity, olfactory structures and ventral forebrain, we hypothesized that structural abnormalities of the nasal cavity might represent disease-associated markers of embryological dysmorphogenesis. Method: a measurement of nasal volume was acquired by acoustic rhinometry for 56 schizophrenia patients (44 men, 12 women) and 37 healthy controls (24 men, 13 women). Nasal volume was segmented into three areas: total (0.0–5.5 cm), anterior (0.0–3.5 cm) and posterior (3.5–5.5 cm). Results: an overall MANOVA demonstrated a significant diagnosis by sex by nasal compartment interaction [$F(2,172) = 2.92, P = 0.05$]. Decomposition of this interaction revealed that male patients had significantly smaller left [$F(2,126) = 6.26, P < 0.003$] and smaller left posterior volume reductions [$F(1,63) = 4.87, P = 0.031$]. Female patients, on the other hand, showed a slight non-significant increase (11%) of total nasal volume. Conclusions: these findings suggest a specific craniofacial abnormality indicating potential disruption of first trimester embryological development in male patients with schizophrenia.

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Poster: Human Olfaction: Pathology

Olfactory testing differentiates idiopathic Parkinson's disease from essential tremor

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Olfactory dysfunction exists in idiopathic Parkinson's disease (IPD) while preliminary studies on essential tremor (ET) patients indicate normal sense of smell. ET may mimic IPD, the two may co-exist, and occasionally ET may progress to IPD. Two olfactory tests were carried out: (i) the 40 odorant University of Pennsylvania Smell Identification Test (UPSIT); and (ii) the olfactory event related potential (OERP). This was undertaken with a Burghart OM2 olfactometer. We used a 200 ms pulse of H₂S gas at a concentration of 2 p.p.m. Latency was measured to the main negative peak (P2) at PZ and amplitude from N1 to P2. The control group for UPSIT consisted of 195 subjects aged 17–90 years. Forty-two ET patients, aged 16–84 years, and 54 IPD patients, aged 21–83 years, were tested using both the UPSIT and OERP tests. All had a Mini-Mental Test score of at least 27/30. Potential patients or controls

with metabolic disease such as diabetes, thyroid disease, local nasal disease or depression were excluded. Significant values adjusted for age are all >0.05 on Student's two-tailed *t*-test. The mean UPSIT score for controls was 33.5 and for ET 32.2, which are not significantly different. For IPD the mean score was 18.1, which is significantly different from controls and ET. OERP showed no difference for P2 latency between controls and ET patients (mean latency 628.5 and 641.3 ms respectively). In 21 PD patients the evoked response was absent. In the remaining 33 the mean latency was 962 ms, which is significantly prolonged compared with controls and ET. In all three groups amplitude measurements did not differ significantly. A normosmic patient with tremor is more likely to have ET than IPD, while someone with tremor and impaired olfaction may have IPD or a related syndrome.

Poster: Human Olfaction: Pathology

Memory for emotional and neutral odors and amygdala: electrophysiological recordings in patients with epilepsy

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It is known that emotionally arousing events are remembered better than neutral ones, but this has not been explored in olfaction. Increasing evidence indicates that the amygdala plays key roles in emotion and memory, and electrophysiological studies show that the human amygdala is implied in recognition memory for odors. In the present study we assessed memory for emotional and neutral odors in patients with temporal-lobe epilepsy undergoing intracranial recordings and examined the related olfactory evoked potentials (OEPs) in amygdala. Behavioral and electrophysiological results were obtained in eight patients implanted in the amygdala (six bilaterally, one left, one right). Patients' behavioral data were compared with those of 10 healthy control subjects. Memory for pleasant, unpleasant or non-emotional odors was tested 24 h after incidental encoding. Odor recognition was similar in patients and controls. OEPs were observed in the amygdala, composed mainly of a large positive peak occurring at a mean latency of 300 ms. An effect of odorant's emotional arousal was found in the right amygdala, with shorter latencies for emotional than for neutral odorants. This effect was specific to the amygdala, as similar OEPs were not found in the hippocampus. The results provide electrophysiological evidence for the postulated role of amygdala in memory for emotionally arousing material. The significance of the laterality of this effect will be explored with respect to the healthiness of left versus right amygdala in individual patients.

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Poster: Human Olfaction: Pathology

Olfactory dysfunction in degenerative ataxias

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Several lines of evidence suggest that the cerebellum may play a role in higher-order olfactory processing. In this study, we administered the University of Pennsylvania Smell Identification Test (UPSIT), a standardized test of olfactory function, to patients with ataxias primarily due to cerebellar pathology (spinocerebellar ataxias and related disorders) and to patients with Friedreich ataxia, an ataxia associated mainly with loss of afferent cerebellar pathways. UPSIT scores were slightly lower in both patient groups than in the control subjects, but no differences were noted between the scores of the Friedreich and the other ataxia patients. Within the Friedreich ataxia group, the smell test scores did not correlate with the number of pathologic GAA repeats (a marker of genetic severity), disease duration, or categorical ambulatory ability. UPSIT scores did not correlate with disease duration, although they correlated marginally with ambulatory status in the patients with cerebellar pathology. This study suggests that olfactory dysfunction may be a subtle clinical component of degenerative ataxias, in concordance with the hypothesis that the cerebellum or its afferents plays some role in central olfactory processing.

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Slide: Human Olfaction: Pathology

Diagnostic options and limits in patients with olfactory dysfunction after head injury

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Objective: in this study, patients with impaired olfaction after head trauma were investigated using smell tests, immunohistochemistry and cell cultures of biopsies of the olfactory epithelium to trace specific changes. Methods: we investigated the sense of smell with Sniffin' Sticks, chemosensory evoked potentials and biopsy of the olfactory epithelium using neurofilament and β -tubulin type III to detect immature neurons on frozen sections and cultures. The results were compared with age- and gender-matched normosmic controls. Results: in five patients complete anosmia was found in the Sniffin' Sticks test, but only in three patients olfactory evoked potentials were missing completely. The biopsies showed intact olfactory epithelium with immature neurons in some cases, cell adhesion was found in 25–100% of the cultures and neurogenesis could be induced under the influence of growth factors. Conclusions: head trauma can cause comprehensible olfactory impairment; there are different possible sites of a lesion. Olfactometry shows an intact neural transmission to the brain. Biopsies of the olfactory epithelium reveal the regeneration capacity of the receptor neurons.

This study was supported by the Deutsche Forschungsgemeinschaft HA-3447/1-1, Germany and by the Garnet Passe and Rodney Williams Memorial Foundation, Australia.

Slide: Human Olfaction: Pathology**Olfactory deficits in sinonasal disease**

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Background: chronic sinonasal disease is a common cause of smell loss but the pathophysiology is unclear. Human biopsy specimens indicate direct inflammatory changes in the epithelium, although it remains unclear how this mediates clinical olfactory deficits. It has been suggested that inflammatory cytokines may alter secretion and/or affect neuronal viability. Under normal conditions, olfactory sensory neurons undergo apoptotic cell death at a baseline rate matched by the regeneration of mature OSNs from precursors in the epithelium. The current study examines normal and diseased olfactory mucosa for evidence of a disturbance in this balance, which result in a net loss of OSNs. Study design: histologic analysis of human and animal olfactory tissue. Methods: normal and inflamed human and animal olfactory mucosa was assessed for immunohistochemical evidence of apoptosis. Results: increased activity of the apoptotic effector enzyme caspase-3 was demonstrated in diseased olfactory mucosa in comparison with normal controls in both human and animal tissue. Conclusion: these results support the hypothesis that olfactory deficits in sinusitis result from increased apoptotic OSN death apparently not matched by regeneration. Interference with the apoptotic pathway is currently the subject of intense pharmaco-therapeutic research. Potential treatment options will be discussed.

Supported by the Department of Otolaryngology-HNS.

Poster: Human Olfaction: Pathology**Chemosensory changes from exposure to formaldehyde in anatomy labs**

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More than 10% of the US workforce (~14 million people) has daily occupational exposure to chemicals or particulates, and as many as 40% of these individuals report symptoms of chronic nasal inflammation or rhinitis that are known precursors of olfactory loss. Moreover, evidence suggests that occupational chemical exposure increases the risk of age-associated olfactory disorders. Despite this, the evaluation of olfactory function in susceptible occupational cohorts is rarely performed and consequently, little is known about the mechanisms underlying chemical-induced olfactory changes. This study evaluated the chemosensory impact of repetitive exposure to formaldehyde among veterinary students enrolled in anatomy labs, using a comprehensive assessment of threshold, secretory and inflammatory parameters. Personal exposures for each participant were collected using passive dosimeters in order to evaluate observed changes in olfactory performance as a function of exposure concentration. Consistent with studies in formaldehyde-exposed animals, mucociliary clearance time increased with exposure duration for the students, but not unexposed controls, an outcome which may predispose individuals to both perceptual changes and nasal health effects following chemical exposures.

Supported by NIH P50 P50 DC00214.

Poster: Human Olfaction: Pathology**Qualitative olfactory dysfunction: frequency and prognostic significance**T. Hummel¹, H. Maroldt¹, J. Frasnelli¹, B.N. Landis², K. Hüttenbrink¹ and S. Heilmann¹¹ORL, University of Dresden Medical School, Dresden, Germany and²ORL, University Hospital of Geneva, Geneva, Switzerland

This study aimed to investigate frequency and prognostic significance of qualitative olfactory dysfunction (parosmia, phantosmia). A total of 868 patients were included; 160 of these patients (18%) complained of parosmic sensations, 59 (7%) mentioned odor phantoms. In patients without qualitative olfactory dysfunction, smell loss was most likely due to trauma (30%), infection of the upper respiratory tract (23%), sinonasal disease (17%) or it was idiopathic (22%). In patients with parosmia these figures were 7, 70, 8 and 12%; in patients with odor phantoms they were 20, 54, 15 and 17%. Thus, parosmias and phantosmias were most frequently encountered in olfactory loss following URTI. Among hyposmic patients those with parosmia exhibited a better discrimination of odorants but were less proficient in odor identification when visiting first. However, at second visit there was no significant difference between patients with ($n = 67$) or without parosmia ($n = 223$). In patients with parosmia decreased olfactory function was found in 22%, improvement in 39%. Similar figures were seen in patients without parosmia (21 and 40%). When visiting first patients with phantosmia were significantly worse in terms of odor identification compared with patients without phantosmia. At return visit in 38% of the phantosmic patients overall olfactory function was decreased; improvement was found in 41% of the patients. In conclusion, parosmias are found most frequently in olfactory dysfunction following URTI. Apparently, the presence of parosmia is not a predictor of the prognosis of olfactory dysfunction. In contrast, phantosmias appear to indicate a higher likelihood for a further decrease of olfactory function.

Poster: Human Olfaction: Pathology**High incidence of functional anosmia in the general population**B. Landis¹, C. Konnerth², K. Hüttenbrink³ and T. Hummel⁴¹University Hospital of Geneva, Geneva, Switzerland, ²ENT, University Hospital of Dresden, Dresden, Germany, ³Otorhinolaryngology, University of Dresden Medical School, Dresden, Germany and⁴University of Dresden Medical School, Dresden, Germany

Objective: to assess the incidence of anosmia and hyposmia in the general population. Study design: open prospective study ruled out on a population based sample representative for the German population. Methods: a total of 1240 subjects were included. Participants received an ENT examination and olfactory testing. Patients reporting sinu-nasal disorders were excluded. Olfactory testing was done by means of the Sniffin' Sticks. Results: in 5% of the subjects, nasal polyposis was diagnosed in the absence of sino-nasal complaints, confirming the high incidence of nasal polyposis previously reported. In the remaining 1182 subjects (603 men, 579 women), functional anosmia was detected at a frequency of 4.7%, whereas incidence increased with age (Figure 1). Hyposmia was found in 16% of the subjects. Most

subjects with functional anosmia were unaware of their poor performances; accordingly, they could not indicate an origin of the anosmia. No significant sex-related difference in functional anosmia rate was detected. Similar to other authors, we found age to be a main factor leading to functional anosmia. However, the present data also revealed functional anosmia to occur in up to 5% of subjects under 65 years of age. Conclusion: the current findings suggest that severe olfactory alteration occurs at a much higher frequency than previously assumed, especially among younger people. Since olfactory deficits have been shown to occur in several general pathologies, and especially in neurodegenerative diseases in their early phase, anosmia must be considered more than just a symptom of chronic sinu-nasal disease and deserves the general physicians attention. The present data also show the need of further research into treatments of olfactory disorders.

Poster: Human Olfaction: Pathology

Estrogen replacement therapy: does it affect smell function in post-menopausal women?

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Decreased smell function is among the first signs of Alzheimer's disease. Although controversial, there is evidence that estrogens mitigate verbal memory and several other cognitive deficits that are associated with Alzheimer's disease. Thus, the question arises as to whether estrogens mitigate olfactory deficits observed in the older female population. This retrospective study focuses on estrogen replacement therapy (ERT) and its possible role in decreasing olfactory loss in post-menopausal women. Currently, 257 women of an anticipated 600 women have participated in the study. Preliminary analyses have focused on olfactory scores of women during the first ten years after menopause. Within this subset of our sample population, there is a tendency for women who are currently on ERT to have higher olfactory scores in comparison to women who are not currently taking ERT. Within this limited age range, no meaningful association between prior estrogen use and olfactory test scores has yet emerged. Additional preliminary analyses suggest there may be a correlation between ERT use and decreased verbal cognitive deficits. Future analyses will explore the effect of ERT on age-related olfactory and memory deficits beyond the first ten years after menopause.

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Slide: Human Olfaction: Pathology

Modeling of airflow and odorant delivery pattern in a pre- and post-operative nasal cavity: a quantitative evaluation of surgical intervention

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Mechanical obstruction of odorant flow to olfactory receptor sites due to inflammation or polyps is a primary cause of olfactory loss in nasal-sinus disease patients. In these cases, surgical intervention (e.g. removal of polyps) can effectively facilitate recovery of olfactory ability. Using computational fluid dynamics (CFD) techniques, we are able to quickly convert nasal CT scans from an individual patient at a given time point (e.g. pre- or post-surgery) into anatomically accurate 3-D numerical nasal models that can be used to predict nasal airflow and odorant delivery patterns. Our goal is to correlate the patient's olfactory recovery with improvement of odorant delivery rate to receptor sites at various times during the treatment. In this preliminary study, we followed the treatment of a patient who had lost most of her orthonasal and all of her retronasal olfactory ability, but regained it after surgical treatment of polyps. CFD modeling of this patient's nose before and after surgery showed significant improvement in ortho and retronasal odorant delivery and suggested that remodeling the airway was a significant factor leading to the recovery of olfactory function. In the future, such modeling techniques may serve as a quantitative evaluation of surgical procedures and an important pre-surgical guide to the optimization of airflow and odorant delivery in the human nose.

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Poster: Human Olfaction: Pathology

Diagnosis and surgical treatment of parosmia

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The purpose of this study was to determine whether transnasal endoscopic excision of the olfactory mucosa is an effective and safe treatment in patients diagnosed with unilateral parosmia and to learn more of its pathogenic features by examining the histological characteristics of the excised mucosa. The two patients (patients A and B) who were subjected to the surgery both had complete and permanent resolution of their parosmia. Ninety days after surgery, olfactory assessment revealed no change (in comparison to preoperative status) in olfactory ability on the operated nostril in patient A, while improved in olfactory ability in patient B. No changes in olfactory capacity were noted in the contralateral (unoperated) nostrils. Patients A and B both had unremitting parosmia, for 3 and 20 years, respectively. The excised olfactory mucosa showed abnormal histological features that would suggest some pathological condition in the peripheral olfactory system. To the best of our knowledge, this is the first report that has demonstrated effective treatment of patients who suffer from parosmia through surgical technique. Furthermore, the abnormal histological features of the excised olfactory mucosa and its excision in the treatment of the parosmia suggest that there is a peripheral pathophysiological mechanism.

Poster: Human Olfaction: Pathology

Odorant-induced exacerbation of burning mouth syndrome

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Burning mouth syndrome (BMS) presents with symptoms of burning, pain, phantageusia. We present three patients, exposed to odorants precipitated severe burning exacerbation. (i) An 83-year-old woman, 2 years burning, salty phantageusia, onset immediately following application of nitroglycerin. Burning worsened with ingesting hot or spicy food, stress, and lidocaine application. Acute severe exacerbation of BMS precipitated with exposure to carbinol in the olfactory test of Amoore. (ii) A 57-year-old male, 1 year sudden onset of idiopathic metallic phantageusia and BMS. Burning worsened after consuming coffee, chocolate and cola, reduced with chewing gum or eating salty food. Upon exposure to aromas of shampoo or soap, the BMS and associated phantageusia were exacerbated. (iii) A 59-year-old woman, 1 year of gradual onset hypo-geusia, metallic and spicy phantageusia, salty and spicy dysgeusia, and BMS. Burning worsened with eating spicy food; reduced with sucking sweet candy and drinking diet Coke. When presented with odors of gas or garage, severe burning mouth symptoms recurred. Possible mechanisms of odorants: stimulate hunger, thus precipitate salivary flow, change chemical content of the mouth, which hypersensitive pain fibers interpret as pain; induce eating memories, stimulate gastroesophageal reflux, change in mouth PH causing pain; induce cephalopancratic reflex, causing decreased blood sugar allowing dysfunctional pain nerve fibers to fire; trigeminal component of odors may act on tongue to precipitate prolonged, intense burning; stressors which worsens BMS. BMS may represent a form of synesthesia, seen in neurological conditions such as odor-sensitive migraine or post-amputation phantom limb, in which otherwise innocuous stimuli precipitate pain.

Poster: Olfactory Regeneration

Aprin in rat olfactory epithelium

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Aprin (androgen-induced prostate proliferative shutoff associated protein AS3) inhibits cell proliferation in the prostate and protects against carcinogenesis. Although proliferation in the olfactory epithelium (OE) continues during adulthood, OE does not develop tumors. We asked whether aprin is expressed in the rat OE. We report here the sequence of rat aprin (Accession # AY388627) and the expression of aprin mRNA in the olfactory epithelium of postnatal rats using RT-PCR and (competitive) duplex PCR. Semiquantitative estimates reveal that aprin mRNA expression level in the OE is lower (~8 times) than that in testis. Although proliferation dramatically decreases in rat OE postnatally, the expression level of aprin does not change much. Besides the OE, aprin mRNA is also expressed in other neuronal (olfactory bulb, visual cortex, cerebellum, eye, adrenal gland) and non-neuronal tissues (testis, kidney, heart, lung, and very weak expression in muscle, intestine, liver), with the highest expression in the testis. No differences between males and females were observed in aprin expression in OE. Aprin function is discussed in relation to cell turnover and neuronal survival.

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Poster: Olfactory Regeneration

Reduced target ablation-induced macrophage recruitment and activation in MIP-1 knockout mice is restored by MIP-1 injection

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The chemokine macrophage inflammatory protein (MIP)-1 α (CCL3) recruits macrophages (m ϕ s) to sites of epithelial remodeling. We showed that MIP-1 α mRNA and protein levels in the olfactory epithelium (OE) increase significantly at 3 days post-olfactory bulbectomy (OBX). Our specific aim was to investigate the effect of lack of MIP-1 α on m ϕ infiltration after OBX in MIP-1 α knockout (KO) mice compared with wild-type (wt) mice and to test if function was restored in KO mice by MIP-1 α injection. OBX was performed on wt and MIP-1 α KO mice; all received six injections at 12 h intervals of either 10 μ g/ml MIP-1 α protein in carrier (0.5% BSA in sterile saline) or carrier alone for 3 days. M ϕ infiltration was evaluated with antibodies to CD68 for resident m ϕ s and F4/80 for activated m ϕ s. Compared with wt mice, CD68⁺ m ϕ numbers in the OE were 59% less than in carrier-injected KO mice and only 28% less in MIP-1 α -injected mice ($P < 0.0005$, t -test), and F4/80⁺ m ϕ numbers were 75% less than in carrier-injected KO mice but were increased by 33% in MIP-1 α -injected mice ($P < 0.0001$). Both suggest functional restoration in OE by MIP-1 α injection. There were no significant differences between numbers of CD68⁺ or F4/80⁺ m ϕ s in livers of carrier- versus MIP-1 α -injected mice. These results confirm the role of MIP-1 α in recruiting and activating m ϕ s in regenerating OE and serve as the basis for a genomic analysis of roles of MIP-1 α and m ϕ s in olfactory neurogenesis.

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Poster: Olfactory Regeneration

Bioinformatic analysis of stem/progenitor cell gene regulation in murine olfactory mucosa following target ablation

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The sequence of genomic events underlying regeneration of the olfactory epithelium (OE) following olfactory bulbectomy (OBX) is linked to the regulation of stem/progenitor cell genes juxtaposed between degeneration of olfactory receptor neurons (ORN) and cell cycle progression leading to neurogenesis. The specific aim was to investigate regulated expression of known stem/progenitor cell

genes at short time intervals following induction of ORN apoptosis by OBX at 2, 8, 16 and 48 h compared with shams. Total RNA was isolated from three male littermate mice per time point and hybridized with Affymetrix GeneChips, three per time point. Signals were analyzed with MicroArray Analysis Suite 5.0, SAS, S-Plus 6 and GeneSpring. Based on a screen of 230 genes associated with stem cell regulation, we identified 19 present stem/progenitor cell genes (8%) that were significantly different from controls ($P < 0.05$) at each time point. Thirteen (68%) of the 19 genes have not been identified previously in target-ablated OE. Six genes were significantly regulated as early as 2 h post-OBX and 12 were differentially regulated, i.e. met the false discovery rate criterion set at 10%, at 48 h post-OBX. Differential expression of seven genes was validated with the SuperArray Mouse Stem Cell Gene Array and of two genes with real-time RT-PCR. Our results characterize the temporal pattern of stem/progenitor cell gene regulation shortly after OBX, which may provide a regulatory link between the degeneration of ORNs and the earliest stages of stem/progenitor cell proliferation leading to neurogenesis.

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Poster: Olfactory Regeneration

Differential responses to bullectomy and minocycline-HCl in bax deficient and wild type mice

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In contrast to wild type mice, bax-deficient mice demonstrate an early but short-lived wave of olfactory sensory neuron (OSN) apoptosis in response to unilateral bullectomy that gives way to apoptosis resistance, at least up to 9 days post-surgery. In order to elucidate early events in OSN apoptosis following bullectomy we compared changes in gene expression and epithelial thickness in wild type and bax deficient mice and in mice treated with minocycline-HCl. The antibiotic minocycline-HCl has been shown to have an anti-apoptotic effect in other systems and is in clinical trials for therapeutic use in Parkinson's disease. In other systems, the mechanism of minocycline-HCl apoptosis inhibition is believed to be at the level of inhibition of cytochrome c release from the mitochondria. This inhibition would suppress activation of caspase-9 and the downstream caspases, including caspase-3. Given that bax promotes cytochrome c release, it was anticipated that apoptosis in the *bax* knockout mouse may be further suppressed by minocycline-HCl. By epithelial thickness measurement there appears to be an initial inhibition of apoptosis in wild type mice receiving minocycline-HCl. In contrast, the initial apoptosis following bullectomy typically observed in bax-deficient mice was not ameliorated. This lack of additional apoptosis inhibition in minocycline-HCl-treated bax-deficient mice may be explained by our results from cDNA expression arrays that focused on apoptotic gene expression. Bax deficient mice show a dramatic increase in caspase-3 mRNA by 8 h post-bullectomy that was absent in wild-type mice.

Supported by the Department of Otolaryngology-HNS.

Poster: Olfactory Regeneration

OEC dynamics in the olfactory system of methimazole-lesioned and control mice

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Olfactory sensory neuron (OSN) axons are held in juxtaposition by specialized glia, the olfactory ensheathing cells (OECs), as they extend towards the olfactory bulb (OB). It has been suggested that OECs provide a favorable substrate for axon outgrowth and potentially assist with targeting and/or glomerular recognition. Damage to the olfactory system (OS) results in incorrect target recognition by the OSNs; the effect of injury on the OECs is unknown. One hypothesis is that OECs fail to correctly ensheath and/or sort OSN axons following lesions. The effect of growth/guidance molecules associated with OECs on the olfactory projection after injury remains ambiguous. Despite the apparent inability of newly developed OSN axons to accurately target following lesions, they nonetheless do innervate glomeruli in the OB, albeit in an indiscriminate manner. What happens to the OECs during this process? We used established immunohistochemical protocols with antibodies to tyrosine hydroxylase (TH), GAP43, NCAM and S100 to evaluate changes in the mouse OS during degeneration (5–10 days), reinnervation (30–60 days) and recovery (90 days) following exposure to the olfactoxin methimazole. Our data demonstrate that TH expression is initially decreased in the OB but recovers by 90 days post-lesion, whereas GAP43 expression is markedly upregulated at 10 days post-lesion before returning to baseline. In addition, we addressed OEC turnover using both BrdU and a marker for apoptosis (NeuroTACS II) and report evidence of both OEC genesis and death in control tissue. Thus, OECs appear to be dynamic agents in the normal adult OS.

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Poster: Olfactory Regeneration

Early olfactory enrichment decreases TUNEL-positive cells in olfactory bulbs of neonatal rats

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A proportion of rat olfactory bulb interneurons normally undergo postnatal cell death, as is evident by both a decrease in the total numbers of interneurons after the second postnatal week, and the presence of TUNEL-positive cells in most layers of the main olfactory bulb at that time. In the current study, we determined whether increased olfactory experience could save olfactory bulb cells from an early death. To that end, we examined the effects of early olfactory enrichment on cell death in the main olfactory bulb. Rats were exposed continuously to a battery of natural and artificial odorants that were changed daily from postnatal days (PNDs) 1–15. On PND 16, TUNEL-positive cells were quantified in both the granule cell layer and the glomerular layer of the main olfactory bulb. Early olfactory enrichment resulted in a statistically significant decrease in the number of TUNEL-positive cells present

in both of these layers. In addition, we examined the effects of odor enrichment on the neural response to odorants using [^{14}C]2-deoxyglucose. Odor enrichment during the first three postnatal weeks resulted in a statistically significant increase in activity in the glomerular layer in response to odorants. These data suggest that olfactory enrichment during the early postnatal period can save olfactory bulb cells from dying, and can also enhance the glomerular response to odorants.

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Poster: Olfactory Regeneration

Caspase 8 activates orn apoptosis following deafferentation and excitotoxic lesion of mouse olfactory bulb

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Our laboratory has been testing how signals initiated at the olfactory bulb mitral cell synapse may regulate the apoptosis of olfactory receptor neurons (ORNs). We have previously shown that, following olfactory bulbectomy, ORNs undergo apoptosis driven by caspase-9 and 3-mediated retrograde axonal signaling. Because a bulbectomy is also a distal axotomy, we modified our lesion model to remove only the ORN target neurons (mitral and tufted cells) in the olfactory bulb using NMDA-mediated excitotoxicity. We find that NMDA infused directly into the olfactory bulb effectively kills off the majority of bulbar mitral and tufted cells within 48 h of being administered. Surprisingly, most OMP-positive mature ORNs do not immediately undergo target deprivation-induced apoptosis following NMDA infusion, and despite the loss of functional glomeruli, a significant population of their axons persist for up to 8 days in the nerve fiber layer. A delayed form of apoptosis does appear to eventually occur (by 4 days after loss of postsynaptic target) in a sub-population of vulnerable ORNs in NMDA-lesioned mice. We have identified caspase 8 as the apical caspase first activated at the glomerular synapse. We demonstrate (by co-immunoprecipitation) that Dynactin, a DED (Death Effector Domain)-containing protein, is a retrograde motor complex protein that can regulate the transport and/or activation of caspase 8 in ORNs following target lesion. Finally, by using taxol to inhibit axonal microtubule motors following bulbectomy, we can delay caspase-mediated retrograde apoptosis in ORNs.

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Slide: Odorant Receptors

Comparative genomics of olfactory receptor gene clusters

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Comparison of the nearly complete genomes of rat, mouse, and human reveals extensive lineage-specific change in the olfactory

receptor (OR) gene repertoires of these species. The primary contributor to repertoire change has been recent duplications that create new genes. Genes have also been lost through deletion and mutation. Only ~400 human genes appear to be functional, compared with ~1210 for the mouse and ~1390 for the rat. Our cDNA screen of olfactory neuroepithelium confirmed expression there for over one third of the intact mouse OR genes and also revealed extensive alternative splicing within the 5'-UTRs of OR transcripts. We observe up to a 300-fold difference in mRNA levels among mouse OR genes, which appears to be due to differences in both the number of expressing cells and the number of transcripts per cell. The family expanded primarily through local duplications in all three species, but inter-chromosomal duplications also occurred in both the human and rat lineages. Intriguingly, these inter-chromosomal duplications involved large genomic segments (up to 800 kb) containing primarily OR pseudogenes. The complex structures and evolutionary relationships among these regions suggest that they are subject to ongoing gene conversion-like ectopic exchange subsequent to the original duplication. The propensity of these segmental duplications to undergo recombination can also lead to gross allelic variation among normal individuals and/or the formation of chromosome abnormalities associated with disease.

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Poster: Odorant Receptors

Hands off my endangered species: low allelic variation of sea turtle or genes supports importance of olfactory sense

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How to determine the importance of a given sense to the life history of an endangered species when government regulations prohibit direct physiological analysis? Estimate the evolutionary selective pressure acting on the sense. Clone an 'important' gene and examine its allelic variation within a population—low variation suggesting selection is strong and that the sense is important to the life history of the animal, high variation suggesting selection is low and that the sense is less important. Cloning and characterizing genes of endangered species is feasible because archives of DNA samples OR genes were cloned from three sea turtles (loggerhead, green, leatherback), four freshwater or terrestrial turtles (musk, painted, box, gopher tortoise) and the American alligator. These genes are more similar to the mammalian than to the fish OR gene subfamily. Sea turtles have a reduced number of OR genes and a greater number of OR pseudogenes (internal stop codons) than freshwater or terrestrial turtles. Population analysis of five sea turtle genes indicates a strong dominance of a single allele for conserved genes, and a surprisingly low level of allelic variation among pseudogenes. These findings suggest olfaction is important and that pseudoization may be a relatively recent event if not an ongoing process. Turtles have similar sized OR and VNO systems; VNO neurons are thought to convey aquatic signals. The presence of strongly selected OR genes suggests that airborne odors may be important signals for sea turtles.

Funding was provided by NOAA.

Poster: Odorant Receptors**Expression of candidate gustatory receptor genes in *Anopheles gambiae***

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The ability to detect and discriminate between chemical cues in the environment is key to successful host finding in mosquitoes. The recent availability of the genome sequence of *Anopheles gambiae*, the principle vector of human malaria in Africa, has allowed for the identification and annotation of the genes believed to encode odorant (*AgOr*) and gustatory (*AgGr*) receptor genes in the mosquito. These genes are members of the G-coupled protein receptor (GCPR) superfamily of chemoreceptors, characterized in part by their seven-transmembrane domains. Phylogenetic analysis reveals that only a few orthologous pairs of the genes have been conserved between *Drosophila* and *Anopheles*. Otherwise, most Ors and Grs form species-specific gene subfamilies. We have begun studies of the expression patterns of the putative AgGr genes, based upon an RT-PCR assay. In this way, we can examine the conservation of expression of Grs in *A. gambiae* over the roughly 250 million years since it diverged from the lineage containing *Drosophila melanogaster*. Patterns of similarity across the two species are apparent. Three of the genes (*AgGr22*, *AgGr23*, *AgGr24*) with high sequence similarity to two genes in *Drosophila melanogaster* (*DmGr21a* and *DmGr63a*) are expressed in antennae and maxillary palps, which matches the expression patterns seen in *Drosophila*. Interestingly, *DmGr21a* has recently been identified by Dr Marien de Bruyne, at the Freie Universität Berlin, as a candidate carbon dioxide receptor in *Drosophila*. We are in the process of determining whether the closely related anopheline genes share the same function.

This research is funded by NIH RO1AI056081.

Poster: Odorant Receptors**Expression of an *Anopheles gambiae* candidate odorant receptor in a subset of distinct sensilla on the proboscis indicates a potential olfactory function**

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The biting behavior of *Anopheles gambiae* is largely influenced by olfactory cues emanating from host animals. The strong preference of the *A. gambiae* s.s. species for human hosts contributes significantly to the transmission of human malaria in sub-Saharan Africa. The family of odorant receptor genes in *A. gambiae* encodes G-protein-coupled receptors for which some ligands have been identified. One of these genes, *GPRor7*, is highly conserved with respect to single genes from many insect orders. Immunocytochemistry demonstrates that its protein product is localized to most sensilla of olfactory organs of *A. gambiae*. By RT-PCR analysis, *GPRor7* is also expressed in the proboscis of *A. gambiae*, a known gustatory organ in other mosquitoes. Immunocytochemistry indicates that *GPRor7* is found within each of a small subset of sensilla on the

labellar lobes, a pair of bulbous organs at tip of the proboscis. Scanning electron micrographic analysis reveals that the *GPRor7* expressing sensilla are short, grooved hairs residing in a socket on the cuticle surface and that there are ~25 of these hairs per labellar lobe. Their morphology is reminiscent of other grooved olfactory sensilla, and the presence of an odorant receptor within them indicates either a potential olfactory function for them, or a potential gustatory function for *GPRor7*.

This work was supported by grants from the National Institutes of Health: the NIDCD and the NIAID.

Poster: Odorant Receptors**Olfactory coding in peripheral organs of *Anopheles gambiae***

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A principal goal in neuroscience is to understand how sensory information is processed and integrated to control a suitable behavioral output in a nervous system of an animal. Of these, olfactory cues are primarily used in an insect to find a host, mate, and oviposition site. Therefore, it is important to understand how olfactory coding occurs a peripheral olfactory organ as well as in central nervous system. In insects, olfactory transduction is initiated by G-protein-coupled receptors in the cell membrane, which have been characterized in different insect species so far. In this study, using an important malaria vector mosquito, *A. gambiae*, from which 79 different G-protein-coupled receptor genes have been found, we focus on functional analysis of olfactory information processing in antenna as well as proboscis where an odorant receptor gene (*AgOR7*) is also expressed. With analysis of gene expression in these appendages, here we aim to characterize central processing and olfactory coding in a primary olfactory and gustatory organ, antenna and proboscis, using electrophysiological methods and back-filling techniques. Recording from epithelia of antenna and proboscis was employed with several key mosquito odorant components. Here we report that proboscis shows olfactory responses to an odorant chemical. This result supports the idea that proboscis contributes olfactory perception in accordance with gene expression of the odorant receptor in proboscis. More detailed evidence of neural anatomy and single sensillum recording will be presented.

Supported by NIH grants A1056402 and DC04692.

Poster: Odorant Receptors**Molecular analysis of *Drosophila* odorant receptors**

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The *Drosophila* olfactory system is a powerful model to determine how neuronal circuits represent the external world in the brain. The peripheral circuitry has a striking stereotypical spatial organization. Olfactory sensory neurons (OSNs) express only one of ~60 odorant

receptors (ORs) along with Or83b, a broadly expressed member of the OR gene family. The axons of OSNs expressing the same OR converge on specific glomeruli in the antennal lobe. Each class of OSN also displays distinct spiking properties in response to odor stimulation, suggesting that both temporal and spatial patterns of neuronal activity contribute to the representation of odor identity. ORs are polytopic membrane proteins that lie at the interface between the odorous environment and neuronal activity patterns. Little is known about their ligand specificity, what second messenger cascade couples receptor activation to neuronal spiking, or the mechanisms by which their activity and localization are regulated. Genetic analysis of candidate downstream signaling components has failed to reveal the involvement of a single canonical pathway, suggesting that multiple and/or novel cascades are involved. ORs are not expressed on the surface of heterologous cells, indicating that OSNs possess unique trafficking pathways integral to the localization and function of ORs. To dissect OR function and regulation, we have initiated a combination of molecular and transgenic approaches. We are using the yeast two-hybrid system to identify cytoplasmic factors that associate with their intracellular loops, and have generated a panel of epitope-tagged ORs for biochemical analysis *in vivo*.

Funding was from EMBO, NIH and NSF.

Poster: Odorant Receptors

The sperm 'nose': key role of particulate adenylate cyclase

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Besides their 'conventional' role in olfaction, odorant receptors have long been suggested to function in mammalian sperm physiology and fertilization. Recently, Spehr *et al.* (2003) identified and characterized a human testicular odorant receptor, hOR17-4, which is activated by certain floral odorants (e.g. bourgeonal, scent of lilies of the valley) In additional behavioral bioassays, bourgeonal was found to act as a strong chemoattractant to navigating human sperm. Nevertheless, the molecular mechanisms that link hOR17-4 activation to sperm responses remain obscure. In imaging experiments as well as behavioral assays, we show here that a membrane-bound AC (mAC) couples OR activation to changes in sperm swimming behaviour, such as chemotaxis, chemokinesis, and hyperactive flagellar beating. Introducing a new approach of protein identification in mature sperm by mass spectrometry (mudpit) we provide evidence for expression and participation of specific receptors, G-proteins and mACs in the underlying signaling cascade. Spatial distribution patterns of the identified signaling components largely correspond to the spatiotemporal character of odorant-induced Ca²⁺ changes viewed via single cell high-resolution imaging techniques. Taken together, our data show that mAC activation is linked to sperm chemotaxis and hyperactivity and provide a basis for further investigations in the field of fertility and sterility.

This work was generously sponsored by the Heinrich and Alma Vogelsang Foundation (to K.S.).

Poster: Odorant Receptors

Chemical communication and the language spoken by sperm and eggs

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Chemical communication between sperm and egg is a critical factor mediating sexual reproduction. Sperm attractants may be significant evolutionarily for maintaining species barriers, and important ecologically for increasing gamete encounters. Still unresolved, however, are the functional consequences of these dissolved signal molecules. Here, we provide the first experimental evidence that sperm chemoattraction directly affects the magnitude of fertilization success. The recent discovery of L-tryptophan as a potent attractant to red abalone (*Haliotis rufescens*) sperm offered the opportunity to quantify how navigation affects gamete interactions. Sperm behavioral responses to manipulations of the natural tryptophan gradient around individual eggs revealed that both chemotaxis and chemokinesis significantly promote contacts. Our results showed further that attractant release via diffusion effectively doubles the target size of red abalone eggs, which in turn significantly increases fertilization success. Although long theorized as potential barriers to hybridization, species-specific sperm attractants in red and green abalone (*H. fulgens*) are only minor contributors to maintaining reproductive isolation. Because abalone typically live in dense, multi-species aggregations, chemically mediated navigation would prevent sperm from pointlessly tracking heterospecific eggs. Thus, even though reproductive isolation fundamentally resides at the level of membrane recognition proteins, species-specific sperm attractants may have evolved to locate the right target within mixed gamete suspensions of closely related species.

Poster: Odorant Receptors

Mouse testicular olfactory receptors: expression pattern, odorant responsiveness, and regulation of sperm motility

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Although a subset of olfactory receptor (OR) gene family is expressed in mammalian testis, developmental expression pattern and physiological function of testicular ORs have not been fully characterized. To clarify testicular OR function in mice, we first analyzed expression pattern of mouse OR genes in testis and found that ORs were expressed stage-specifically in round spermatids during spermatogenesis. We next conducted functional analysis of MOR23, a mouse testicular OR, that had been shown to recognize a floral odorant, lylal, in the olfactory system. Lylal elicited Ca²⁺-increases in a fraction of spermatids and mature sperm in a dose-dependent manner. Comparison of lylal-responsiveness

of germ cells derived from transgenic mice expressing MOR23 with that of wild type mice provided evidence that MOR23 mediated lylal-induced Ca^{2+} -increases in spermatids and spermatozoa. Finally, lylal induced sperm accumulation in chemotaxis assay, suggesting that MOR23 functioned as a chemosensor in mature sperm, and that the internal Ca^{2+} -increase via MOR23 affected the sperm motility. The MOR23-expressing transgenic mouse line generated in this study will be a powerful tool to identify endogenous ligand(s) that may be structurally related to lylal, with implications for physiological roles of mouse testicular OR(s).

Slide: Odorant Receptors

Discovery of acetals, alcohols, and esters as isovaleric acid odor blockers

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Isovaleric acid (IVA) is probably a potent agonist for one or more of the ~350 human olfactory receptors (Zozulya, 2001). To find an odor blocker, we hypothesized a simple binding pocket based on known GPCR receptors and then designed antagonist candidates that would fit into this binding pocket. Each candidate was tested for its ability to reduce the perceived odor intensity of a 56 p.p.m. solution of IVA in water (Target). The evaluation of blocking utilized a cross adaptation protocol in which the subject (i) sniffed the target solution with both nostrils and scored its odor intensity on a Labeled Magnitude Scale (LMS); (ii) waited 1 min; (iii) sniffed a 1% v/v solution of the candidate in diethyl phthalate twice with each nostril; and (iv) sniffed the target again with both nostrils. The reduction in IVA intensity between the first and third sniffing was used to calculate a percent malodor reduction (MOR) for each candidate. The compounds that worked best have a hydrocarbon tail similar to that of IVA and a polar head. Of the homologous series tested, the acetals worked best with 2-isobutyl-[1,3]dioxane, giving a MOR score of 97%. For the alcohols, 2-methylcyclopropane methanol provided the best blocking, with a MOR of 85%. For the ester series, the ethyl and methyl esters of IVA gave MOR scores of 94 and 92% respectively. The magnitude of blocking was much higher than the 25–30% odor reduction generally reported in the cross adaptation literature. These results suggest that we have identified antagonists for the primary IVA receptor.

Slide: Odorant Receptors

HeLa cells designed for functional genomics of odorant receptors and pheromone receptors

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Odorant receptors (OR) of the main olfactory epithelium and pheromone receptors (VR) of the vomeronasal organ are the largest group of orphan G-protein-coupling receptors (GPCR), with a small number of ligands identified out of thousands of odorants and hundreds of pheromones. De-orphanization of OR was hampered by their combinatorial odorant coding, suboptimal plasma membrane expression and lack of olfactory-specific signal transduction in recombinant cell systems. Heterologous functional expression of VR has not been reported, so far. We have achieved stable reconstitution of OR-specific signaling in HeLa cells, via G-protein α_{olf} and adenylyl cyclase type-III to the olfactory cyclic nucleotide-gated CNGA2 channel. In these cells, receptors of the V1R-type inhibit, via G-protein α_i , the cAMP pathway. In another cell line, we established stable expression of a TRP channel that can be activated by pheromones via V1R and phospholipase C- β 2. CNGA2 or TRP channels translate changes in intracellular cyclic nucleotides into changes in Ca^{2+} -influx that can be monitored by means of fluorescence imaging multiwell-plate reader techniques. This allows us to functionally characterize the de-orphanized OR and V1R by EC₅₀-ranking odorant profiles.

Poster: Odorant Receptors

Mechanism for olfactory receptor–odorant interactions

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Objective of the study: to study the interactions between olfactory receptors (OR) and odor molecules through molecular dynamics simulations. Methods: 10 odorant ligands, eight carbon atom chain aldehydes with variable branches, side chains and unsaturation, were docked into the binding region of rat olfactory receptor I7, the first olfactory receptor identified by Buck and Axel in 1991 and modeled by Singer (2000). The experimental binding of the ligands with I7 has been previously reported by Araneda and co-workers. (2000). We used the Singer OR model, and the lowest energy configuration for each docked ligand. Each system was first minimized, and molecular dynamics simulations were carried out for 500 picoseconds. Results: our results for *in vacuo* molecular dynamics simulations indicate that the ligand attempts periodically to exit and re-enter the OR binding region. There is an exit channel in I7 irrespective of whether the loops of the helical domains are included in the model. The exit events were strongly correlated with significant structural changes within the binding pocket. Conclusions: the exit–re-entry events may be related to the binding strengths of the ligands with the OR. This previously unreported exit–re-entry behavior in OR–odor dynamics is an important consideration in the study of the mechanism of olfaction at the molecular level.

This work was supported by the Human Brain Project and the National Library of Medicine.

Poster: Odorant Receptors**Can two nostril sniffing help electronic noses?**

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There is worldwide interest in development of devices to detect and identify airborne chemical compounds. Currently, techniques such as gas chromatography and mass-spectroscopy are expensive and difficult to deploy in a portable package. Portable sensing devices have a wide range of applications, such as monitoring of food and drug processing, manufacturing, dangerous substance identification, land mine detection and medical diagnostics. However, chemical sensor technology is new and applications are often limited by sensor sensitivity, selectivity, discrimination, drift and durability. A new theme in electronic nose research is to use the mammalian olfactory system as a model to address these issues. This direction is focusing on using multiple sensors and sophisticated multi-variant statistical models (PCA, learning machines, etc.). However, there has been little attention to sampling technique. Often, sampling is done by flowing the carrier gas over the sensor or exposing the sensor to an aqueous solution containing the odorants. These methods are the easiest to perform, but may not be the most effective. In the mammalian olfactory system, there are two separate inputs—the left and the right nostrils. Each nostril receives slightly different flow rates, and this results in odorants having different solubility characteristics between the two nostrils. In essence, the olfactory system receives two offset samples in a single sniff. Here we explore the possible benefits of using mammal-like sampling strategies for electronic nose technologies.

Poster: Odorant Receptors**Characterization of the mechanism of odor sensing in novel DNA-based fluorescent sensors**L.B. Williams¹, J.S. Kauer¹ and J.E. White¹¹*Neuroscience, Tufts University, Boston, MA, USA*

Our laboratory has developed an artificial nose that exploits in principle, some 22 attributes of the biological olfactory system. One important feature is the use of broadly tuned sensor arrays to achieve odor detection. In the first such experiments of which we are aware, we show that 20–30 base single-stranded DNA–Cy3 (ssDNA) conjugates can respond to odorant molecules in our artificial nose. DNA–Cy3 conjugates have the combinatorial potential to provide large arrays of novel sensors. The mechanism by which these sensors operate is as yet unknown. Preliminary work has produced a set of nine DNA–Cy3 sensors. Using this sensor set, the following odorant response observations have been made: (i) sensor responses return to baseline when no longer exposed to odorant, suggesting that the interactions between the odorant and the sensor are rapidly reversible and therefore non-covalent. (ii) Change in fluorescence intensity (ΔF) can be either positive or negative, suggesting that a simple quenching process is unlikely to be the mechanism. (iii) Sensors tested so far (dried from water based buffers) do not respond as well to non-polar odorants as to polar odorants, (iv) nor do they function well at <15% humidity, suggesting a role for residual water (solvent). (v) Odor detection increases in a rela-

tively linear manner with increasing concentration of odor. Based on these data, we believe that the ΔF seen in the presence of odors may be due to odorant molecules dissolving into the hydration layer surrounding the DNA and exerting solvent effects on Cy3. These are modulated by DNA sequence mechanisms under investigation that involve tertiary structure.

Supported by grants from NIDCD and NSF.

Slide: Taste Genetics and Physiology**Associations between PTC/PROP gene, 6-n-propylthiouracil bitterness and alcohol intake**V.B. Duffy¹, A. Davidson², J. Kidd², K. Kidd³, W. Speed², A. Pakstis², D. Reed⁴, D. Snyder⁵ and L. Bartoshuk⁵¹*Dietetics Program, University of Connecticut, Storrs, CT, USA,*²*Genetics, Yale University, New Haven, CT, USA,*³*Psychiatry, Yale University, New Haven, CT, USA,*⁴*Monell Chemical Senses Center, Philadelphia, PA, USA and*⁵*Surgery, Yale University, New Haven, CT, USA*

Nontasters [6-n-propylthiouracil (PROP) is weakly bitter] report less negative (bitterness, irritation) and more positive (sweet) sensations from alcoholic beverages than do supertasters (PROP is strongly bitter). Younger (23 F, 26 M) and middle-aged (30 F, 5 M) adults rated bitterness of five PROP concentrations (0.032–3.2 mM) on the general Labeled Magnitude Scale. Alcoholic beverage intake was assessed by a frequency survey; adults stated drinking at least once per year. DNA was analyzed for the PTC/PROP gene (TAS2R38) that codes for molecularly different receptors depending on specific amino acids at three positions in the protein. The common forms are AVI (alanine, valine, isoleucine) and PAV (proline, alanine, valine) (Kim *et al.*, 2003). Via repeated measures analysis of variance (ANOVA), PROP bitterness varied significantly across genotype groups [AVI ($n = 26$) less than PAV/AVI ($n = 38$) less than PAV ($n = 22$)] but not enough to explain supertasting. Age group (younger > middle-age) and 3.2 mM PROP bitterness (greater bitterness, less intake) contributed significantly to predicting alcohol intake via multiple regression analyses. With ANOVA, alcohol intake varied significantly across genotype groups (AVI > PAV/AVI > PAV) yet significance was primarily in younger adults. These data support genetic taste effects on alcohol intake. Phenotypical and genetic markers of taste may be necessary to study orosensory effects on diet across aging.

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Slide: Taste Genetics and Physiology**Genetics of PTC taste sensitivity in humans**D. Drayna¹, U. Kim¹, S. Wooding², L. Jorde³, W. Floriano⁴ and W.A. Goddard⁴¹*National Institute on Deafness and Other Communication Disorders,**National Institutes of Health, Rockville, MD, USA,*²*Genetics, University of Utah, Salt Lake City, UT, USA,*³*Human Genetics, University of Utah, Salt Lake City, UT, USA and*⁴*Chemistry, California Institute of Technology, Pasadena, CA, USA*

The inability of some individuals to taste phenylthiocarbamide (PTC) was discovered >70 years ago and since that time it has been the subject of detailed studies in genetics, anthropology and sensory physiology. We have identified the major gene underlying this trait as TAS2R38, a G-protein-coupled bitter taste receptor located on chromosome 7q. The taster and non-taster forms of this protein differ at three amino acid positions, and we have identified other alleles of this gene that encode various combinations of these three variant amino acids, at least some of which produce intermediate PTC sensitivity. We have used population genetic methods to study the paradoxical high frequency of the non-taster form of this gene. Our analyses indicate that both the major taster and major non-taster alleles have been maintained by balancing natural selection. Since bitter taste is thought to protect individuals from ingestion of toxic substances in our diet, this raises the question of what selective benefit is provided by the non-taster allele. We hypothesize that the non-taster form of the protein serves as a functional receptor for a different toxic bitter substance not yet identified. We have also employed molecular structure prediction techniques to determine the 3-D structure of the taster and non-taster forms of this receptor, along with PTC ligand binding sites. Our results indicate that PTC binds to both forms of the receptor with equal affinity, and that the non-taster form of the protein does not signal due to a failure of G-protein activation.

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Slide: Taste Genetics and Physiology

Genetic control of lick rate in mice

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Licking is a highly stereotyped behavior that is thought to be determined in part by a central pattern generator (CPG). Lick rate has been shown to differ among inbred strains of mice, and may play a role in apparent differences in gustatory sensitivity. We used a 20 min access procedure to quantify lick rate in several strains of water-deprived mice. Inbred strains could be classified as fast, intermediate or slow lickers based on the median value of the inter-lick interval (ILI) distribution. With virtually no overlap among individual distributions, C57BL/6J (B6; $n = 36$) mice were classified as slow lickers (average median ILI = 122.8 ms) whereas DBA/2J (D2; $n = 27$) mice were fast lickers (average median ILI = 101.1). Mice from an F1 generation ($n = 14$) possessed an intermediate phenotype (112.8 ms). Fast/slow lick rate phenotypes were static over an extended time period, and were also evident in non-deprived tests with sucrose. Further testing of an F2 generation ($n = 62$) resulted in an estimate of broad-sense heritability = 0.54, with no fewer than two polymorphic genes influencing the ILI phenotype. Initial QTL mapping analysis using a panel of BXD RI strains indicates that at least two and possibly as many as four QTL with comparatively large effects on the ILI are segregating in this cross. These data will soon be complemented with a genome scan of a panel of ~150 F2 mice that are now being typed using a panel of ~70 microsatellite markers. Additionally, we show

how differences in lick rate between B6 and D2 strains may exert a non-gustatory influence in lick ratio measurements of sucrose sensitivity.

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Slide: Taste Genetics and Physiology

Intake of sweet and bitter solutions: variation in inbred strains of golden hamsters

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Intake of sweet and bitter solutions by seven inbred strains of golden hamsters (*Mesocricetus auratus*), a principal species for studies of mammalian gustatory systems, was measured. Two concentrations of sucrose, maltose, D-Phe and Na saccharin (which are sweet) and quinine-HCl, L-Phe, caffeine and sucrose octaacetate (SOA) (which are bitter to humans) were tested. Difference scores, solution intake minus mean baseline water intake in ml (DIF), were evaluated by analysis of variance ($\alpha = 0.05$). Compared with ACN, CN, APA, APG and CBN (five strains with similar DIF for all tested solutions), the strains ACNT and GN (an ACNT ancestral strain) preferred sucrose, caffeine and SOA more strongly; ACNT also preferred saccharin and maltose more strongly and rejected quinine more strongly. There were no strain differences in DIF for D-Phe or L-Phe. Narrow sense heritabilities for the six compounds for which strain differences were revealed ranged from 0.31 to 0.57. Genetic correlations indicated the strain variations in intake of sucrose, saccharin, SOA and caffeine were coupled, suggesting an association with several possible interpretations. The genetic differences that influence taste behaviors in existing strains of hamsters may help identify relevant genes.

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Slide: Taste Genetics and Physiology

Responses to ethanol in wild type and α -gustducin knockout mice

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Although it has been shown that C57BL/J mice prefer ethanol, it is not known what kind of taste nerve responses ethanol elicits. We have shown in primates that ethanol stimulates chorda tympani (CT) and glossopharyngeal (NG) taste fibers and the response depends on type of taste fibers. Our purpose was to study taste responses to ethanol in mice. We also investigated if α -gustducin is involved in transduction of ethanol taste. Methods: CT and NG responses and results of two bottle preference tests (TBP) with

ethanol (0.5–5 M) were recorded in wild type (WT) and knockout (KO) mice. Results: (i) in WT only high concentrations of ethanol elicited CT responses. This contrasts NG effects in which low concentrations elicited significant responses. (ii) In both nerves the responses developed slowly. (iii) In KO ethanol did not produce CT responses at any concentration. The NG responses did not differ between the two groups. (iv) In contrast to WT, KO did not prefer ethanol at any concentration and rejected higher concentrations in TBP tests. Conclusion: ethanol is not an effective stimulus in WT mice in contrast to primates. Absence of preference and CT responses to ethanol together with previously demonstrated decrease of responses to sweeteners in KO mice suggest that ethanol has a sweet taste component, which may be the cause for its consumption by WT. Thus α -gustducin is important in transduction of ethanol taste and consumption. Rejection of ethanol by the KO might be explained by the significant responses in the NG, which may originate from non-taste fibers or non-sweet fibers.

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Slide: Pheromones

A *Drosophila* odorant-binding protein mediates responses to a pheromone

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Pheromones are chemosensory cues released by animals to influence the behavior of other members of the same species. In *Drosophila*, a male-specific lipid, 11-*cis*-vaccenyl acetate (VA), mediates olfactory-based social aggregation. OBPs are a large family of proteins found in all terrestrial species and have been suggested to transport odorants. Here we show that the odorant binding protein 76a (OBP76a), an extracellular protein secreted into the fluid bathing a subset of olfactory neurons, is required for behavioral attraction to VA. This phenotype is due to a complete loss of VA-evoked activity in pheromone sensitive neurons. These defects are reversed by germline transformation with a cloned, wildtype copy of *obp76a* or, importantly, by introducing recombinant OBP76a protein into mutant sensilla. Remarkably, spontaneous activity of the pheromone-sensitive neurons is reduced over 400-fold in the absence of the binding protein. This implicates the binding protein as a direct activator of the pheromone-sensitive neurons. These studies directly link odorant binding protein expression with activation of a specific subset of olfactory neurons and pheromone-induced behavior. We suggest OBP76a is an extracellular modulator of neuronal activity that translates the presence of pheromone to neuronal activation.

Slide: Pheromones

Pheromone regulation of a transcription factor in the honeybee brain

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Honeybee social organization is predominantly regulated by chemical communication. Using honeybee cDNA microarrays, we have begun to analyze the molecular mechanisms of pheromonal regulation of honeybee behavior by using queen mandibular pheromone (QMP), one of the primary pheromonal regulators of physiology and behavior of worker bees. One of the genes QMP regulates in the bee brain is the transcription factor, Kr-h1. Kr-h1 is expressed primarily in the mushroom bodies, the region of the insect brain associated with multimodal integration and learning, and its expression is regulated by QMP especially strongly in this region. Furthermore, Kr-h1 expression is correlated with foraging in bees, and seems to be activated prior to the transition to the foraging behavioral state. Previous work has demonstrated that honeybee mushroom bodies undergo a period of synaptic remodeling and expansion prior to the initiation of foraging, and synaptic remodeling continues as bees gain foraging experience. The specific localization and timing of Kr-h1 expression suggests that it may be involved in this process.

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Slide: Pheromones

Chemical communication in zebrafish: how pheromones affect female mate choice

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In contrast to males, female fitness can decrease significantly by inappropriate matings. In species in which females usually receive no resources from a male besides his sperm, females might choose mating partners whose genes will confer greater fitness on her offspring. While several studies have shown how pheromones trigger and synchronize mating behavior in fish very little is known if pheromones can be used as signals to assess mate quality. In odor choice tests we analyzed the olfactory preference of female zebrafish for (i) males of different relatedness and (ii) males of different social rank. A single female zebrafish was exposed to stimulus water of different males on either side of a flume. The time a female spent on either side was determined. As odor stimuli, holding water of males that were either related (brother) or unrelated to the female was used. In the second experiment two male zebrafish were isolated in a 9 l tank and observed for agonistic interactions. After 2 days the dominant and subordinate males were separated into single tanks and their holding water was used as stimuli. The results can be summarized as follows: (i) adult females preferred the odor of unrelated males. (ii) Females preferred odor cues of dominant over subordinate males. We conclude that kin recognition mechanisms such as phenotype matching occur in zebrafish that enables females to avoid inbreeding. In addition, dominant males seem to release a specific odor cue that made them distinguishable from subordinate males. Our successful breeding of a zebrafish mutant without a nose and an olfactory bulb provides a unique opportunity for further analyses of the effect of pheromones on mate choice.

Slide: Olfactory Development, Disease, and Plasticity**It came from the sea—olfactory adaptations for a terrestrial life in the robber crab (*Birgus latro*)**M.C. Stensmyr¹, S. Erland², P. Greenaway³ and B.S. Hansson¹¹Swedish University of Agricultural Sciences, Alnarp, Sweden,²Ecology, Lund University, Lund, Sweden and ³Biological Sciences, University of New South Wales, Sydney, New South Wales, Australia

An important step in the evolution of the olfactory sense has been the transition from sea to land. Although terrestrial and aqueous olfactory systems share many characteristics, marked differences exist. These distinctions likely reflect the differences of the ligands that the systems need to detect, air borne, mostly hydrophobic volatiles on land and water-soluble molecules in the sea. The olfactory system of land crabs, whose terrestrial existence is a comparatively recent evolutionary development, represents an excellent opportunity to investigate the effects of the sea to land transition. Have land crabs come to the same solutions as other terrestrial animals, or is their olfactory sense characterized by unique innovations? Here we show that the terrestrial robber crab (*Birgus latro*) has evolved an olfactory sense displaying a high degree of resemblance to the insect system. The similarities extend to physiological, behavioural and morphological characters. The insect nose of the robber crab is a striking example of convergent evolution, nicely illustrating how similar requirements result in similar end-products.

Slide: Olfactory Development, Disease, and Plasticity**Examination of circadian rhythms in the antenna of the moth *Manduca sexta***M. Stengl¹, C. Flecke², J. Schuckel² and K.K. Siwicki³¹University of Marburg, Marburg, Germany, ²Biology, Philipps-University of Marburg, Marburg, Germany and ³Biology, Swarthmore College, Swarthmore, PA, USA

A coupled network of circadian pacemakers orchestrates physiological processes in a 24 h rhythm. In *Drosophila melanogaster* circadian pacemakers in the antenna contain the circadian protein PERIOD (PER) and appear to drive 24 h rhythms of olfactory sensitivity. In the hawkmoth, *Manduca sexta*, a circadian rhythm of octopamine was measured in the hemolymph and octopaminergic neurons project into the antenna. Because octopamine is known to sensitize pheromone-perception in different moths apparently via cAMP-dependent mechanisms we wanted to know whether this also applies to the night-active *M. sexta*. In addition, we tested whether also in *M. sexta* olfactory receptor neurons (ORNs) are PER-immunoreactive. In extracellular tip-recordings from single sex-pheromone-dependent ORNs responses to the pheromone-component bombykal were tested under constant light at two different time points with or without the presence of 8-bromo-cAMP in the recording electrode. During control recordings pheromone-dependent action potential responses showed a decline from ZT 1–4, but not at ZT 8–11. This decline was not seen in the presence of 8-bromo-cAMP. Thus, possibly circadian adaptation of the action potential generator occurs during the day which is counteracted by

octopamine-dependent cAMP-rises at night. Antibodies against the circadian clock protein PER stained ORNs and other antennal cells. Future studies will examine whether PER-antibodies delete the circadian decline in the sensitivity of ORNs.

Supported by DFG grant STE531/13-1 to M.S.

Slide: Olfactory Development, Disease, and Plasticity**Life stage and odorant-induced changes in olfactory sensitivity in coho salmon, *Oncorhynchus kisutch***A. Dittman¹, D. May², D. Baldwin¹ and N. Scholz¹¹Northwest Fisheries Science Center, NOAA-Fisheries, Seattle, WA, USA and ²School of Aquatic and Fishery Science, University of Washington, Seattle, WA, USA

Over the lifetime of an organism, the sensitivity of the olfactory system to specific odors may change in response to developmental changes, hormones, environmental stimuli and odorant exposure. Salmon provide an excellent model for studying such changes because almost every aspect of their lives is influenced by olfaction and they experience dramatic developmental and environmental transitions (smolting, maturation, freshwater versus oceanic) as part of their migrations from freshwater to oceanic feeding grounds and back. Furthermore, these homing migrations are governed by olfactory discrimination of home stream odors that juvenile salmon learn (imprint to) prior to their seaward migrations. Our previous studies demonstrated that salmon imprinted to the odorant phenylethyl alcohol (PEA) developed a long-term sensitization of peripheral olfactory neurons to this odorant. To further examine this imprinting phenomenon, we exposed juvenile coho salmon, *Oncorhynchus kisutch*, to three distinct classes of odorants (amino acids, bile acids and PEA) during smolting, the presumptive sensitive period for imprinting. To assess life stage and olfactory imprinting associated changes in the sensitivity of the olfactory system, we recorded electrical field potentials (electro-olfactograms) generated in response to these three classes of odorants and ovarian fluid at four distinct life stages: oceanic juvenile, maturing adult, immature adult and mature. Our results suggest that olfactory sensitivity changes over the lifetime of the salmon and previous odor exposure can influence olfactory responses.

Funded by BPA grant 199305600.

Slide: Olfactory Development, Disease, and Plasticity**Defective olfactory development in 3GnT1 null mice**G. Schwarting¹, D. Raitcheva², T. Hennet³ and T. Henion¹¹University of Massachusetts Medical School (Worcester), Waltham, MA, USA, ²Shriver Center, Waltham, MA, USA and ³University of Zurich, Zurich, Switzerland

Subsets of olfactory neurons in mice express unique lactosamine-containing glycans (LCGs) that react with the monoclonal antibody, 1B2. We have previously shown that LCGs are expressed by neurons in all zones of the embryonic OE but project axons preferentially to the ventral OB, suggesting that these glycans may participate in axon guidance mechanisms. The gene encoding an

enzyme critical for synthesis of LCGs was recently isolated. *In situ* hybridization reveals that this enzyme, β 1-3-*N*-acetylglucosaminyl-transferase-1 (β 3GnT-1) is expressed by neurons in the OE beginning at very early embryonic stages. We show here that mice deficient in β 3Gnt-1 fail to express LCGs during embryonic and early postnatal olfactory development. β 3Gnt-1 null mice have severe defects in formation of connections between the OE and OB at birth. Between P1 and P10, many OMP+ glomeruli are absent from the glomerular layer of the OB of β 3GnT-1 null mice. This defect in glomerulogenesis is accompanied by increased neuronal cell death in P1 mice followed by an increase in neurogenesis at P10. In addition, the expression of some odorant receptors, such as P2, are significantly down-regulated in β 3Gnt-1 null mice. Although OBs of β 3GnT-1 null mice are ~25% smaller than control littermates, overall structure and layering of these OBs is relatively normal. In summary, mice that do not express LCGs in the embryonic and early postnatal OE fail to form normal axonal connections with the OB, suggesting that LCGs play an important role in axon growth and guidance in the developing mouse olfactory system.

Supported by DC00953.

Slide: Olfactory Development, Disease, and Plasticity

Effect of air pollution on olfactory function in residents of Mexico City

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To our knowledge there has been no study of the effect of everyday air pollution on olfactory function. It was therefore the aim of this study to compare the olfactory performance of long-term residents of Mexico City (MC)—an environment with high air pollution—with the olfactory performance of residents of the Mexican state of Tlaxcala (Tx)—a region culturally and geographically similar to MC but with low air pollution. Healthy volunteers (MC $n = 82$, Tx $n = 86$), from 20 to 63 years of age and balanced for gender, were tested for the perception of the odors of everyday beverages presented in squeeze bottles. When tested with ascending concentrations of stimuli in a three-way oddball paradigm, residents of Tx detected the odor of an orange juice preparation (Clight) and of Nescafe at significantly lower concentrations than residents of MC. They could also attribute a quality to and then finally correctly identify the stimuli at lower concentrations. However, differences between the groups decreased across the three tasks, suggesting the increasing participation of central, cognitive processes unimpaired by pollution. Residents of Tx also performed significantly better in discriminating between the two similarly smelling Mexican beverages of horchata and atole in oddball tests. Significant differences between the two populations were apparent even in the youngest subjects. No significant differences were found between sexes. Thus, air pollution in Mexico City appears to have a substantial impact on peripheral olfactory function, even in young adults.

Slide: Olfactory Development, Disease, and Plasticity

Olfactory dysfunction occurs in transgenic mice overexpressing human tau protein

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Disorders of olfaction are among the first clinical signs of neurodegenerative diseases such as Alzheimer's disease (AD) and idiopathic Parkinson's disease (PD). In this study, we evaluated the olfactory function of Ta1-3RT transgenic mice that overexpress tau, a key pathogenic protein in AD, and compared such function to that of wild type controls who do not express this protein. The Ta1-3RT mice, but not the controls, exhibited responses indicative of decreased olfactory function. These data (i) lend support to the notion that tau may be involved in the pathogenesis of the olfactory dysfunction of some neurodegenerative diseases and (ii) demonstrate, for the first time, that olfactory function is present in a transgenic mouse model of neurodegenerative tauopathies including the filamentous tau tangles seen in AD. Future studies need to similarly assess other pathogenic markers, as well as their distribution within various sectors of the brain, to determine the specificity of this phenomenon.

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Slide: Olfactory Development, Disease, and Plasticity

Predator and non-predator odors: similarities in spectral and behavioral patterns

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Previous research has suggested that olfactory structures respond to predator odors with bursts of oscillatory activity in the beta frequency band (15–40 Hz). However, those same oscillation bursts were seen in response to some non-predator odors. We compare physiological and behavioral responses to six monomolecular odorants (toluene, amyl acetate, benzaldehyde, acetone, indole, vanillin) with responses to two predator odors [trimethyl thiazoline (TMT) and fox urine]. Olfactory bulb (OB) local field potential (LFP) responses were recorded during five consecutive 2 min presentations of odorants in a closed chamber, and behavior was recorded by webcam. Theta (3–15 Hz), beta (15–40 Hz), low gamma (35–60 Hz) and high gamma (60–115 Hz) oscillation patterns are examined in response to each odor. We find similarities between predator and non-predator odors across frequency bands, as well as some differences between the two predator odors tested. Behavioral responses, such as freezing and attentive sniffing, are strongly correlated with concurrent oscillatory activity. Our results suggest that variations in oscillatory patterns can be attributed primarily to behavioral

variations. We also find that isoamyl acetate (IAA) produces frequency responses different from all the other monomolecular odors tested. This suggests that IAA, which is used as a control odor in many olfactory studies, may be inappropriate for this purpose.

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Poster: Cell Biology of the Olfactory Epithelium

Tyrosine hydroxylase promoter-driven reporter gene expression in olfactory epithelium of transgenic mice

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Transgenic mice expressing either green fluorescent protein (GFP) or lacZ reporter genes driven by 9 kb of tyrosine hydroxylase (TH) promoter have been extensively used in characterizing the CNS dopaminergic phenotype. To investigate whether TH promoter driven reporter gene expression occurred in the olfactory epithelium (OE), TH/GFP and TH/lacZ transgenic mouse strains were examined using immunohistochemical or X-gal histochemical methods. The antibodies used were chicken anti-GFP, rabbit anti-TH, rabbit anti-βgal and goat anti-olfactory marker protein (OMP). Both GFP-immunoreactive (IR) and βgal-IR cells localized to the superficial OE. The cells showed a sparse distribution that was similar to the zone 1 pattern of olfactory receptor neurons (ORN). Morphologically, the cells resembled ORNs with a dendritic and an axonal process directed towards the nasal cavity and the lamina propria, respectively. Axonal processes did not enter the OB. Double label confocal analysis showed that OMP, a marker of mature ORNs, was not coexpressed with either GFP or βgal. X-gal staining confirmed the cellular morphology and distribution of the transgene expressing cells in the OE. The vomeronasal organ contained a few βgal-IR cells. Reporter gene expression was negatively correlated with age with cell number highest at postnatal day 2 (P2), the first age examined, and declining until few cells could be detected at P22. These studies suggest that some cells in the OE transiently exhibit low level expression of dopaminergic properties during early postnatal development.

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Poster: Cell Biology of the Olfactory Epithelium

The fine structural distribution G-protein receptor kinase 3, beta-arrestin-2, Ca²⁺/calmodulin-dependent protein kinase II, and phosphodiesterase PDE1C2 in olfactory epithelia

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The sequentially activated molecules of olfactory signaling onset are mostly concentrated in the long thin distal parts of olfactory epithelial (OE) receptor cell (ORC) cilia (*Chem Senses* 22: 295,

1997). Is this also true for the sites of signal termination? G-protein receptor kinase 3 (GRK3) is thought to act at the level of the odor receptors, whereas beta-arrestin-2, Ca²⁺/calmodulin-dependent protein kinase II (CAMKII) and PDE1C2 are thought to act at the level of adenylyl cyclase. We used four antibodies to GRK3, two to beta-arrestin-2 (one, courtesy Dr Lefkowitz, Duke University), five to CAMKII (one to both the alpha and beta form, and two each specific to α- and β-CAMKII) and two to PDE1C2 (courtesy Dr Beavo, University of Washington). Earlier, light microscopic, studies showed that antibodies to all of these molecules labeled the OE luminal border that includes ORC cilia (*Science* 259: 825, 1993; *J. Biol. Chem.* 41: 25425, 1997; *Neuron* 21: 495, 1998; *Proc. Natl Acad. Sci. USA* 94: 3388, 1997). However, the current, fine structural, data indicate that none of these antibodies labeled ORC cilia exclusively, and included other ORC structures, such as dendritic endings, as well. Significantly, the antibodies also labeled apices and microvilli of neighboring OE supporting cells and some bound to luminal regions, including cilia, of the respiratory epithelium adjacent to the OE. Thus, among others, the observations suggest that neuronal ORCs and neighboring OE supporting cells share at least some molecules of signal transduction. In the supporting cells, these molecules occur in addition to those of biotransformation and transepithelial transport.

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Poster: Cell Biology of the Olfactory Epithelium

Immunocytochemical localization of 11 beta-hydroxysteroid dehydrogenase in the mammalian olfactory mucosa

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Mineralocorticoid (type I) receptors have been identified in the olfactory mucosa by a number of approaches. In immunohistochemical studies, mineralocorticoid receptor immunoreactivity was localized to the supranuclear region of sustentacular cells, as well as the acinar cells of the Bowman's glands. Functioning of the mineralocorticoid (type I) receptor is enhanced by the action of the enzyme 11-beta-hydroxysteroid dehydrogenase type II (11 beta HSD2). 11 beta HSD2 catalyses the inactivation of glucocorticoids, thus playing a major role in the protection of the mineralocorticoid (type I) receptor. In previous studies using histochemical and biochemical approaches, 11 beta HSD was identified in the olfactory mucosa. The objective of this current study was to identify the corticosteroid inactivating (type II) form of the 11 beta HSD by use of a monoclonal antibody specific to 11 beta HSD2. When we incubated sections from the olfactory mucosa with this antibody, 11 beta HSD2 immunoreactivity was found associated with the acinar cells of Bowman's glands and the supranuclear region of sustentacular cells. This distribution corresponds with the location of mineralocorticoid (type I) receptors reported in earlier studies and the histochemical location of 11 beta HSD. This study provides further evidence that mineralocorticoid hormones may be involved in the modulation of olfactory function.

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Poster: Cell Biology of the Olfactory Epithelium

Localization of retinoic acid receptors in mouse and human nasal epithelium

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All-*trans* retinoic acid (ATRA), a metabolite of vitamin A, binds to retinoic acid receptors (RARs) to mediate gene-transcription in target cells. We previously found that an ATRA supplement enhanced olfactory recovery rate in adult mice after olfactory bulb (OB) deaf-ferentation. In this study, we examined the localization of RAR α , RAR β and RAR γ after injury and ATRA treatment using immunocytochemistry. Mice received a left olfactory nerve transection (LNX) with the right side serving as control. One day after LNX, the mice were given either ATRA mixed with sesame oil or just sesame oil. In the control OB, RAR α immunoreactivity (ir) was observed in periglomerular and granule cells, while we did not detect immunostaining for RAR β or RAR γ . In the oil-treated right olfactory epithelium (OE), RAR α -ir was found in NST- and SUS4-negative microvillar-like cells located in the supporting cell layer, in cells in the lamina propria, and in some respiratory cells. RAR β -ir was localized only in the respiratory cells, while no RAR γ -ir was observed in the OE. Surprisingly, the density of RAR α -ir microvillar-like cells was higher in the transected OE and highest in transected OE with ATRA treatment, suggesting these cells are non-neural. We also examined RARs-ir in human nasal tissue and found a similar cellular localization. These findings suggest that microvillar cells, a population about which comparatively little is known, are targets for ATRA modulation of gene expression in the OE and may play a role in OE recovery following injury.

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Poster: Cell Biology of the Olfactory Epithelium

Immunolocalization of Bex proteins in the mouse brain: colocalization with OMP

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Bex proteins are a family of 'Brain expressed X-linked genes' that are closely linked on the X-chromosome. Bex1 and 2 have been characterized as interacting partners of OMP. Here we report the development and characterization of an antibody to mouse Bex1 protein that also cross-reacts with Bex2 (but not Bex3) and its use to determine the first comprehensive distribution of Bex proteins in the murine brain. Immunoblots and immunocytochemical analyses of cells transfected with either Bex1 or Bex2 have shown that the antiserum reacts with both Bex1 and Bex2. Antibodies pre-absorbed with recombinant Bex2 still recognize Bex1 while blocking with Bex1 totally eliminates all immunoreactivity to Bex1 and Bex2. Bex protein immunoreactivity (ir) was primarily localized to neuronal cells within select regions of the brain, including the olfactory bulb, epithelium, peri/paraventricular nuclei, suprachiasmatic nucleus, arcuate nucleus, median eminence, lateral hypothalamic

area, thalamus, hippocampus, and cerebellum. Bex protein-ir was broadly present throughout the rostral-caudal aspects of the hypothalamic region. Further studies, using double-label immunocytochemistry, indicate that Bex-ir is colocalized with OMP in mature ORNs and in the OMP-positive subpopulation of hypothalamic neurons. This is the first anatomical demonstration of the comprehensive mapping of Bex proteins in the mouse brain and their colocalization with OMP in ORNs and hypothalamic neurons.

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Poster: Cell Biology of the Olfactory Epithelium

Reduced olfactory epithelium mitotic rate in streptozotocin-induced diabetic rats

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Individuals who suffer from diabetes frequently develop anosmia for unknown reasons. The site(s) of disfunction in the olfactory pathways is (are) not known. We used immunohistochemistry to compare the numbers of mitotically active cells in the main olfactory epithelium (OE) of normal ($n = 4$) male Wistar rats and of males ($n = 4$) that suffered from streptozotocin (STZ)-induced diabetes. Insulin-dependent (type 1) diabetes was induced by i.v. injection of 50 mg/kg bw STZ. After 8 weeks, each animal was injected with bromodeoxyuridine (BrdU) (50 μ g/g body wt). An hour later, the animals were deeply anesthetized with pentobarbital and perfused with buffered 4% paraformaldehyde. After paraffin embedding, 7 μ m transverse sections were mounted on slides and hydrated. BrdU(+) cells were counted in eight nonserial sections from three regions dorsal to ventral per animal. The length of epithelium containing those cells was measured using Spot Advanced software. Counts were rendered as BrdU(+) cells/mm epithelium. Analysis by Student's *t*-test using SAS 8.2 software indicated a significant difference in mitotic rates ($P < 0.0001$). ANOVA analysis suggested no significant differences within groups ($P > 0.05$). These data indicate that, after 8 weeks of STZ-induced type 1 diabetes in Wistar rats, mitotic rate in the main OE is lower in diabetics compared with normal controls.

Poster: Cell Biology of the Olfactory Epithelium

Qualitative and quantitative study of cytochrome oxidase staining pattern in olfactory epithelium of neonatal rat

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Newborn rats are capable of olfaction and their olfactory epithelium (OE) contains functionally mature olfactory receptor neurons (ORNs), although total ORN number is <10% of adult. Cytochrome oxidase (CO) staining density is positively correlated to levels of metabolic/neuronal activity. We stained newborn rat OE to characterize

its staining pattern qualitatively and quantitatively. Staining densitometry was carried out using MCID software to gauge the luminescence of the sample over a range of 0.0–1.0 (higher numbers indicated lesser CO staining). A differential banding pattern of CO staining was observed corresponding to specific OE layers and ORN cellular sites, indicating the parts of the ORNs that were most actively involved in neuronal activity and transduction. Zones corresponding to ORN dendrite and knob exhibited high CO activity (0.34). Staining was heterogeneous within and along the length of dendrites, darker in the apical part, implying higher neuronal activity in this region than the deeper part of the dendrite, closer to the soma. A light staining band (0.40), between the two dark bands, corresponded to the overlapping presence of supporting cell cytoplasm and nuclei. Areas of OE close to the basal lamina also stained lightly (>0.44), implying lack of mitochondria and neuronal functional activity in the basal and immature neurons. The findings confirm that some ORNs are metabolically mature and functional in neonatal rats, capable of neuronal function.

Support: University of Illinois Research Funds.

Poster: Cell Biology of the Olfactory Epithelium

Functional characterization of CUB-serine protease in the spiny lobster's olfactory organ

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Several serine proteases and protease inhibitors have been identified in the olfactory organ of decapod crustaceans (Hollins *et al.*, 2003, *J. Comp. Neurol.* 455: 125–138; Stoss *et al.*, 2002, *Chem. Senses* 27: A45), including a CUB-serine protease (Csp; Levine *et al.*, 2001, *J. Neurobiol.* 49: 277–302). The function of these proteases in the olfactory organ is unknown. To examine directly the functional activity of proteases in the olfactory organ of the Caribbean spiny lobster, *Panulirus argus*, we used soluble and membrane tissue fractions from the lateral flagellum in a spectrophotometric enzyme activity assay with a variety of protease substrates and inhibitors to demonstrate trypsin-like serine protease activity. Csp accounts for at least 40% of the serine protease activity of the membrane fraction of the olfactory organ, based on immunoprecipitation of Csp. Serine protease activity follows a developmental pattern: it is lowest in the proximal zone, which lacks aesthetascs, and in the proliferation zone, where olfactory receptor neurons and associated cells are born, and highest in aesthetascs of the senescence zone, which has the oldest olfactory tissue. Csp activity is present at approximately the same level in each of the developmental zones. Currently we are determining if Csp's functional activity changes due to damage of olfactory tissue.

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Poster: Cell Biology of the Olfactory Epithelium

Exocrine glands containing serine protease are associated with olfactory sensilla in the spiny lobster, *Panulirus argus*

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In decapod crustaceans, the olfactory organ is constituted by specialized setae—aesthetascs—located on the lateral flagella of the first antennae. In an attempt to identify molecular components of the aesthetascs, diverse genes have been cloned from the lateral flagella of the spiny lobster, *Panulirus argus*, among them a gene encoding a CUB-serine protease (Csp) (Levine *et al.*, 2001, *J. Neurobiol.* 49: 277–302). We found that an antibody against Csp specifically labels cells that are closely associated with but not a component of the aesthetascs. These cells are arranged in a rosette-like pattern of 5–7 cells around a duct with a terminal swelling revealed by labeling with phalloidin, an actin marker. The arrangement and phalloidin-reactivity are typical for 'rosette glands', an exocrine epithelial gland of decapods (Talbot *et al.*, 1991, *Zoomorphology*, 110: 329–338). SEM imaging revealed that in *P. argus* the gland openings are a field of 50–70 dome-shaped cuticular structures (~1 µm in diameter) with crescent-shaped pores proximal to each row of aesthetascs. Similar small pores have been found at the base of aesthetascs of other decapod crustaceans, suggesting that the association of exocrine rosette glands with aesthetascs is a common feature. From the presence of Csp among the excreted substances, we hypothesize that the rosette glands protect the aesthetascs from microbial fouling.

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Poster: Cell Biology of the Olfactory Epithelium

Functional studies of a serine protease and an amine mono-oxygenase specific to the lobster olfactory organ

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Olfactory specific proteins probably participate in critical functions of the olfactory system. We previously identified olfactory enriched transcript-03 (OET-03), which is expressed only by collar cells associated with the inner dendrites of the olfactory sensory neurons of *Homarus americanus* (Hollins *et al.*, 2003, *J. Comp. Neurol.* 455: 125). We now report the complete cDNA sequence of OET-03, a serine protease. Confirmation that OET-03 is a protease was obtained by assaying protease substrates and inhibitors. Removal of the presumed catalytic domain eliminated activity. Preliminary evidence indicates that OET-03 is functionally related to the chymotrypsin family. In addition, full-length OET-03 specifically reduces proliferation of transfected HEK293 cells. We also report the complete sequence of OET-02, expressed only by outer auxiliary cells ensheathing the proximal regions of the inner dendrites of the sensory neurons. OET-02 has a unique double mono-oxygenase structure in which the two domains characteristic of dopamine beta-hydroxylases are repeated. Preliminary assays of olfactory tissue have not identified a biogenic amine product of this enzyme, so tests with recombinant OET-02 are planned. An antiserum raised against OET-02 confirms the large size of this protein (155 kDa) and its restricted expression to the outer auxiliary cells.

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Poster: Olfactory Bulb: Neurophysiology**Quantification of taurine-synthesizing enzyme mRNA in olfactory structures with real-time RT-PCR**

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The olfactory epithelium (OE) and olfactory bulb (OB) are rich in the amino acid taurine, which is synthesized in liver and nervous tissue via the enzyme cysteine sulfinate decarboxylase (CSD). Our previous studies have shown that (i) taurine, acting on GABA receptors, inhibits mitral/tufted cells and their synaptic responses to olfactory nerve input; (ii) immunoreactivities for taurine and CSD are located in olfactory receptor cells and most OB neurons; and (iii) CSD mRNA is expressed in the OE and OB. In the present study, gene expression of CSD in the rat OE and OB was assessed quantitatively using the real time reverse transcription-polymerase chain reaction (RT-PCR) in the LightCycler. With this method, a PCR product is detected after each amplification cycle by measuring fluorescence emission of a reporter dye, whose release is proportional to the amount of the product. A calibration curve (a regression line with a correlation coefficient of 0.99) showing the relationship between a starting copy number of CSD mRNA and a threshold cycle number within a range of 200–20 000 000 mRNA copies was obtained first. Values of the threshold cycle number for tissue samples were then detected and a copy number of CSD mRNA was calculated using a regression equation. The level of CSD mRNA in both OE and OB was ~1 000 000 copies/mg tissue. A higher amount of ~100 000 000 CSD mRNA copies/mg tissue was detected in the liver. The results confirm that CSD mRNA is expressed in the OE and OB and provide its quantitative evaluation.

Supported by NIDCD grant DC04083.

Poster: Olfactory Bulb: Neurophysiology**Kv1.3-null mutation alters scaffolding proteins, olfactory bulb biophysics, and glomeruli size/abundance**R.M. Perkins¹, K. Tucker², M. Meredith² and D. Fadool²¹*Department of Biological Science, Florida State University, Tallahassee, FL, USA* and ²*Program in Neuroscience & Molecular Biophysics, Florida State University, Tallahassee, FL, USA*

Previously we demonstrated that Kv1.3-null (KO) mice had smaller sized and more abundant glomeruli independent of coronal position in the olfactory bulb (OB). To test whether projections to the OB were altered, we generated Kv1.3^{-/-} mice with a targeted P2-IRES-taulacZ mutation (KvP2). Coronal cryosections (12 μm) and whole mounts of OB and olfactory epithelium of KvP2 and P2 mice (*n* = 3–5) were photographed using a Zeiss AxioCam digital camera with Axiovision software. The location of P2 receptors along the olfactory epithelium and known P2 glomeruli in the OB showed no noticeable differences between P2 and KvP2 mice. In spite of a demonstrated lack of change in axonal connectivity, action potentials recorded from mitral cells of KO mice

exhibited increased hyperpolarization, 10–90% rise time and width at half-maximum amplitude. Current injection elicited higher spiking frequency with less variance in interpulse interval. OB neurons are no longer modulated by activation of receptor tyrosine kinases (insulin or TrkB receptor) typically associated with the Kv1.3 channel. SDS-PAGE indicates the up-regulation of Ca²⁺ channels, two tyr kinases and six adapter proteins in the OB of KO mice. The electrical properties in mitral cells from Kv1.3 KO mice could produce rapid synchronous firing to strengthen connections to the more numerous glomeruli. This interpretation would be consistent with other experiments in our laboratory that indicate an increased olfactory ability in the mice.

Support: NIH DC03387 (NIDCD) and Florida Foundation.

Poster: Olfactory Bulb: Neurophysiology**Multiple roles of TrkB receptor in modulating Kv1.3 ion channel in the olfactory bulb**

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Previously we have shown that olfactory bulb neuron (OBN) current properties are modulated by activation of the neurotrophin receptor TrkB, by brain-derived neurotrophic factor (BDNF), to increase tyrosine phosphorylation of the potassium channel Kv1.3. Here we made Y to F mutations in the Kv1.3 channel to test whether removal of the Y phosphorylation motif disrupted BDNF-induced current suppression in Kv1.3 + TrkB transfected HEK293 cells. Tyrosines in the N and C termini (111–113, 137, 449) were found to be the molecular targets for current modulation via phosphorylation as demonstrated by patch-clamp electrophysiology and phosphorylation assays using various mutant channel constructs compared with the wildtype channel. Unexpectedly, we found a 2.1-fold increase in channel protein expression (*n* = 4) and a 2-fold increase in peak current amplitude in HEK293 cells co-transfected with channel + TrkB versus channel alone. This phosphorylation-independent up-regulation of Kv1.3 was not observed using a GFP-tagged Kv1.3 that likely disrupts an N-terminal ER retention motif. Kv1.3 can be immunoprecipitated with TrkB in the presence of the adaptor protein nShc and the described up-regulation is disrupted by substituting trkB K538N or Y490F, which are trkB mutants that lack kinase activity or association with nShc adaptor respectively. These results suggest that TrkB receptor may alter the excitability of OBNs by both phosphorylation-dependent and -independent mechanisms involving Kv1.3 ion channel.

Supported by NIH R01 DC03387 (NIDCD).

Poster: Olfactory Bulb: Neurophysiology**GABAergic periglomerular cells presynaptically inhibit on input to themselves**Z. Shao¹, G. Szabo², A.C. Puche¹ and M.T. Shipley¹¹*University of Maryland at Baltimore, Baltimore, MD, USA* and²*Department of Gene Tech. and Developmental Neurobiology, Institute of Experimental Medicine, Budapest, Hungary*

Olfactory nerve axons terminate in olfactory bulb glomeruli and form excitatory synapses onto the dendrites of mitral/tufted (M/T) and periglomerular (PG) cells. ON terminals express dopamine D2 and GABAB receptors, and there is evidence that DA and GABA presynaptically inhibit the release of glutamate from ON synapses. It is thought that ON excitation of GABAergic PG cells releases GABA back onto ON terminals, but there is no direct evidence for this. To investigate this question we used whole cell patch clamp recordings of identified GABAergic PG cells in a line of mice expressing green fluorescent protein under the control of the glutamic acid decarboxylase 65 kDa gene (GAD65-GFP). GAD65-GFP+ GABAergic PG cells are excited by ON stimulation; 30–40% receive monosynaptic ON input and the remainder have variable-latency polysynaptic responses to ON stimulation. For those that receive monosynaptic input, paired-pulse ON stimulation caused a reduction of EPSC amplitude in response to the second pulse. This paired-pulse inhibition was abolished by addition of selective GABAB receptor antagonists. This indicates that ON terminals targeting GABAergic PG cells are subject to GABAergic presynaptic inhibition. When step-depolarization of the cell was used to evoke GABA release prior to stimulation of ON, the magnitude of the ON-evoked EPSC was reduced by ~30%. The reduction was prevented by application of selective GABAB receptor antagonists. This demonstrates that a GABAergic PG cell can presynaptically inhibit the ON terminals targeting that same PG cell. GABAergic PG cells, therefore, may provide feedback, presynaptic inhibition that limits subsequent glutamate release from ON terminals.

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Poster: Olfactory Bulb: Neurophysiology

Intraglomerular synchronous calcium oscillations of periglomerular cells in the mouse olfactory bulb

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Synchronous neuronal oscillations have been widely considered to be an important mechanism for coding and processing odor information. Oscillations of different frequency ranges have been reported in the olfactory bulbs of a wide range of vertebrate species. Olfactory sensory neurons expressing the same odorant receptor project to a few glomeruli in the mammalian olfactory bulb. Thus, glomeruli are key for the neural basis of olfactory coding. In this study we have used optical imaging methods to map neuronal synchrony among periglomerular (PG) cell populations that either share a common glomerulus or belong to different glomeruli. An ester form of calcium-sensitive dye was used to load hundreds of neurons in the olfactory bulb slices. Calcium imaging was performed for 30–150 s of to analyze the dynamic structure of PG-cell spontaneous activities by a cooled CCD camera. We observed novel slow (<1 Hz) calcium oscillations in PG cells. Cells connected to the same identified glomerulus were more likely to develop spontaneous synchrony than cells connected to different glomeruli. Each glomerulus could have its own phase and pace for synchrony. Application of APV (50 mM) or CNQX (30 mM) separately was not able to block the intraglomerular synchronized calcium oscillations, whereas different gap junction blockers (octanol, 1 mM; β -glycyr-

retinic acid, 10 mM; carbenoxolone, 100 mM) could eliminate the oscillations. These results indicated that at least some forms of glomerular synchrony did not require the activation of NMDA receptors, whereas electrical coupling appeared to be essential.

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Poster: Olfactory Bulb: Neurophysiology

Rat olfactory bulb neurons express functional calcium-fluxing AMPA receptors

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The AMPA subtype of glutamate receptors (AMPA) plays an important role in processes such as glomerular synchronization, correlated spiking, and the gating of recurrent and lateral inhibition in the olfactory bulb (OB). AMPARs are heterotetrameric ligand-gated ion channels formed by various combinations of the GluR1–4 subunits. The GluR2 subunit is perhaps the most significant of these as it controls ionic selectivity; AMPA receptors lacking the edited form of GluR2 are highly permeable to Ca^{2+} (CaAMPA). A combination of whole cell recording, pharmacology and histological techniques were used in cultured neurons to determine whether OB AMPA receptors flux calcium. Coapplication of NAS, a selective antagonist of CaAMPA, inhibited kainate-activated AMPAR-mediated currents to various degrees and in a voltage-dependent manner. Current-voltage relationships (in 100 mM extracellular calcium) further suggest that some OB AMPARs flux calcium. Application of kainate plus cobalt revealed cobalt labeling in some neurons, which was not present in neurons treated with cobalt alone or cobalt plus kainate plus CNQX (an AMPAR antagonist). Collectively, these results suggest that some OB neurons express functional CaAMPA. Calcium entry through CaAMPA could influence odor information processing through activation of Ca^{2+} -dependent K^+ channels and/or CaMKII, modulation of NMDA receptors or via additional second messenger effects. CaAMPA also may contribute to synaptic vesicle fusion, thus, influence synaptic events important to OB function such as reciprocal transmission and feed-forward excitation.

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Poster: Olfactory Bulb: Neurophysiology

Rhythmic excitation of EPL interneurons via AMPA/kainate receptors

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Interneurons in the external plexiform layer (EPL) of the olfactory bulb receive type 1 synapses from mitral and tufted (M/T) cells and form type 2 synapses onto the M/T cells. To study the functions of these synapses, recordings were obtained from cell bodies in the

EPL of mouse olfactory bulb slices. Biocytin-filling confirmed that interneurons, tufted cells and astrocytes were recorded. The interneurons had finely branched, varicose dendrites, and those located close to the glomerular layer (GL) appeared to bridge two glomerular widths. Recordings from the interneurons were readily identifiable by the low resting membrane potential (~ 80 mV) and high frequency (~ 100 Hz) of spontaneous EPSP/CS that increased rhythmically at a rate of ~ 0.5 Hz, similar to the action potential burst frequency of some tufted cells. The EPSP/CS were largely eliminated by TTX and by the AMPA/kainate receptor antagonist CNQX. Healthy interneurons produced few action potentials spontaneously, but readily produced them in response to depolarizing current pulses and olfactory nerve (ON) stimulation. The response latencies indicated that the ON responses were polysynaptic. Cells expressing green fluorescent protein under control of the mouse 65 kDa glutamic acid decarboxylase (GAD65) promoter also exhibited these properties, providing evidence that the interneurons are inhibitory. Our results suggest that EPL interneurons are rhythmically excited by M/T cells via AMPA/kainate receptors and in turn inhibit the M/T cells within spatial domains that are topographically related to adjacent pairs of glomeruli.

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Poster: Olfactory Bulb: Neurophysiology

Olfactory nerve-evoked metabotropic glutamate receptor-mediated responses in rat olfactory bulb mitral cells

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The group I metabotropic glutamate receptor (mGluR), mGluR1, is highly expressed on mitral cell (MC) apical dendrites, and thus may be activated by glutamate from the olfactory nerve (ON) and/or mitral/tufted cells. We have shown that mGluR1 agonists potently excite MCs. Here we investigated if ON stimulation engages mGluR-mediated responses in MCs in rat olfactory bulb slices. In voltage clamp recordings, EPSCs evoked by single ON shocks were unaffected by the potent, non-selective mGluR antagonist LY341495 (50–100 μ M). Single ON shocks in the presence of ionotropic glutamate and GABA_A receptor antagonists (CNQX, APV, Gabazine) did not evoke EPSCs, except at high intensities (>600 μ A). In the same cell (in CNQX, APV, Gabazine), brief high frequency ON trains (six pulses at 10–200 Hz) induced long duration EPSCs that were blocked by TTX or by low calcium ACSF. Application of glutamate uptake inhibitor (TBOA, 100 μ M) and a glutamate transporter inhibitor (THA, 300 μ M) increased the amplitude and duration of responses to single or trains of ON stimuli. These agents also unmasked 'latent' responses to single ON stimuli in some cells. An additional application of LY341495 (50–100 μ M) reduced or completely blocked ON-evoked responses observed in the presence CNQX, Gabazine and APV. These results demonstrate that ON stimulation evokes frequency dependent excitatory responses in MCs that are mediated, in part, by activation of

mGluRs. Such responses are maximally engaged during high frequency ON input, as occurs during odor stimulation.

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Poster: Olfactory Bulb: Neurophysiology

Metabotropic glutamate receptors enhance synaptic interactions among juxtglomerular neurons in olfactory bulb glomeruli

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Metabotropic glutamate receptors (mGluRs) are present on juxtglomerular (JG) cells and may regulate dendrodendritic synapses. We investigated the actions of mGluR agonists in identified JG cells in slices. Group I (DHPG) and group II (CCGI) mGluR agonists, but not group III mGluR agonists (L-AP4), increased the number of spikes/burst in external tufted (ET) cells in the presence of synaptic blockers. In the presence of TTX and synaptic blockers, DHPG and CCGI directly excite and induce an inward current in ET cells. They also increased the frequency of spontaneous and miniature IPSCs in ET cells. This effect, abolished by the Ca²⁺ channel blocker, cadmium, may be due to increased ET and/or mitral cell dendritic excitability and/or a direct, mGluR-mediated excitatory effect on the dendrites of GABAergic periglomerular (PG) cells. DHPG and CCGI also increased the frequency of miniature EPSCs in PG and short axon (SA) cells but not in ET cells, suggesting that activation of mGluRs on ET cell dendrites, increases glutamate release onto PG and SA cells. In the presence of fast synaptic blockers and the Na⁺ channel blocker QX-314 in the pipette, DHPG induced in ET cells rhythmic events associated with membrane current oscillations (~ 2 Hz) that are possibly due to gap junctions among ET cells. mGluR agonists also decreased olfactory nerve-evoked EPSCs in ET and mitral cells via a GABA_B-mediated presynaptic mechanism. Thus, mGluR agonists directly activate rhythmically bursting ET cells and enhance glomerular dendrodendritic interactions.

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Poster: Olfactory Bulb: Neurophysiology

In vivo mouse preparation for olfactory bulb electrophysiology

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The objective of this study was to develop an *in vivo* mouse preparation for recording from the main olfactory bulb (MOB). An aim was to establish a protocol using an injectable anesthetic such as chloral hydrate. The anesthetic plane was evaluated using the EEG and the hindpaw withdrawal response. Single-unit recordings measured the spontaneous activity of neurons in the MOB. The amount of chloral hydrate required to maintain a surgical plane of anesthesia was linearly correlated with a decrease in MOB spontaneous activity ($P = 0.2$; $n = 13$), and could be lethal. Therefore,

analgesic supplements to chloral hydrate anesthesia were investigated. Supplementation with buprenorphine, a synthetic μ -opioid receptor agonist, was problematic. Buprenorphine at doses of 0.02, 0.05 and 0.2 mg/kg reduced spontaneous activity of bulbar neurons by 19, 45 and 54%, respectively. In contrast, ketoprofen, a non-steroidal anti-inflammatory with reported analgesic activity, did not inhibit spontaneous activity at doses of 100 or 200 mg/kg. Importantly, mice given 100 mg/kg ketoprofen at the beginning of an experiment, before surgery, required significantly less chloral hydrate than control animals ($P = 0.05$; $n = 18$). Animals in both groups were maintained at a surgical plane of anesthesia. Lastly, administration of ketoprofen during an experiment changed the EEG indicative of a deeper plane of anesthesia. Thus, chloral hydrate supplemented with ketoprofen in the mouse provides an *in vivo* preparation for stable, long-term recording of neurons in the MOB.

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Poster: Olfactory Bulb: Neurophysiology

Spontaneous activity of main olfactory bulb neurons in the rat

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Neurons in the main olfactory bulb (MOB) are spontaneously active, with rates above 30 Hz reported. In this study, the sources of this activity were investigated. We specifically tested the hypothesis that the spontaneous activity of bulbar neurons is at least partly due to spontaneous activity in olfactory receptor neurons (ORNs). This study was conducted using anesthetized rats breathing clean air. The conduction of ORN action potentials was blocked by cooling the olfactory nerve rostral to the cribriform plate. To assess the efficacy of the cold block, a bipolar stimulation electrode was positioned rostral to the cold probe, and field potentials were recorded from the MOB while cooling the nerve. Cooling completely and reversibly abolished the field potentials. The spontaneous activity of single units was recorded from the same region of the MOB, and the recording sites subsequently marked with dye. Mitral and tufted cells were further identified by antidromic activation from the lateral olfactory tract. To date, for every cell recorded that exhibited spontaneous activity (from glomerular, external plexiform, and mitral cell layers of the MOB), cold decreased this activity. This study suggests that ORNs are spontaneously active in the rat. Thus, in the absence of odor stimulation, ORN spontaneous activity, directly and/or via bulbar circuits, at least in part sets and maintains neuronal activity in the MOB.

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Poster: Olfactory Bulb: Neurophysiology

Multiunit and field potential recordings in rat olfactory bulb

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To test the relationship between olfactory bulb slow waves and single cell responses, we conducted recordings with silicon based multiple electrodes. Field potential recordings are used to calculate one- and two-dimensional current source density in rats anesthetized with urethane (1.5 g/kg body wt) or chloral hydrate (0.4 g/kg body wt). Antidromic stimulation of the lateral olfactory tract is used to localize the mitral cell layer and in some cases to identify output cells. Under both anesthetics a number of odorants can evoke spike responses and waves in the 30–60 Hz range at concentrations in the range of 10^{-3} times saturated vapor in our preliminary results. The field responses evoked by odorants are more obvious in the voltage records than in the current density calculations. This agrees with our findings that the voltage oscillations are not well localized in the olfactory bulb. We failed to find spikes in either the mitral or granule layers that are strongly synchronized with slow waves. On the other hand it has been common to see strong inhibition or excitation of spikes in the mitral cell layer during bursts of oscillations. While some of these cells may be too strongly inhibited to show oscillations, preliminary attempts to uncover the underlying oscillation by reducing odorant concentration have been unsuccessful. While the prominence of these oscillatory phenomena in the bulb suggests that they represent a strong synchronization of inhibitory interneurons, our results suggest that it will be difficult to show simple relationships with spike activity.

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Poster: Olfactory Bulb: Neurophysiology

Nitric oxide modulates antennal lobe neuron activity in the moth, *Manduca sexta*, through soluble guanylyl cyclase-dependent mechanisms

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Gaseous messengers like nitric oxide (NO) have been implicated in a number of physiological processes including the modulation of olfactory representations in the brain. NO and its signaling components have been studied in the olfactory systems of several species, but the function of NO in olfactory processing remains elusive. In the moth *Manduca sexta*, NO synthase (NOS) was found in olfactory receptor neurons (ORNs), while a well-characterized target of NO, soluble guanylyl cyclase (sGC), was found in the post-synaptic targets of ORNs in antennal lobe (AL). This expression pattern, along with preliminary imaging data showing increases in fluorescence of an NO-sensitive dye in odor-activated glomeruli, led to a hypothesis that NO is released focally when odor is present, and then modulates signaling in the activated glomeruli by acting on the sGC-containing neurons in the AL. This hypothesis was tested with multi-channel and intracellular recording methods coupled with pharmacological manipulation of NO levels and the activity of sGC in the AL. Blocking NO production with L-NAME resulted in an overall decrease of spontaneous activity in AL neurons, and this effect was mimicked reversibly by the sGC inhibitor ODQ. Odor-evoked responses were also diminished when NO-dependent signaling was blocked. These results suggest that NO

is an important modulator of odor responsiveness, and that sGC-dependent signaling is at least partially responsible for the observed changes in glomerular activity levels.

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Poster: Multimodal Integration

Behavioral responses of the crayfish *Procambarus clarkii* to single compounds

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We investigated chemosensory behavior of the crayfish *Procambarus clarkii* in response to glutamate and glucose (not stimulatory to leg chemoreceptors in physiological tests; *J. Chem. Ecol.* 28: 1117–1130), ammonium, glycine, maltose and trehalose (physiological stimuli of varying efficacies), and a blank. Each animal was acclimated to a 1.5 l testing chamber for >2 h and was stationary in the chamber's shelter when tested. Individual compounds were injected in 10 ml aliquots into a 149 ml/min carrier flow and diluted to 200 μ M–2 mM within the testing chamber according to conductivity measurements. Each animal was tested once. Responses were filmed, and the time spent probing with pereopods, the time spent exploring, and the number of dactyl clasps were quantified by observers blind to the stimulus. ANOVA followed by Fisher's PLSD tests indicated the following elicited responses that differed significantly from that evoked by the blank: glucose for the time spent probing; glucose, maltose and glycine for exploration time; glucose, maltose and trehalose for the number of dactyl clasps. Ammonium's ineffectiveness parallels its weakness as a physiological stimulus. Trehalose was the most potent physiological stimulus tested, but it was no more effective in evoking behavioral responses than glucose or maltose. Glucose was a potent behavioral stimulus, but earlier physiological tests failed to show responses to glucose from leg chemoreceptor cells. Either receptor cells for glucose are elsewhere or they are on the legs in small numbers and carry a disproportionate influence on behavior.

Poster: Multimodal Integration

Dual intracellular recordings from single parasol cells reveal impulse burst initiation site and dendritic trajectories

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Parasol cells are multimodal sensory interneurons in the crustacean forebrain. They exhibit complex and interesting forms of electrical activity, investigations of which may reveal novel concepts of dendritic integrative mechanisms. We used dual intracellular recordings from individual parasol cells to examine impulse and impulse burst initiation sites and propagation within the dendritic tree. Previous findings (Mellon, 2003, *J. Neurophysiol.* 90: 2465) indicated that impulses are initiated at a low threshold region on the dendritic trunk adjacent to the basal branch points. Dual simultaneous recordings from different basal branches of the same neuron

support this interpretation and, furthermore, suggest that impulse bursts arise at a trunk region distant from (and more ventral to) the initiation zone of single spikes. Orthodromic stimulation via the olfactory globular tract or antidromic stimulation via the parasol cell axon terminals generates slightly different impulse latencies in simultaneous recordings from basal branches on opposite sides of the dendritic tree. Impulse bursts, evoked either by photic stimulation of the compound eyes or by post-hyperpolarization-activated I_h , invade both recording sites and exhibit latency differences identical with those of individual spikes following orthodromic or antidromic stimulation. These findings support conclusions regarding a trunk site for impulse initiation and provide evidence for bursts also being initiated on the dendritic trunk.

Supported by NSF.

Poster: Multimodal Integration

Tuning of mechanoreceptors in the lobster antennule

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The American lobster, *Homarus americanus*, is capable of tracking an odor to its source in a turbulent plume. In such a plume an animal encounters hydrodynamic and chemical signals. Concurrent reception of odor and flow could be an important cue for odor plume tracking. The lateral antennule contains many chemoreceptor neurons and is important for successful tracking. We now want to determine the bandwidth in which the lobster uses lateral antennule mechanoreceptors to monitor the hydrodynamic properties of the plume. Under oscillatory flow conditions the antennule has a resonance of frequency of 5–12 Hz; antennular mechanoreceptors could also be expected to respond optimally in this range. In this experiment frequency synchronization is used to determine the types of hydrodynamic stimuli to which the antennule is capable of responding. The distal end of the antennule was fixed in a speaker driven oscillatory flow chamber to stimulate whole antennule movement. We recorded extracellular responses from antennular axons to oscillatory antennule movement over a frequency range of 1–128 Hz in an amplitude range of 16–125 μ m. Responses were recorded from 30 cells and synchronization coefficients were computed for each stimulus presentation. As a population these cells were capable of synchronizing to the stimulus at frequencies between 4 and 64 Hz. Cells with low to no background spiking synchronized best at lower frequencies of 4–16 Hz, while cells with high background spiking synchronized best at higher frequencies between 16 and 64 Hz. This frequency range may have significance in monitoring turbulent plume dynamics.

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Poster: Multimodal Integration

Octopamine-immunoreactive neurons in the olfactory and gustatory centers in the brain of *Manduca sexta*

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Octopamine (OA) is a neuroactive monoamine that is found in the nervous systems of many invertebrates and plays a key role in different aspects of behavior, including associative learning. In this study, we used a monoclonal antibody against OA (gift of H.-J. Agricola) to examine the distribution of OA-immunoreactive (OA-ir) cells in the olfactory and gustatory neuropil centers that mediate olfactory conditioning in the hawkmoth, *Manduca sexta*. OA-ir processes were observed in many regions of the brain with the distinct exception of the upper division of the central body. We found 11 ventral unpaired median (VUM) cell bodies in the subesophageal ganglion that express OA-ir. Two particularly elaborate cells project bilaterally: one interconnects the antennal lobes and the calyces (input region) of the mushroom bodies, and a second cell innervates the gamma-lobe of the mushroom bodies. The branching patterns of *Manduca* VUM neurons are therefore similar, but not identical to those in other insects, including the uniquely identifiable VUMmx1 neuron in the honeybee.

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Poster: Multimodal Integration

Temporal interaction of olfactory and trigeminal processing

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Aim: in everyday life, olfactory and trigeminal sensations hardly ever occur separately. On the contrary, most odors in our environment will elicit excitation of both olfactory receptor cells and trigeminal neurons. However, the temporal resolution of this interaction has not been studied in detail yet, mostly due to technical difficulties. The aim of this study was to analyse the interaction of both systems using intensity estimates following olfactory and trigeminal stimuli with different interstimulus intervals. **Methods:** participants were 11 women and 12 men (age 21–29 years). Trigeminal (50% v/v CO₂) and olfactory (5 p.p.m. H₂S) stimuli were presented at different interstimulus intervals (0, 700, 1200, 2200, 4200 and 8200 ms). One group of subjects received first the olfactory stimulus, followed by a trigeminal stimulation (OT). A second group received the stimuli in reversed order (TO). Intensity estimates were recorded using a visual analogue scale. **Results:** OT resulted in a significant enhancement of perceived intensity of the following trigeminal stimulus for up to 1000 ms. When the stimulation sequence was reversed (TO), perceived intensity of the olfactory stimulus was significantly reduced for up to 2000 ms. **Summary:** with regard to the present experimental conditions, the olfactory and trigeminal systems interact within a time frame of 1–2 s. It appears that trigeminal activation affects the processing of other chemosensory stimuli for a longer period than olfactory excitation.

Poster: Multimodal Integration

PROP taster status versus texture and flavor sensations for low-fat semi-solid foods

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The hypothesis was tested that sensitivity to 6-n-propylthiouracil (PROP) was related to texture and flavor perception, and preference for 16 commercial vanilla custard desserts (mostly starch-based; fat levels between 0.1 and 4%). A group of 175 naive consumers rated the intensity of a PROP stimulus presented on a filter paper using a gLMS. The group was divided into 25% non-tasters (NT, rating <40% of LMS scale), 50% medium tasters (MT, 40–86%) and 25% super tasters (ST, ≥86%). PROP status affected ratings for creaminess, fattiness, thickness, melting, heterogeneity, airiness, stickiness, roughness and preference significantly ($P < 0.001$). Flavor attributes were not affected. Ratings for thickness, fattiness and stickiness increased significantly with PROP sensitivity. Ratings for preference and airiness decreased significantly, whereas the other attributes showed most either highest or lowest ratings for consumers with medium PROP sensitivity. Partial Least Square analysis indicated that creaminess, a complex attribute composed of several other attributes, was related to different attributes for the PROP groups. Creaminess ratings of consumers with low PROP sensitivities were well predicted by a combination of all texture attributes, whereas creaminess ratings of the other consumers were well predicted by fewer texture attributes plus flavor/taste attributes. In general, the largest variations in attribute ratings with PROP status were found for males and for young consumers.

Poster: Multimodal Integration

Effects of *Ginkgo biloba* on chemosensory function

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Diminutions and losses of chemosensory function are common. While diagnostic procedures have advanced markedly, there are few known methods to enhance chemosensory function. *Ginkgo biloba* reportedly enhances cerebral blood flow, neurogenesis in the olfactory epithelium, neurotransmission and cognition. Improved odor recognition has been noted in rats administered the extract. This trial examined the effects of acute and chronic extract administration on taste and smell threshold sensitivity and odor recognition in healthy humans. A randomized, double-blind, placebo-controlled trial was undertaken with 30 individuals meeting strict eligibility criteria including non-use of medications, nutraceuticals or supplements that can affect sensory function. Active treatment consisted of a daily 0.87 mg/kg dose (2.35% *G. biloba* extract standardized to 24% flavanoids and 6% terpenes, %w/w) administered TID for 13 weeks. Forced-choice, staircase thresholds for sucrose, NaCl and butanol and odor identification were measured at weeks 0, 1, 5, 9 and 13 following a standardized meal. No significant treatment or treatment × time interactions were observed for the olfactory indices. Taste thresholds were significantly higher with active treatment over the trial. This trial revealed no beneficial effects of *Ginkgo biloba* on taste or smell in healthy adults. Whether benefits may be realized by individuals with diminished chemosensory function is not known.

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Poster: Multimodal Integration

Influences of antihypertensive and antihyperlipidemic drugs on the senses of taste and smell: an overview

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According to the *Physician's Desk Reference* (PDR), 36% of modern antihypertensive and antihyperlipidemic drugs produce untoward alterations in chemosensory perception. Such disturbances can adversely affect the quality of life, produce noncompliance to medication schedules, and may result in decreased food intake, loss of appetite, weight decrement and depression. This abstract lists the primary antihypertensive and antihyperlipidemic drugs that adversely alter chemosensory function. Among these, angiotensin-converting enzyme (ACE) inhibitors and antihyperlipidemic drugs are the worst offenders, as seven of the 10 (70%) ACE inhibitors and seven of the 10 (70%) antihyperlipidemic drugs included in the PDR are listed as having chemosensory side effects. Of the 15 calcium-channel blockers in the PDR, eight (53%) have been reported to induce taste or smell disturbances. Moreover, chemosensory dysfunctions have also been reported with use of angiotensin II antagonists and diuretics. This abstract also provides information on better defining the nature of the dysfunction, outlines testing strategies and available tests that could be used to better define the prevalence of the dysfunction, and summarizes means for mitigating such alterations.

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Poster: Multimodal Integration

Repeatability and intercorrelations of sensory and cognitive tests in unmedicated elderly subjects

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The purpose of the study was threefold: (i) to determine if age-related losses in various sensory functions are correlated; (ii) to determine if sensory acuity is related to cognitive performance; and (iii) to determine if practice effects occur with repeated testing over a 6 month period. Elderly individuals between 65 and 85 years of age in good health on no medications (other than hormone replacement) were tested on the same battery of cognitive and sensory tests measuring taste, smell, vision, hearing, and touch. There were a total of 71 subjects (29 male, 42 female), with a mean age of 72.6 years and mean length of education of 16 years. Subjects were tested at the following time points: baseline, at 3 weeks, at 2 months and at 6 months. Data for each time point were collected during two 2 h sessions performed within 1 week of each other. The results over the four sessions show considerable variability. Data analysis indicates rather low correlations between tests of each sensory modality and with cognition. Repeated testing of elderly subjects over the 6 month period showed the following trends: (i) self-rated perception of taste and smell ability decreased; (ii) cognitive performance improved slightly; and (iii) olfactory identification and odor mem-

ory scores decreased slightly. These findings suggest that repeated testing is necessary to evaluate accurately some sensory and cognitive functions in the elderly.

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Poster: Multimodal Integration

Hedonic contrast and hedonic discrimination

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Stimuli are rated as less intense when compared with very intense context stimuli than when presented alone or with less intense context stimuli (Frederiksen, 1975), a phenomenon called contrast. In audition, loud contextual sounds engender a reduction in intensity discrimination, perhaps by changing the psychophysical loudness function (Parker *et al.*, 2002). We recently demonstrated contrast with hedonic judgments (Zellner *et al.*, 2003). Here we look for a reduced hedonic discrimination accompanying hedonic contrast.

In Experiment 1 two groups of subjects rated the pleasantness of two diluted fruit drinks (Test drinks). Group 1 drank and rated the Test drinks. Group 2 rated four other full-strength fruit drinks (Context drinks) before the Test drinks. Group 2 rated the Test drinks as less pleasant ($M = -22.22$) than did Group 1 ($M = +8.44$) [$t(32) = 2.63$, $P = 0.013$]. Thus, hedonic contrast occurred. Experiment 2 investigated whether reduced hedonic discrimination accompanies that hedonic contrast. Subjects rated how much more they liked the preferred Test drink than the other one. On the 10-point rating scale, 1 meant 'slightly more' and 10 meant 'very much more'. Group 1 drank only the diluted Test drinks and rated their difference in likeability. Group 2 drank and rated them after exposure to the four full-strength Context drinks. Group 2 rated the two Test drinks as hedonically less different ($M = 1.31$) than did Group 1 ($M = 3.63$) [$t(30) = 2.69$, $P = 0.012$]. These results demonstrate reduced hedonic discrimination accompanying hedonic contrast, paralleling phenomena seen in studies of loudness. Perhaps the psychophysical growth of pleasantness is malleable.

Poster: Multimodal Integration

Memantine and mecamylamine alter nicotine perception in man

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NMDA receptors are present at different regions of the olfactory system and NMDA-knockout mice were profoundly impaired in olfactory discrimination. Memantine, a non-competitive, selective NMDA antagonist with a voltage-dependent, fast unblocking kinetic can be used in man. Neuronal nicotinic acetylcholine receptors (nAChRs) are present at the nasal mucosa. Mecamylamine, an acetylcholine receptor antagonist, blocked painful responses to nicotine following chemical stimulation of the tongue. In the present

study we investigated (i) the influence of Memantine (oral application) and of (ii) mecamlamine (local application) on nicotine perception using (i) a placebo-controlled double-blind parallel group and (ii) a twofold crossover design. Nicotine perception was studied under Memantine/placebo (steady-state condition, $n = 15$) and under Mecamlamine/placebo (following local application, $n = 15$) following chemical stimulation of the nasal cavity with olfactory and trigeminal stimulus concentrations. Memantine significantly reduced the olfactory intensity estimates of S(-)-nicotine and Mecamlamine significantly reduced trigeminal intensity estimates of nicotine enantiomers. Thus, acetylcholine receptor- and NMDA antagonists are effective tools to influence nicotine perception in man acting with different specificity.

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Poster: Multimodal Integration

Multiple short-term effects of environmental tobacco smoke and propionic acid

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As part of a research program to better understand short-term responses of humans to environmental chemical exposures, we are measuring multiple responses/impacts from 20 normal (no evidence of chemosensory deficits or of chemical hypersensitivity) participants (Ps) in a previously described (AChemS 2003) 10 m³ environmental chamber. Ps are tested in pairs and are given one 100 min test session with each of eight stimulus conditions. By varying the proportion of total chamber flow (fixed at 1000 l/min) derived from an antechamber in which one or two smokers puff in response to signal lights, we approximate three target concentrations of environmental tobacco smoke (ETS): 15, 100 and 800 mg/m³ of ETS-attributable respirable suspended particulate. As a control, smokers puff on unlit cigarettes. Four concentrations (0, 1, 10, 15 p.p.m.) of propionic acid (PA) are generated by varying the proportion of chamber flow passed over liquid PA held in glass saturators. Except with 15 p.p.m. PA (where the plateau is held for minutes 20–30), concentrations of PA and ETS rise during minutes 11–20 and then are held through minute 70, when an exponential decline begins. Sensory/symptom, breathing, eye-blink rate, psychological state and information processing speed responses/impacts are measured at various times over the course of the session. As a first step toward an improved understanding of the effects of various environmental contaminants on short-term responses in humans, our analyses evaluate stimulus type, concentration and time as determinants of the magnitude of the change in each endpoint.

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Poster: Taste: Umami

Comparison of the tastes of MSG and L-alanine

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Monosodium glutamate (MSG) is an amino acid that occurs naturally in protein rich foods such as meat and dairy products. Chaurdhari *et al.* (1996) showed that MSG activates taste-mGluR4 receptors. Nelson *et al.* (2002) found that the receptor T1R1/T1R3 responds to many amino acids including MSG, but not to stimuli perceptually sweet to humans. Alternatively, the T1R2/T1R3 receptor responds to sugars and artificial sweeteners but not to umami substances. Nevertheless, conditioned taste aversion (CTA) studies report that rats perceive similarities in taste between MSG and several sweet substances when the sodium taste is reduced by amiloride, a sodium channel blocker (Heyer *et al.*, 2003). Several L-amino acids, such as L-alanine (ALA), elicit a variety of taste qualities including 'sweet' in humans (Schiffman *et al.*, 1981). Two questions were addressed in this study: do ALA and MSG share taste qualities, and, if so, can rats distinguish between the two substances? CTA experiments were done with water-deprived rats conditioned to either ALA or MSG, and then tested against three concentrations of the opposite stimulus, the conditioned stimulus and control substances. The results revealed a strong perceptual similarity between MSG and ALA. To assess the strength of similarities in these tastes, discrimination experiments were also conducted. Rats can discriminate between the two substances but the discrimination is significantly difficult when amiloride or amiloride + NaCl is added to control for the taste of Na⁺ in MSG. These results suggest that MSG and ALA may share similar but not identical afferent signaling.

Poster: Taste: Umami

L-Serine, glycine, and MSG tastes in rats: generalization of conditioned taste aversion

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Recent molecular studies suggest that L-amino acids and substances that elicit an 'umami' taste may be detected by T1R1/T1R3 heterodimeric receptors in taste receptor cells, whereas 'sweet' substances are detected by T1R2/T1R3 receptors. Monosodium glutamate (MSG) is a naturally occurring amino acid found in most protein-rich foods such as meats, dairy products and some vegetables, and is a prototypical substance that elicits an 'umami' taste. In humans, L-amino acids can elicit quite different tastes (Schiffman *et al.*, 1981). However, other than preference measures there is little behavioral research on the taste qualities of amino acids in non-human mammalian species. In this study, conditioned taste aversion (CTA) methods were used to see if aversions to L-serine and glycine, both common and naturally occurring amino acids, would cross-generalize to L-MSG in short duration, reactive tests. In contrast to other CTA studies, amiloride was present to reduce the taste of the sodium ion. A lithium chloride-induced aversion to 100 mM MSG generalized to L-serine and glycine at concentrations as low as 10 mM when amiloride was present in all solutions. Similar generalization to MSG was detected when the rats were conditioned with L-serine and glycine. These results suggest that MSG shares taste qualities with L-serine and glycine, possibly through common afferent signaling mechanisms.

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Poster: Taste: Umami**Polymorphisms of known monosodium glutamate taste receptors and bimodal distribution of sensitivity to glutamate savory taste**

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Monosodium glutamate (MSG) elicits a unique taste quality, often labeled savory, brothy or umami. This quality is thought to underlie the protein and amino acid tastes, which convey a preferred flavor in many foods. But a small subset of the population (~6%) is insensitive to this taste quality. Subjects were screened for their ability to distinguish 29 mM NaCl versus 29 mM MSG in a forced-choice triangle test. The few subjects who could not discriminate these stimuli also demonstrated an insensitivity to glutamate on a concentration–intensity scaling task. Furthermore, the synergy of savory taste that occurs for most subjects when MSG is mixed with 5′ ribonucleotides, such as GMP or IMP, is lacking in selected individuals and can occur despite normal sensitivity to MSG savoriness. Therefore, there are semi-independent mechanisms underlying sensitivity to MSG and its interactions with IMP and GMP. At present, there are two putative savory taste metabotropic receptors identified: human taste GRM4 [6p21.3] and the receptor dimer human TAS1R1-TAS1R3 [1p36.23; 1p36.33]. Taste GRM4 (an orally located splice variant of the CNS GRM4, the group III inhibitory glutamate receptor) was sequenced and several identified exonic polymorphisms were not correlated with savory taster status. The common polymorphism in TAS1R3 (A5T) was also not correlated with savory taster status. TAS1R1 contains eight less common polymorphisms, including a nonsense mutation, comprising several haplotypes in our subjects. TAS1R1 also has four splice variants. These studies will help identify the receptors responsible for this quality of taste in humans.

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Poster: Taste: Umami**Glutamate- and inosine-induced calcium responses in mouse taste receptor cells**Y. Maruyama¹, N. Chaudhari² and S.D. Roper²

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L-Glutamate (L-Glu) is a naturally occurring amino acid in protein-rich food and elicits umami taste. Umami taste is synergistically enhanced by nucleotides, including inosine 5′-monophosphate (IMP). Hypotheses proposed to explain nucleotide synergy include: (i) separate receptors for L-Glu and nucleotide are expressed in distinct cells and integration occurs at a subsequent stage, e.g. post-synaptically; (ii) each receptor is expressed in the same taste receptor cells; and (iii) L-Glu and nucleotide interact with the same receptor. To examine whether the same taste cells respond to L-Glu and IMP, we measured intracellular Ca²⁺ responses to L-Glu, IMP and a mixture of both tastants in gustatory sensory cells loaded with

Calcium Green-1 dextran and imaged in slices of circumvallate papillae with confocal microscopy. Monopotassium glutamate (MPG, 500 mM) alone, as well as IMP (1 mM) alone, applied focally to taste pores, induced transient [Ca²⁺]_i responses in some taste receptor cells. To date, MPG-responsive cells have also responded to IMP, suggesting that glutamate and nucleotides act on the same receptor. Alternatively, the cognate receptors are expressed in the same cell and the receptor(s) do not obligatorily require a mixture of IMP and glutamate. Mixtures of MPG and IMP elicited ~3-fold larger responses than the summation of individual responses to MPG and IMP alone. These data indicate that nucleotide synergy of glutamate taste is detectable at the level of Ca²⁺ signals in individual taste cells.

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Poster: Taste: Umami**Behavioral taste responses to monosodium glutamate and sodium chloride in four species of nonhuman primates**L. Hernandez Salazar¹ and M. Laska²

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Taste responses of six squirrel monkeys, five pigtail macaques, four olive baboons and four spider monkeys to monosodium glutamate (MSG) and to sodium chloride were assessed in two-bottle preference tests of brief duration (2 min). When given the choice between tap water and defined concentrations of the two tastants dissolved in tap water, the animals were found to significantly discriminate concentrations of MSG as low as 2 mM (spider monkeys and olive baboons), 50 mM (pigtail macaques) and 300 mM (squirrel monkeys) from the solvent. With sodium chloride, taste preference thresholds were found to be 1 mM (spider monkeys), 20 mM (pigtail macaques), 50 mM (olive baboons) and 200 mM (squirrel monkeys), respectively. Across-species comparisons of the degree of preference for MSG and sodium chloride displayed by the four primate species showed the same order of spider monkeys > olive baboons > pigtail macaques > squirrel monkeys. When presented with equimolar concentrations of different tastants, all four species preferred sucrose as well as a mixture of sucrose and sodium chloride over MSG, and—at least at one concentration—they preferred MSG over sodium chloride. The results support the assumption that the taste responses of the four primate species to MSG and sodium chloride might reflect an evolutionary adaptation to their respective dietary habits.

Poster: Human Olfactory Performance**Source memory for odors and objects in children and young adults**E. Pirogovsky¹, P. Gilbert², S. Addie¹, K. Stull¹, T. Ricardo¹, E. Viera¹ and C. Murphy¹

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Recall and recognition memory for odors is poorer in children than in adolescents. In addition, children perform worse than young adults in source memory tasks using visual and auditory stimuli. However, source memory for odor stimuli has not been examined in children. The present study investigated source and item memory for odors and objects in children (7–10 years) and young adults (18–24 years). During the study phase, one male and one female experimenter (sources) randomly presented either 16 odors or 16 objects to the participant. Presentation alternated between sources so that each source presented eight stimuli. Once the 16 stimuli were presented, the sources exited and a third experimenter began the test phase. To assess item recognition memory, a stimulus from the study phase and a novel stimulus were presented to the participant who was asked to choose the stimulus presented in the study phase. Source memory was assessed with the eight stimuli not used in the item memory task. The experimenter presented a stimulus and asked whether the male or female experimenter had previously presented the stimulus. Results indicate that for objects, source and item memory were similar for children and adults. For odors, item memory was similar for the two groups; however, source memory for odors was significantly poorer in children than in young adults. It has been suggested that the frontal lobes play a critical role in source memory and odor memory and that this brain region continues to develop into adolescence. Children's poor performance on the source memory task for odors may be due in part to the immaturity of the frontal lobes.

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Poster: Human Olfactory Performance

Age-related changes in source and item memory for odors and objects

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Source memory is more affected by aging than item memory. However, little research has investigated age-related changes in source and item memory for olfactory stimuli. The present study compared source and item memory for odors and objects in healthy elderly and young adults. During the study phase, either 16 odors or 16 objects were randomly presented by a male and a female experimenter (sources) to the participant. Presentation alternated between sources so that eight stimuli were presented by each source. Once the stimuli were presented, the test phase was conducted by a third experimenter. To assess source memory, a stimulus from the study phase was presented to the participant who was asked to indicate whether the stimulus was presented by the male or female experimenter. To assess item recognition memory, a stimulus from the study phase and a novel stimulus were presented and the participant was asked to indicate which stimulus was presented during the study phase. Source and item memory for odors and objects were similar in young adults. In elderly adults, source memory was impaired relative to item memory for odors and objects. However, the impairment in source memory relative to item memory for odors was significantly greater than for objects. The data suggest that source memory for olfactory stimuli may be particularly sensitive to age-related changes in the brain.

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Poster: Human Olfactory Performance

Age dependent odour memory and odour identification: differential effects of gender

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Objective: to investigate the decline of odour memory and odour identification with age. Method: 10 different well-known food odours were presented in a 4AFC identification task to 279 subjects (78 males) younger and 229 subjects (73 males) older than 50 years of age. Half of the subjects in each age group were cued to remember three of the 10 odours, whereas the other half were not informed about the following memory task. Memory for the same target odours in the two groups were tested with a 3AFC procedure, where each target odour was to be detected among two distractor odours. Results: Mann–Whitney tests of overall memory find no significant differences between males and females in the groups below and above 50 years of age. If subjects are divided by the age of 60 (202 subjects older than 60, 68 males) elderly women outperform elderly men significantly ($P < 0.035$), whereas there is no gender difference in the group younger than 60 years of age. Dividing subjects by the age of 65 (179 subjects, 60 males) again demonstrates superior odour memory by women ($P < 0.046$). ANOVAs of the identification data find no significant difference between men and women younger than 50 years of age, whereas women in the group older than 50 outperform men significantly ($P < 0.009$). We find a similar significant gender difference when subjects are divided by age 60 and 65. Conclusion: despite having provided subjects the opportunity of coding odours semantically, the differential gender effects on odour memory and odour identification provide further support for the hypothesis that odour memory is not a semantically based memory system.

Poster: Human Olfactory Performance

Memory for emotionally arousing odors: subjective ratings and autonomic responses

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Memory for odors is more emotional and lasts longer than memory for other types of sensory information. It is also accepted that emotionally arousing events are remembered better than neutral ones. Surprisingly, this memory enhancing effect of emotional arousal has never been investigated with odors. Here, we examined whether emotionally arousing odors are remembered better than non-emotionally arousing ones. We measured subjective emotional reactions, skin conductance and heart rate variation during an incidental odor memory task. At encoding, subjects smelled 21 odors twice; first while skin conductance and heart rate were recorded, and second while they rated the odors for intensity, pleasantness, familiarity and degree to which the odors were emotionally arousing. A week later they received an unexpected yes–no memory test (21 targets,

21 foils), during which skin conductance and heart rate were again recorded. We found that memory was superior for emotionally arousing unpleasant odors than for emotionally arousing pleasant or non-arousing odors [$F(2,34) = 3.45, P = 0.04$]. Moreover, skin conductance variation was greater for unpleasant than for pleasant or non-arousing odors [$F(1,17) = 4.51, P = 0.03$]. These results demonstrate, for the first time, that emotional and autonomic arousals modulate odor memory strength. Since similar results have been obtained with visual and verbal stimuli, our findings suggest that the enhancing effect of emotional arousal on memory is not modality specific.

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Poster: Human Olfactory Performance

Subjective and objective assessments of odor-influenced memory

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This study of odor-influenced memory investigated (i) recent versus childhood memories and (ii) objective measures, including number of emphasis, cognitive and emotion words, word count and grade level, versus subjective measures, including self-reports of the emotionality, vividness of the memories, and frequency of recall. One hundred and ten participants ($n = 55$ men, 55 women) were exposed to either a control (no odor) or an ambient odor (one of three childhood, three environmental or three floral odors) while writing childhood and recent memories. All odors increased the number of emphasis words used in both memory types for both genders; women used more emphasis words [$F(1,102) = 7.06, P < 0.009$]. The floral odors increased emotion words in recent memories for both genders [$F(3,102) = 3.46, P < 0.019$]. Men's childhood memories [$F(1,102) = 3.83, P < 0.05$] and women's recent memories [$F(1,102) = 4.08, P < 0.046$] contained more emotion words. Childhood odors decreased grade level for men for recent memories compared with women; environmental odors decreased grade level for women for recent memories compared with men [interaction: $F(3,102) = 3.39, P < 0.02$]. Overall, ambient odor did not influence subjective ratings. However, women reported recalling both childhood [$F(1,102) = 8.53, P < 0.004$] and recent [$F(1,102) = 4.42, P < 0.038$] memories more frequently. Thus, objective measures are well suited for studying the effects of odors and odor/gender interactions on memory. Both recent and childhood memories reveal odor effects.

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Poster: Human Olfactory Performance

Odors and memories: the Proust phenomenon revisited

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The Proust phenomenon refers to the common belief that odor cues elicit vivid and emotional childhood memories. To test the validity of

this phenomenon, we investigated the effects of odors alone, in the absence of semantic or visual cues, on autobiographic memory. One hundred and ten participants ($n = 55$ men, 55 women) were exposed to either a control (no odor) or an ambient odor (one of three childhood, three environmental or three floral odors) while writing childhood and recent memories. ANOVAs compared emotion words, sensory references and emphatic language across odor categories. We found no evidence for a Proust phenomenon. There was no significant difference in emotion, sensory qualities or emphatic language in childhood memories as a function of odor condition. When odor effects were obtained, they were effects of floral odors on recent memories, not childhood memories (all reported effects, $P < 0.05$). Recent memories included more positive emotion words in the floral odor condition as compared with all other conditions. Women reported less negative emotion in the floral condition for recent memories and men reported more sensory qualities in the floral odor condition. Thus, (i) childhood odors had no special effect on childhood memories; (ii) there was no overall odor effect on childhood memories; and (iii) odors did not affect childhood memories more than recent memories. In contrast, recent memories were more affected by odor than childhood memories.

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Poster: Human Olfactory Performance

Odor categorization in the absence or in the presence of odor names

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In order to study the top-down influence of names on odor categorization, we compared situations allowing perceptual categorization which is supposed to be mainly driven by stimulus properties, with semantic categorization supposed to be driven by our knowledge. Two groups of 20 subjects were asked to form categories among 16 odors according to their similarity: group 1 was provided odor vials without any information on the name, group 2 was provided the same odor vials labeled with a name. A third group categorized only the labels, without olfactory stimuli, so relying on their memory to judge similarity. After the categorization task, subjects evaluated the 16 odors again on intensity, pleasantness, familiarity and typicality. We stated the hypothesis that when judging real odors, the main dimension for odor categorization would be the hedonic valence, and that valence would no longer dominate when categorizing from memory. The results confirmed the hypothesis: hedonic ratings were correlated with the main dimension for groups 1 and 2, not for group 3. Results also show that the presence of names reduces the number of categories and that these categories tend to be hierarchically organized, which is not the case for the group 1.

Poster: Human Olfactory Performance

Can your nose shine an attentional spotlight?

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Directed spatial attention in vision and audition enables heightened sensory acuity for events at known spatial locations. We asked whether a similar phenomenon exists in chemical sensing. Initially, we asked if humans have access to spatial information through chemosensation. We tested 33 subjects on a 16 trial left versus right localization task. The odorants rose and strawberry (diluted 1:4 into propionic acid) were generated by an air dilution olfactometer, and delivered via a divided nasal mask with separate left and right entry ports. As a group, subjects were slightly but significantly above chance in their ability to spatially localize the odorants (accuracy = $57.8 \pm 2.7\%$, $t = 2.841$, $P < 0.01$). This group effect was carried by five subjects. To ask whether these subjects have directed spatial attention in chemosensation, we compared their odorant recognition accuracy and latency under two conditions: (i) cued trials where a pre-trial monitor arrow indicated the side (left versus right) from which the odor was presented; and (ii) uncued trials where no spatial cue was provided. In contrast to our expectation, we did not find a significant increase in accuracy (accuracy increase = 5% , $t = 0.711$, $P = 0.51$) or decrease in reaction time on trials preceded by a spatial cue (latency decrease = -169 ms, $t = 0.447$, $P = 0.677$). This null result may reflect one of two alternatives: (i) that spatial attention does not exist in olfaction; or (ii) that our experimental design failed to detect the attentional modulation that was there. To address the latter, we are repeating the experiment using modified temporal task parameters.

Poster: Human Olfactory Performance

Intensity of retronasal and orthonasal odorants: time-intensity tracking

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Using odorant presentation containers (OPC; Pierce and Halpern, 1996, *Chem. Senses* 21: 529–543), subjects each selected concentrations of odorants (alcohol-free food-grade liquid extracts of plant materials) for which air-phase orthonasal (ortho) or retronasal (retro) intensities matched ortho or retro air-phase intensity of a standard odorant. In a later session, subjects used a computer mouse to indicate smelled intensity (I) over time on a computer display of a 11 cm vertical line, viewed from ~46 cm, labeled 'Minimum Smell Intensity' at the lower end and 'Maximum Smell Intensity' at the upper end, in response to OPC-presented randomized air-phase ortho or retro odorants, or ortho and retro odorant pairs. Trials, 30 s in duration, started with the mouse cursor at 'Minimum Smell Intensity' when a noseclip was removed. Natural breathing was done; sniffing was not permitted. For odorant pairs, subjects either initially exhaled (retro 1st) or inhaled (ortho 1st). Results: peppermint odorant: ortho I-reaction time (I-RT) = 3.93 ± 1.09 s (median \pm SIR), time to maximum I (t -max) = 5.67 ± 7.98 s, final I = $99 \pm 5.5\%$ of max-I; retro, I-RT = 4.35 ± 1.6 s, t -max = 7.87 ± 7.55 s, final I = $100 \pm 5.75\%$ of max-I. Strawberry odorant: ortho I-RT = 3.49 ± 0.89 s, t -max = 6.49 ± 0.933 s, final I = $99 \pm 4.75\%$ of max-I; retro, I-RT = 4.19 ± 1.16 s, t -max = 11.87 ± 7.7 s, final I = $96 \pm 4.88\%$ of max-I. High t -max SIR indicates large individual differences.

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Poster: Human Olfactory Performance

Discriminating deuterated from undeuterated acetophenone: comparing humans and a dog

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To determine if deuterated compounds can be distinguished from their undeuterated counterparts through smell, we set out to replicate a previous report: 31 subjects performed a one-trial same/different discrimination on two jars containing deuterated (D) and undeuterated (H) acetophenone. Twenty-four subjects correctly chose different. However, when performing the same task using two identical samples of H, 23 subjects erroneously chose different. Thus, a bias existed toward answering different. To avoid the bias, we used an olfactometer to test 38 subjects in a 32 trial same/different forced choice design. Mean accuracy was not different from chance ($52.5 \pm 3.5\%$, $P > 0.1$). To eliminate possible habituation effects, we repeated the task in 14 subjects with only eight trials. Mean accuracy was not different from chance ($60 \pm 23\%$, $P > 0.1$). To test a different paradigm, an additional 10 subjects performed a three-alternative forced choice identification task. Mean accuracy was not different from chance ($38.5 \pm 22\%$, $P > 0.1$). Humans thus far unsuccessful, we set out to see if a dog could discriminate H from D. A German shepherd dog was trained to recognize H. He then did seven trials of a three-alternative forced-choice identification task at 100% accuracy ($P < 0.001$). To address the concern that the dog was making an intensity rather than identity judgement, we conducted an additional 12 trials of the same task, using different intensities of the target and distracters. The dog was at 100% accuracy ($P < 0.0001$). Thus we conclude dogs can distinguish deuterated from undeuterated acetophenone ($P < 0.0001$).

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Poster: Human Olfactory Performance

Restrained eaters show smaller N1P3 amplitudes and suppression of attention to chocolate odor

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The present study examined sensory and cognitive information processing of restrained and unrestrained eaters using the olfactory event-related potential (OERP) technique. Seventeen restrained eaters and 18 unrestrained eaters participated. OERPs were recorded to a food and a non-food odor, which were presented in an air stream that was presented through an olfactometer. Each participant completed four conditions, two with a food odor (chocolate) and two with a non-food odor (geraniol). For each odor, participants completed an attended condition where they paid attention to the odor presented and an ignored condition where they performed a distraction task. OERP amplitudes and latencies

were analyzed with an ANOVA, with food restraint (restrained, unrestrained) as a between-subject factor and attention (attended, ignored), odor (food, non-food) and electrode site as within-subject factors. N1P3 amplitude to the food odor in the attended condition was larger in unrestrained eaters. Restrained eaters did not show differences in ERP amplitudes regardless of their attentional allocation, suggesting that they paid as much attention in the ignored condition as they did in the attended condition. The data suggest that restrained eaters were able to suppress attention to the food odor. The difference in the N1P2 inter-peak amplitude of OERPs suggests the possibility of altered sensory processing of food odor between restrained and unrestrained eaters. The results suggest that differences in sensory and cognitive information processing of food-related odors exist between restrained and unrestrained eaters.

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Poster: Human Olfactory Performance

Mixture segmentation: are two nostrils better than one?

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In his model of olfactory processing, Hopfield (1999) described how a system that compared population responses across multiple sniffs could successfully separate the components of a binary odor mixture. Given that humans have two nostrils that maintain different flow rates, we hypothesized that humans would be able to perform this separation with only one sniff by comparing odor information simultaneously across two nostrils. To address this, nine subjects were presented 10 different mixtures three times under each of three conditions—two nostrils, one sniff (2N1S); two nostrils, two sniffs (2N2S); or one nostril, two sniffs (1N2S)—for a total of 90 presentations. In agreement with our hypothesis, we found that two nostrils were better than one [1N2S mean = 9.00 ± 3.87 , 2N2S mean = 11.00 ± 2.12 , $t(8) = -3.06$, $P < 0.016$]. In addition, although recent work in rats shows that only one sniff was needed to perform complex mixture discrimination (Uchida and Mainen, 2003), our findings indicated that two sniffs were better than one at mixture component discrimination [2N1S mean = 8.88 ± 2.55 , 2N2S mean = 11.00 ± 2.12 , $t(8) = -2.60$, $P < 0.032$].

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