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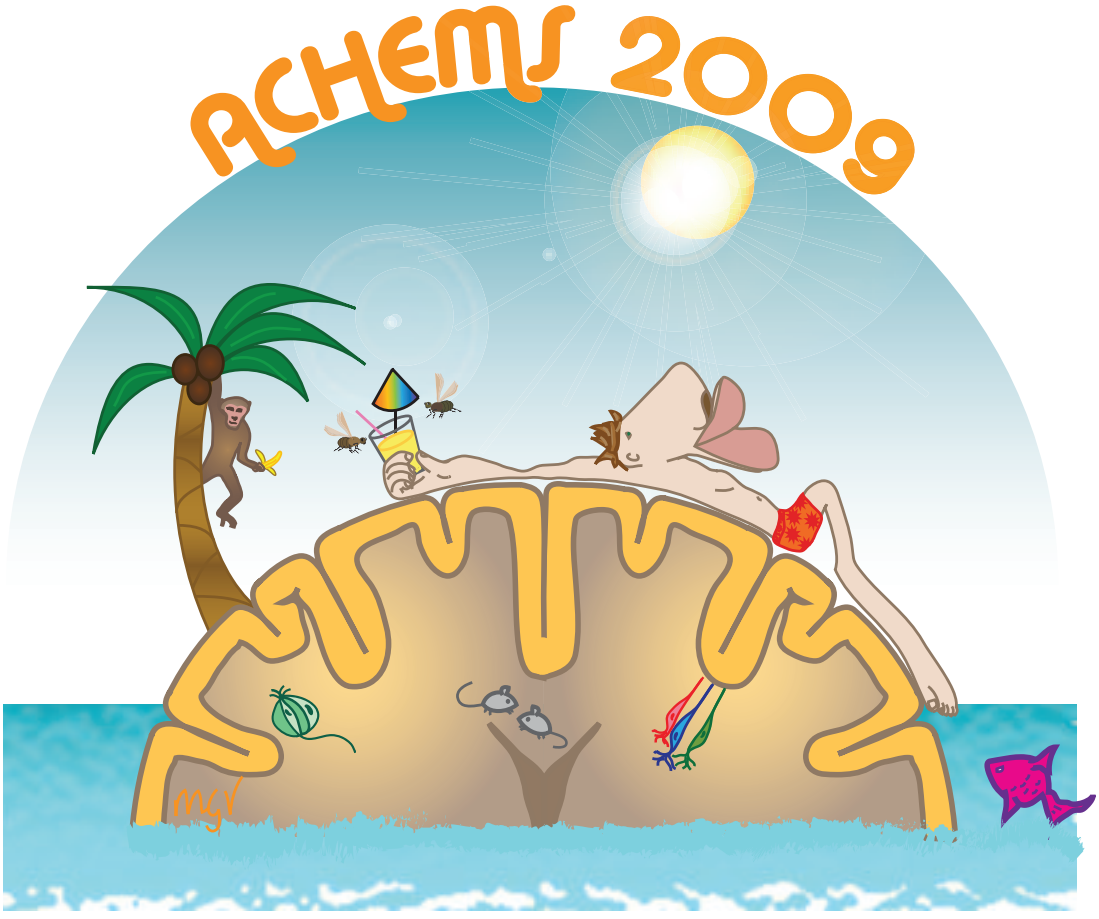
ACHEMS 2009 ANNUAL MEETING

ABSTRACTS



31st Annual Meeting April 22-26
Sarasota, Florida

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2009 Awardees

31st Annual Givaudan Lectureship - Givaudan Corporation
Carla J. Shatz, Stanford University, Stanford, CA

18th Annual Moskowitz Jacobs Award for Research in Psychophysics of Taste and Olfaction
Johan Lundström, Monell Chemical Senses Center

16th Annual Ajinomoto Award to Promising Young Researcher in the Field of Gustation
Alan Carleton, Brain Mind Institute, Ecole Polytechnique, Fédérale de Lausanne

International Flavors and Fragrances Award for Outstanding Research on the Molecular Basis of Taste
Keiko Abe, The University of Tokyo

Max Mozell Award for Outstanding Achievement in the Chemical Senses
Charles Greer, Yale University School of Medicine

The AChemS Young Investigator Award for Research in Olfaction
Nathaniel Urban, Carnegie Mellon University

AChemS Distinguished Service Award
Barry Davis, National Institute of Health

The Don Tucker Memorial Award (2008 Awardee)
Aaron Beyerlein, University of Arizona

AChemS 2009 Logo Contest Award
Maria Veldhuizen, John B. Pierce Laboratory and Yale University School of Medicine

The Polak awards are funded by the Elsje Werner-Polak Memorial Fund in memory of our niece gassed by the Nazis in 1944 at age 7: Ghislaine Polak and the late Ernest Polak
2009 Polak Young Investigator Award Recipients:
Wen Li, University of Wisconsin-Madison
Nathalie Mandairon, Lyon University
Ivan Manzini, University of Göttingen
Koichi Matsumara, Monell Chemical Senses Center
Arie Mobley, Department of Neurosurgery, Department of Neurobiology, Yale University
Sharif Taha, University of Utah School of Medicine
Maria Veldhuizen, John B. Pierce Laboratory and Yale University School of Medicine

We are pleased to announce that five 2009 Polak Junior Scientist Travel Awards were given for this year's meeting.

AChemS Minority/Travel Fellowship Recipients
Funded by a generous grant from the National Institute on Deafness and Other Communication Disorders and the National Institute on Aging, NIH
Juan Aggio, Georgia State University
C. Shawn Dotson, University of Maryland School of Medicine
Wombura Fobbs, John B. Pierce Laboratory and Yale University School of Medicine
Kristina Gonzalez, Clark University
Ernesto Salcedo, University of Colorado Denver
Nhat-Tuan Tran, University of Colorado Denver
Robert Utsman, University of Minnesota

AChemS Student Housing and Travel Award Recipients
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Allana Goodman	Amanda Elson	Kristin Rudenga
Krystin Corby	Sebastian Rasche	Julie Boyle
Faye Pesenti	Amy Gordon	Markus Rothermel
Anna Kleeman	Lian Gelis	Sabrina Baumgart
April Glatt	Matthias Luebbert	Silke Hagendorf
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MEETING EVALUATION

The meeting evaluation is available online this year. Please visit www.achems.org after the meeting to give us your feedback on the meeting. A reminder email will be sent. Your input helps AChemS' leadership continue to offer quality annual meetings and member services.

#1 **Givaudan Lecture**

Tuning up Circuits: Brain Waves, Immune Genes and Synapse Plasticity

Carla J. Shatz
Stanford University Stanford, CA, USA

Connections in adult brain are precise, but they do not start out that way. Precision emerges in development as synaptic connections remodel, a process requiring neural activity and involving regression of some synapses and strengthening and stabilization of others. Neural activity also regulates neuronal genes; in an unbiased PCR-based differential screen, we made the unexpected discovery that MHC Class I genes are expressed in neurons and are upregulated by neural activity and visual experience (Corriveau et al, 1998; Goddard et al, 2007). To assess requirements for MHCI in CNS, mice lacking expression of MHCI were examined. Synapse regression in the developing visual system did not occur and in adult hippocampus synaptic strengthening was greater than normal (Huh et al, 2000), suggesting that MHCI may function in synaptic plasticity. Receptors for neuronal MHCI could carry out these activity-dependent synaptic processes. In a systematic search, PirB, an innate immune receptor, was found to be highly expressed in CNS neurons. Mutant mice lacking PirB function have increased plasticity in visual cortex (Syken et al., 2006), as well as increased synaptic strengthening (LTP) in hippocampus. Thus PirB, like MHCI, appears to function as a "brake" on synaptic plasticity in the CNS. Together, results imply that this family of molecules, thought previously to function only in the immune system, may also act at neuronal synapses to limit how much- or perhaps how quickly- synapse strength changes in response to new experience. These molecules may be crucial for controlling circuit excitability and stability in developing as well as adult brain. Supported by NIH Grants EY02858, MH071666, Mathers Charitable Foundation, Dana Foundation.

#2 **Gustation**

Wnt/ß-catenin Signaling Controls Taste Bud Regeneration in Mice

Linda A. Barlow[1], Fei Liu[2], Shoba Thirumangalathu[1], Elizabeth A. Harvey[1], Ping Wu[1], Sarah E. Millar[3]
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Taste buds are renewed throughout life such that each of the ~3 cell types is continually replenished. However, the molecular mechanisms controlling this process are not understood. Because Wnt/ß-catenin signaling is a key regulator of embryonic taste bud development (Liu et al. 2007; Iwatsuki et al. 2007) we tested the role of this pathway in adult taste cell turnover. Using several independent mouse lines that report Wnt/ß-catenin signal, we discovered that many cells within fungiform and circumvallate taste buds are Wnt-responsive. Double immunostaining for several differentiated taste cell markers revealed that virtually all Wnt activation in fungiform taste buds occurs in NTPDase2-IR type I cells, while in circumvallate papilla, type I and serotonin-IR type III cells are also Wnt-responsive. To determine the functions of Wnt/ß-catenin in taste buds, we used tetracycline inducible systems to either force activation of Wnt/ß-catenin (ac-ßcat) or ectopically express Dkk1, a secreted Wnt inhibitor, in the lingual epithelium of adult mice. After a 2 week drug treatment to induce the transgene, the tongue surface of ac-ßcat mice appeared pebbled, and in sections, both fungiform papilla size and the number of taste bud-like cell clusters within each papilla were increased. Using taste cell type specific immunomarkers, we found that the excess taste cell clusters comprise only type I cells, with no increase in types II or III. By contrast, all 3 cell types were affected in taste buds of Dkk1 overexpressing mice; types II and III were completely lost, and only a few abnormal type I cells remained. We now hypothesize that Wnt signaling is sufficient to drive type I cell differentiation and that Wnt-supported type I cells are required for maintenance of type II and III cells.

#3 **Gustation**

Fatty acids induce increases in intracellular calcium in Type II and a subset of Type III mouse taste cells

Pin Liu, Bhavik Shah, Hala Hadawar, Timothy Gilbertson
Department of Biology and The Center for Advanced Nutrition, Utah State University Logan, UT, USA

Obesity has been shown to be driven, as least in part, by increases in dietary fat intake. Thus, it is important to understand the underlying mechanisms that our body uses to recognize dietary fat. In recent years, a number of studies have demonstrated the ability of components in fats, specifically free fatty acids (FFAs), to activate taste bud cells (TBCs) and elicit behavioral responses consistent with there being a taste of fat. Recently, we have used fura-2 based calcium imaging to explore the ability of FFAs to elicit intracellular calcium ($[Ca^{2+}]_i$) rise in isolated C57BL/6 TBCs. Our data show that FFAs (1-100 µM) elicit robust increases in $[Ca^{2+}]_i$ in approximately 20% of circumvallate TBCs, and 35% of fungiform TBCs. To identify the subtype of FA-responsive TBCs, we have used responses to high KCl solutions as an indicator of type III (presynaptic) cells and responses to a tastant mixture (sweet/bitter/umami) to identify type II (taste receptor) cells. FFAs are primarily able to elicit intracellular Ca^{2+} responses in cells that correspond to Type II cells, however, a small but significant subpopulation of Type III cells also respond to FFAs. Surprisingly, a small number of TBCs responded to all the solutions tested, including high KCl, the tastant mixture and FFAs. This finding questions the utility of being able to use these solutions to identify unequivocally subtypes of cells within the taste bud. Transgenic mice expressing enhanced green fluorescent protein (GFP) under control of the PLCbeta2 promoter (PLCbeta2–GFP) were also used to verify the data showing FFA responsiveness occurs primarily in Type II cells. A model for the transduction of FFAs in TBCs consistent with these findings and our previous data will be presented.

#4 **Gustation**

GPR40 knockout mice have diminished taste responses to fatty acids

Sami Damak[1], Cristina Cartoni[1], Keiko Yasumatsu[2], Johannes le Coutre[1], Yuzo Ninomiya[2]
[1]Nestlé Research Center Lausanne, Switzerland, [2]Kyushu University Fukuoka, Japan

The perception of dietary fat is based mainly on texture, viscosity and olfaction, but there is increasing evidence that the taste system also plays a role. Triglycerides, the main components of oils and other dietary fats are hydrolyzed by the lingual lipase into free fatty acids, which are detected by the gustatory system. GPR40 and GPR120 are G-protein coupled receptors (GPCRs) that respond to medium and long chain fatty acids. By immunohistochemistry, we showed that both GPCRs are expressed in mouse taste cells. GPR120 is expressed in circumvallate, foliate and fungiform papillae, mainly in type II cells. GPR40 is expressed in circumvallate and foliate but very rarely in fungiform papillae, mainly in type I cells. To determine if GPR40 plays a role in fat taste signal transduction, we carried out behavioral and nerve recording studies on GPR40 knockout mice. Compared with control mice, GPR40 knockout mice have diminished preference and intake for intralipid, oleic acid, linoleic acid and lauric acid. Glossopharyngeal whole nerve recordings showed a diminished response to lingual application of lauric acid, oleic acid, linoleic acid, linolenic acid and DHA. The GPR40 knockouts showed residual behavioral and electrophysiological responses to fatty acids and oils. These data show that GPR40 is a fat taste receptor, but other receptors and/or pathways must exist. The residual response of GPR40 knockout mice to lipids may be accounted for by other signaling molecules such as CD36, DRK or GPR120.

#5 **Gustation**

Thermal taste: association with perception of oral sensations and food and beverage behavior

Martha R Bajec, Gary J Pickering
Brock University St Catharines, ON, Canada

Food/beverage flavor and liking strongly influence consumption, and consequently a range of nutritional and health outcomes. Thus, variation in perception of chemosensory stimuli, which form the flavor construct, can influence habitual dietary intake and risk of chronic diet-related disease (Duffy et al 2008). Arguably, the most important factor explaining differences in perception of chemosensory stimuli is genetic variation. Sensitivity to phenylthiocarbamide (PTC) and 6-n-propylthiouracil (PROP) has long been proposed as a genetically mediated correlate of some food behaviors. More recently, a new source of individual differences in oral sensation was identified: thermal taster status (TTS; Cruz and Green 2000). Thermal tasters (TTs), who may constitute up to 50% of the population, perceive phantom tastes when small areas of the tongue are heated or cooled. The phenomenon is linked to the TRPM5 cation channel (Talavera et al 2005). We investigated the hypothesis that (i) TTS predicts responsiveness to multiple oral sensations, and (ii) TTS associates with liking of food/beverages. 73 subjects were classified as TTs or thermal non-tasters (TnTs), and rated the intensity of a range of prototypical taste and other oral stimuli using the gLMS. Subjects also rated liking – a proxy for consumption (Duffy et al 2008) – of 301 food and 61 beverage items, including 43 alcoholic products, using a 7-point hedonic scale. TTs rated most taste and trigeminal stimuli as significantly more intense than TnTs (ANOVA; $p(F)<0.05$), and TTS associated with food/beverage liking. These findings are discussed in the context of the utility of TTS as a potential indicator of general taste responsiveness, food/beverage consumption, and diet-related disease risk.

#6 **Gustation**

TRPA1 Sensory Agonism in Humans: Time Dependence

William S. Cain, Roland Schmidt, J. Enrique Cometto-Muñiz
University of California, San Diego La Jolla, CA, USA

A family of TRP channels, TRPA1, mediates chemesthetic sensations to electrophilic agents present in environmental exposures, e.g., formaldehyde. To activate the channels, the material enters the neuron and can form reversible covalent bonds with cysteine residues. One would expect time-dependence, particularly an increment in response, for breaking the bonds should require more energy than making them. We have charted such increments for ETS and formaldehyde. Novel methodology has allowed us to chart the course of reaction to very low levels of the electrophile chloropicrin. The methodology captured reactions even below the sensory threshold. Ss (n=62) occupied a chamber (1-4 occup.) for exposures to 0 to 150 ppb chloropicrin. Ss rated confidence that chemesthetic sensations (eye, nose, throat) had not or had occurred. At the beginning of exposure, all concentrations seemed alike, without effect. For 50 ppb, Ss gave no evidence of detection until 20 min into exposure. The response manifested itself as a reduction of confidence that any sensation had occurred. For 75 ppb, the same occurred, but earlier, and with a bigger change. For 100 ppb, the progression continued. Only there did responding eventually cross from diminishing uncertainty of no detection to some positive confidence of a reaction. For 150 ppb, responding migrated into the zone of positive reaction after 10 min. These reactions occurred only in the eye, with the nose and throat quiescent. The site-specificity has precedent for other electrophilic agents, including certain tear gases, but requires explanation. Here we have shown that the sensory response can depend as much on time as on concentration, even at the subtlest levels of stimulation that would seem unworthy of note at first.

#7 **Gustation**

Oral Disinhibition Varies With Taster Status: Unilateral Anterior Oral Anesthesia Produces Asymmetric Posterior Taste Loss in Nontasters

Derek J. Snyder[1,2], Frank A. Catalanotto[2], Patrick A. Antonelli[3], Linda M. Bartoshuk[2]
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Four cranial nerves carry oral sensory cues, and mounting evidence suggests that some of these inputs inhibit others centrally. In clinical and experimental studies, psychophysical data from our laboratory indicate that localized oral sensory loss produces compensatory disinhibition at remaining oral loci:

Chorda tympani (CT) anesthesia leads to elevated glossopharyngeal (IX) sensation; among supertasters of 6-*n*-propylthiouracil, it also enhances trigeminal (V) sensation. These findings imply that oral disinhibition occurs in proportion to genetic taste status, and that affected sensations are differentially susceptible to these effects. Supporting this idea, in healthy subjects with low taste function, unilateral anesthesia of either the lingual nerve (i.e., CT and V; $N = 46$) or the chorda tympani ($N = 28$) asymmetrically compromises posterior taste sensation, with the side contralateral to anesthesia showing the least suppression. Lingual nerve block produced posterior asymmetry for all four common taste modalities, but direct CT block did so only for salty stimuli. Previous reports suggest that loss of 2+ oral sensory inputs broadly compromises taste function; these reports also show posterior taste enhancement with CT block, but modality-specific asymmetry may represent mild disinhibition in individuals with low native taste function. Finally, contralateral anterior oral burn remained intact with both nerve blocks, while posterior oral burn was blunted bilaterally; these data imply that disinhibitory trigeminal enhancement is associated with high taster status. In sum, disinhibitory effects of localized oral sensory loss vary based on genetic taste status; posterior taste elevation manifests in nontasters as asymmetric loss, while trigeminal effects occur mainly in supertasters.

#8 Withdrawn

#9 **Gustation**

"Restrained Eaters" Show Abnormal and Differential fMRI Activation to Sucrose and Saccharin

Claire Murphy[1,2], Nobuko Kemmotsu[1,2]
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[2]University of California, San Diego San Diego, CA, USA

Obesity in the US has reached epidemic proportions, affecting present and future health status of millions of Americans. Persons who exercise cognitive restraint over eating behavior may provide insights into underlying mechanisms of obesity. "Restrained eaters" attempt to control weight by cognitive restraint, actively regulating the quantity and quality of food intake. Whether they have altered brain activity to nutritive and non -nutritive taste stimuli is unknown. The present study investigated taste information processing in the central nervous system using the functional MRI technique. Sixteen participants were "restrained eaters" defined by Factor 1 (cognitive restraint) items of the Three Factor Eating Questionnaire (TFEQ; Stunkard & Messick, 1985), and 16 were "non-restrained eaters." The BOLD signal change was investigated when the participants were hungry and satiated with a nutritional preload, in response to sucrose and saccharin. Non-restrained eaters responded with more robust activation to the caloric sweetener. In contrast, the restrained eaters showed a different activation pattern, demonstrating greater response to saccharin than to sucrose. Interestingly, restrained eaters showed decreased activation in OFC and amygdala in response to the saccharin when satiated, the same pattern observed in the non-restrained eaters in response to sucrose. This differential activation to sucrose and saccharin, particularly in reward areas of the brain, suggests altered brain mechanisms in restrained eaters. A better understanding of the development and maintenance of this phenomenon may lead to strategies for prevention of and interventions for obesity.

#10 **Gender effects on olfactory processing**

Axons of gustatory receptor 32a expressing neurons extend their terminal throughout adult lifetime

Tetsuya Miyamoto, Hubert Amrein
Department of Molecular Genetics and Microbiology,
Duke University Medical Center

Courtship is an essential behavior for successful mating. In male Drosophila, chemosensory cues are thought to control many aspects of courtship such as identification of partners of the same species, appropriate sex and suitable mating status. Recently, we report that the gustatory receptor (Gr) gene, Gr32a, plays an important role in male courtship. Gr32a mutant males show normal courtship towards females. However, the courtship index of Gr32a mutant males towards males and mated females, which contain male pheromones, is much higher than that of wild type males. These results indicate that Gr32a is essential for detection of a male inhibitory pheromone, which is necessary for suppression of courtship towards unrewarding potential mates. Gr32a is expressed in chemosensory neurons of labial palp and legs, but leg neurons are sufficient to repress male-male courtship. All gustatory neurons are thought to project to the primary taste-processing center, subesophageal ganglion. However, we occasionally observed that Gr32a leg neurons directly project to the ventro-lateral protocerebrum, which is known as a higher order brain structure as receiving input from multiple sensory modalities, including visual, auditory, and possibly olfactory cues. The morphology of Gr32a neurons outside of the subesophageal ganglion shows a huge diversity among individual and even in either side of the same brain. Their axon ends at various positions, from the subesophageal ganglion to upper end of the ventro-lateral protocerebrum. The number of axon terminal is also widely ranged, from 1 to 20. Strikingly, there is a clear correlation among the probability of axon to reach the ventro-lateral protocerebrum, the complexity of axon terminal and age up to 60 days after eclosion. This is also observed in flies kept in isolation, though less significant than that of flies kept in a group. Our studies suggest Gr32a leg neurons slowly extend their terminals throughout adult lifetime; this phenomenon might lead older, socially experienced flies tend to avoid males and mated females more strictly.

#11 **Gender effects on olfactory processing**

Recognition of Sexual Cues in the Urine by Mouse Vomeronasal Organ

Ron Yu[1,2], Jie He[1], Limei Ma[1], Sangseong Kim[1], Junichi Nakai[3]
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The vomeronasal organs of the mammalian species detect complex chemical signals in the urine that convey information about sex, strain, as well as the social and reproductive status of an individual. How such complex signals are recognized by the vomeronasal organ is not well understood. In this study, we have developed transgenic mice expressing the calcium indicator, G-CaMP2, to analyze the population response of vomeronasal neurons to urine from individual animals. We found that a substantial portion of the cells were activated by either male urine or female urine, but most of them contributed little to sex discrimination. Sex information was represented by a surprisingly

small population of cells responding exclusively to sex-specific cues shared across strains and individuals. Female-specific cues activated more cells and were subject to more complex hormonal regulations than male-specific cues. In contrast to sex, strain and individual information contained in urine was encoded by the combinatorial activation of neurons such that urine samples from different individuals elicited distinctive patterns of activation. Interestingly, mouse urine at different concentrations activates distinct subsets of VNO neurons. Direct investigation of the urogenital area allows pheromones to reach the VNO at a level that provides unambiguous identification of the sex and the strain of animals. Lower concentration urine activates a different set of cells, some of which are masked by high concentration urine. These observations suggest that the vomeronasal circuitry is likely to perform complex computation of pheromone information in a context-dependent manner to trigger innate behaviors and endocrine changes.

#12 **Gender effects on olfactory processing**

Vomeronasal reception of a sex peptide pheromone ESP1 in mice: the receptor, neural circuitry, and behavior

Kazushige Touhara
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We have previously discovered a male-specific putative pheromone, exocrine gland-secreting peptide 1 (ESP1), that is released into tear fluids from the extraorbital lacrimal gland and activates vomeronasal sensory neurons in female mice. Here we show the identification of a specific vomeronasal receptor V2Rp5 for ESP1 and the involvement of TRPC2 in its downstream signal transduction mechanism. The neural pathway beginning with a specific recognition of ESP1 by V2Rp5 was visualized, showing the transmission of the ESP1 signal to the spatially highly-organized population of secondary neurons in the accessory olfactory bulb. ESP1 induced a sexually dimorphic activation pattern at higher-order brain regions. We observed the involvement of ESP1 in a sexual behavior of female mice. Together, we demonstrate that the "labeled-line" pathway from a specific receptor to the brain in a sex-specific manner is a molecular and neural basis for the vomeronasal system that decodes and represents information of a sex peptide pheromone. The present study provides molecular and functional link between a sex peptide pheromone and a selective neural circuitry leading to a behavioral output via a specific receptor in the mouse vomeronasal system.

#13 **Gender effects on olfactory processing**

Differential Sensory Neuron Activation Underlies Gender Dimorphic Aggressive Behavior

Lisa Stowers[1], Pablo Chamero[2], Kelly Flanagan[1], Fabio Papes[3], Darren DW Logan[1], Toby F Marton[4], Angeldeep Kaur[1]
[1]The Scripps Research Institute La Jolla, CA, USA, [2]University of Saarland Homberg, Germany, [3]State University of Campinas Campinas, Brazil, [4]University of California San Diego San Diego, CA, USA

Females do not respond aggressively to the same pheromones that provoke aggression in males. The underlying neural code that results in this gender dimorphic behavior is unknown. Molecular differences in the sensory neurons between male and female mice have not been reported. Recent findings in Drosophila have shown that each gender detects the ligands similarly and known differences in the organization of the responsive neural circuits may lead to their differing innate behaviors (Datta et al., 2008). We now show that while neurons in the male vomeronasal organ are activated in response to MUPs, female sensory neurons are dramatically impaired to all but one identified MUP suggesting a sensory perception difference between males and females. Interestingly, females do not robustly detect the individual MUP that promotes aggression in males. The dimorphic sensory response is not genetic but rather a response to female hormones as juveniles of both sexes and castrated males detect all Mups similarly to intact males with the response extinguishing as females become sexually mature. This response is plastic and rapid; manipulating hormone levels in females leads to a corresponding change in the ability of vomeronasal neurons to detect and respond to Mups. The lack of response to the aggression MUP pheromone by females singularly provides a novel mechanism for this innate gender dimorphism.

#14 **Gender effects on olfactory processing**

Opposite-sex volatile urinary odors detected by the main and processed via the accessory olfactory system contribute to mate recognition in mice

Michael J. Baum[1], Ningdong Kang[1], Kristine M. Martel[1], James A. Cherry[2]
[1]Dept. of Biology, Boston University Boston, MA, USA, [2]Dept. of Psychology, Boston University Boston, MA, USA

We assessed the contribution of main and accessory olfactory signaling to the preference of mice to investigate opposite- versus same-sex urinary odors. Urinary volatiles from the 2 sexes stimulated distinct profiles of glomerular activation in the ventral main olfactory bulb (MOB) of both male and female mice. By contrast, only opposite-sex urinary volatiles stimulated Fos expression in the accessory olfactory bulb (AOB) after their detection by the main olfactory epithelium (MOE) as opposed to the vomeronasal organ. Tract tracing experiments in female mice showed that urinary odors from opposite-, but not same-sex, conspecifics stimulated Fos expression in MOB mitral/tufted cells that project directly to the 'vomeronasal' (medial) amygdala. Opposite-sex urinary volatiles also selectively stimulated Fos expression in medial amygdalar neurons that send centrifugal projections to the AOB of females. Bilateral AOB lesions in either male or female mice attenuated subjects' motivation to investigate opposite-sex urinary volatiles. Opposite-sex urinary volatiles that are initially detected by the main olfactory system gain preferential access to the vomeronasal amygdala and the AOB with a resulting facilitation of mate recognition and reproductive success. Supported by NIH grant HD 044897.

#15 Gender effects on olfactory processing

Neural control of sexually dimorphic behaviors
Nirao Shah
UCSF San Franciscio, CA, USA

Sex specific behaviors such as mating and aggression are innate behaviors in mice as they can be displayed without prior training or experience. Nevertheless, these behaviors are tightly regulated by pheromones as well as by sex steroid hormones. We will present data demonstrating distinct sensory and hormonal control of these behaviors.

#16 Presidential Symposium: On beyond glomeruli

Functional Architecture of Inhibition in the Olfactory Bulb: Glomeruli and Beyond
Michael T Shipley
Department of Anatomy & Neurobiology, Program in Neuroscience University of Maryland School of Medicine Baltimore, MD, USA

Sensory inputs are transformed to outputs via mitral and tufted (MT) cell projections to olfactory cortex (POC). Inhibition shapes this transformation at two levels: Glomerular circuits mediate presynaptic inhibition of olfactory nerve (ON) terminals and postsynaptic inhibition of MT apical dendrites. Infraglomerular circuits exert postsynaptic inhibition at MT lateral dendrites. Glomerular circuits: Two intraglomerular glomerular circuits operate on single glomeruli to mediate tonic/phasic and pre-/postsynaptic inhibition. Interglomerular circuits enhance contrast among hundreds of distant glomeruli and multiglomerular circuits link smaller groups of neighboring glomeruli roughly the size of 'modules' responsive to structurally similar odors. Glomerular circuits lack POC feedback but centrifugal modulatory inputs (5HT, ACh) strongly shape pre- and postsynaptic inhibition. Infraglomerular circuits: MTs reciprocally synapse with granule cells (GC), which mediate recurrent and lateral inhibition of MTs. Infraglomerular circuits are targeted by central modulatory inputs (5HT, ACh, NE). They receive massive cortical inputs, which excite GCs thus inhibiting MTs. How do these inhibitory circuits shape olfactory processing? Modulatory inputs provide behavioral state dependent regulation of both glomerular and infraglomerular circuits. Glomerular circuits are driven by sensory signals. They follow repetitive ON inputs and exert more powerful inhibition of MTs than previously thought. They temporally sharpen MT firing and maintain contrast among glomerular outputs across dynamic changes in odor concentration. Infraglomerular circuits are strongly influenced by cortical feedback, which may regulate changes in MT firing across sniff cycles. Cortical feedback may mediate experience-dependent plasticity.

#17 Presidential Symposium: On beyond glomeruli

Olfactory systems theory
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The idea that glomerular activation patterns underlie odor perception and recognition is analogous to claiming that the inverted image maps of photoreceptor activation identify visual objects; that is, the statement is not clearly wrong, but misses nearly the entirety of the problem. As with any sensory system, the olfactory receptor neuron layer serves the primary purpose of transducing environmental variance into profiles of neural activity. The resulting primary representations are degenerate, occluded, and naïve reflections of the odor environment; nominally identical stimuli emit variable odor signatures, unpredictable mixture elements and background odors irreversibly interfere with the replicability of receptor activation profiles, and there exists no clear means to distinguish important from random sources of sensory variance. Meaningful perceptual information must be constructed from this pool of structured variance by the neural circuitry of the olfactory bulb (OB) and the multiple secondary structures to which it projects. We will outline the computational problems faced by the olfactory system and describe models for how the architectures of its successive layers of neuronal processing contribute to their resolution. Particular attention will be paid to the analogous problems faced by other sensory systems, as well as the differences between them, and to the role of memory in olfactory perception. Expanding upon a history of comparing the OB to the retina, we propose that an amalgam of the retina and primary visual cortex (V1) is a more appropriate visual-system analogy for OB function.

#18 Presidential Symposium: On beyond glomeruli

Oscillatory Modes and the Role of Task Structure in Early Olfactory Processing
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Early stages of processing in the olfactory system are often considered to consist of relatively static mechanisms, dictated by glomerular input patterns, with minor adjustments related to intensity of the input signal and modulation of its strength by central processes. More recently, it has been shown by my lab and others that changes in the temporal precision of mitral cells, signified by the gamma oscillation of the local field potential, are related to processing highly overlapping odorant input patterns. Recent work from my laboratory shows that gamma oscillations are modified dependent on task demands. However, we have also shown that the structure of the task used to determine odor identification can influence the type of oscillatory mode, the involvement of central brain areas in primary odor processing, and the difficulty of the discrimination itself. We show that changing the task from a two-alternative choice to a go/no-go task alters the way in which the olfactory bulb, piriform cortex and hippocampus participate in odor processing. The oscillatory signature changes from a gamma oscillation local to the olfactory bulb, to a beta oscillation that is coherent with activity in these

higher order areas. The beta oscillation mode is thus not just a different frequency but a different network. This larger and more distributed network is associated with more flexibility in learning odor associations.

#19 Presidential Symposium: On beyond glomeruli

Rostral Olfactory Cortex
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In mammals, several cortical areas receive direct input from the olfactory bulb. Although in some species these areas can comprise 10% of total cortical volume, fundamental questions remain regarding their role in olfactory information processing. In this presentation, I will consider the circuitry and function of the rostral olfactory cortical structures, and examine how these regions may contribute to olfaction and odor-guided behavior.

#20 Presidential Symposium: On beyond glomeruli

Olfaction in the wider world: The cortex and beyond
Joel Price
Washington University at St. Louis

The olfactory bulb projects through the lateral olfactory tract onto at least seven "primary" olfactory cortical areas. Although a topographic organization has not been identified, the cortex is dominated by "association" connections from several of the olfactory cortical areas that match the bulbar projection. Throughout the olfactory cortex the fibers from the olfactory bulb end on dendrites in the superficial lamina of layer I, while association fibers end on precisely complementary parts of the same dendrites, and in deeper parts of the cortex. This provides a mechanism for cortex-wide integration of the information coming out of the bulb that is presumably the basis for olfactory discrimination. Many of the olfactory areas send axons into isocortical areas in the caudal orbital and rostral insular cortex. Although these areas could be considered "higher order" olfactory cortex, they are substantially interconnected with adjacent areas in the orbital cortex that receive taste, visceral, visual and somatic sensory inputs. It appears that these areas integrate olfactory information with other modalities to provide assessment of flavor and other characteristics of food including appearance and texture. In addition, this cortex codes for reward or value as well as sensory aspects of the stimuli.

#21 through #27 Development and Plasticity: First Central Chemosensory Relays

Development and Plasticity:
First Central Chemosensory Relays
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There is little information about the nature, timing and extent of dynamic processes that establish and maintain functional central nuclei in chemosensation. Formation of functional groups during development requires timed waves of neuron birth, migration, and differentiation. Neuron clusters then attract and receive sensory input that often dramatically reorganizes the maturing circuit. With data from moth, lobster, chick and rodent, across taste, olfactory and respiratory systems, we will explore how neurons initially come to cluster and receive specific sensory input, and understand feats of plasticity in central chemosensation. The focus is on the first central afferent relays of three systems. After an introductory talk with general principles and mechanisms, there will be three short talks with specific examples of early nucleus development. Then two concluding talks will emphasize plasticity in sensory relay nuclei in developing rostral and caudal rodent brainstem. The symposium will stimulate development of new ideas and questions about formation of chemosensory nuclei — highly plastic central regions. ***C. Krull,*** University of Michigan. Mechanisms to establish functional groups of neurons: Lessons from chick neurogenesis. ***L. Oland,*** University of Arizona. Roles for glia in regulating formation of neuronal groups in moth olfactory lobe. ***M. Schmidt,*** Georgia Sate University. Long life expansion of olfactory brain in spiny lobster by neurogenesis. ***R. Bradley,*** University of Michigan. Establishing the rat taste nucleus of solitary tract (NST): ***A. Erisir,*** University of Virginia. Development and plasticity of neuron and synapse morphology in rat rostral NST. ***D. Kunze,*** Case Western Reserve University. Plasticity in synaptic function following altered chemosensory input to caudal NST.

#28 Polak Young Investigator Award Winners

Nucleotide-mediated signaling in the olfactory epithelium
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Extracellular nucleotides are important signaling molecules that mediate various biological effects via cell-surface receptors termed purinergic receptors. Here we employed functional calcium imaging in acute slice preparations of the olfactory epithelium (OE) to investigate the effect of extracellular nucleotides on the different epithelial cell types of larval *Xenopus laevis*. Nucleotides evoked distinct increases in the intracellular calcium concentration $[Ca^{2+}]_i$ in sustentacular cells (SCs) and basal cells (BCs), the olfactory stem cells of the OE, but not in olfactory receptor neurons. Thereby we were able to show that nucleotides elicit intracellular calcium waves in SCs. The waves initiate in the apical part of the SCs and propagate towards their endfeet in the basal part of the OE (wave velocity 17.10 ± 1.02 µm/s). The characteristic increases in $[Ca^{2+}]_i$ could be evoked in

both the presence and absence of extracellular calcium. In contrast, a depletion of the intracellular calcium stores abolished the $[Ca^{2+}]_i$ responses. Accordingly, SCs and BCs seem to express only metabotropic purinergic receptors. Furthermore, we defined the receptor subtype(s) involved in the nucleotide-induced responses of the two cell types. The determined order of potency of a variety of purinergic agonists and antagonists suggests that multiple P2Y receptor subtypes are involved in the responses of SCs and BCs. Taken together, our findings indicate that nucleotides, most probably locally released in the OE, could stimulate distinctive $[Ca^{2+}]_i$ increases in SCs and BCs. The physiological role of these $[Ca^{2+}]_i$ responses remains to be determined, but our findings suggest to view the OE as a tissue and to start to concentrate on possible functional interactions between the different cell types in the OE.

#29 Polak Young Investigator Award Winners

Hyperpolarization-Activated Cyclic Nucleotide-gated Channels in Olfactory Sensory Neurons Mediate Axon Targeting and Glomerular Formation

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Mechanisms influencing olfactory glomerular development are not fully understood. Odor receptors (OR) play an important role in olfactory sensory neuron (OSN) axon targeting (Mombaerts et al. 1996; Wang et al., 1998; Feinstein and Mombaerts, 2004). Recent work suggests that G-protein activation alone is sufficient to induce OSN axon coalescence (Imai et al., 2006, Chesler et al., 2007). Consistent with a role for activity in glomerular development, axon sorting is perturbed in mice deficient for adenylyl cyclase III, a member of the olfactory signal transduction cascade. In contrast, mice lacking Gαolf or the cyclic-nucleotide-gated (CNG) channel have normal OSN axon coalescence and glomerular formation, suggesting that CNG channels may not be an early target of cAMP. This prompted us to investigate an alternative channel, the hyperpolarization-activated, cyclic nucleotide-gated cation channel (HCN) as a developmental target of cAMP in OSNs. We characterized the spatio-temporal expression of HCN subunits during development using PCR, quantitative immunoblots, and immunohistochemistry. Initially, HCN subunits are present in both "immature" (GAP-43+) and "mature" (OMP+) neurons. By E17, HCN expression co-localizes primarily with mature OSNs. Interestingly, there was quantitative temporal variation in HCN subunit expression, which may affect channel kinetics. Consistent with a role in axon extension, inhibition of HCN channels in primary OSN cultures significantly reduced neurite length and branching. Moreover, $HCN1^{-/-}$ mice show axons overshooting their target at E17, and abnormal glomerular formation from E17 through postnatal ages. These data strongly suggest that HCN channels are present during the relevant period to influence axon outgrowth, targeting and glomerular formation.

#30 Polak Young Investigator Award Winners

Virus infection increases mouse pheromone production

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Mouse mammary tumor virus (MMTV), a retrovirus that can be transferred from the mother mouse to her pups through her milk, causes mammary adenocarcinomas. We previously reported (Yamazaki et al. 2002) that mice infected with MMTV can be discriminated by scent from uninfected, genetically identical control mice long before the development of tumors. In this study, we investigated the chemical nature of the MMTV-related odor signal. We first created MMTV-infected mice by mating female B6 MMTV (RIII) with male B6 mice; infection of offspring was confirmed by PCR. Urine was collected from infected and non-infected adult mice and was chemically analyzed using solid-phase-microextraction, followed by gas chromatography coupled with mass spectrometry. The most striking result was the finding that the amount of 3,4-dehydro-exo-brevicomin (DHB - reported to be a male mouse pheromone [Novotny et al. 1985]) was dramatically increased in urine of infected female mice. DHB in female mouse urine has been reported only once (Andreolini et al. 1987) and its function in females is unknown. We hypothesized that DHB levels might be used to determine infection status. To test this, mice were trained in a 2-choice Y-maze. As expected based on previous work, the trained sensor mice could discriminate between mice with and without MMTV. These mice were then given generalization trials comparing two samples of non-infected mouse urines one of which was spiked with authentic DHB at approximately physiological level. As hypothesized, they responded to the DHB-spiked sample as if it were from an infected mouse. This result suggests that DHB plays an important role in the recognition of MMTV-infected mice and it raises interesting questions about virus interactions with mouse chemosensory communication systems.

#31 Polak Young Investigator Award Winners

Increased inhibition in the olfactory bulb due to newborn neurons allows perceptual learning

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Olfactory perceptual learning is a critical component of adaptation of olfactory function to a changing environment. Perceptual learning can be evidenced by the fact that odor discrimination is enhanced by prior experience. The exact mechanisms of that implicit and robust learning remain elusive. We have previously shown that odor enrichment enhances rats' ability to discriminate between chemically similar odorants. In the present study, mice were exposed to pairs of similar odors for 1-hr over 10 days. We first show that the discrimination of odorants is improved in mice after odor enrichment. More specifically, the perception of odorants is only modulated by enrichment with odorants that activate at least partially overlapping regions of the olfactory bulb. Second, we show that this improvement of perception is accompanied by an increase of inhibition due to an increase of newborn granule cell survival in regions of the

olfactory bulb involved in odor processing. Finally, we show that bulbar neurogenesis is essential for perceptual learning because its blockade during the enrichment period prevents the improvement of discrimination.

#32 Polak Young Investigator Award Winners

Opioid modulation of taste encoding in the amygdala
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Taste plays a crucial role in determining palatability, or reinforcing value, of food items. However, CNS encoding of palatability, and neuromodulators contributing to this encoding, remain poorly understood, particularly in subcortical forebrain targets of ascending taste information. Opioid signaling is thought to play an important role in this encoding, but its role has not been well defined. We investigated the role of the endogenous opioid ligand enkephalin, which is widely expressed in taste and reward circuits, in palatability driven behaviors and taste encoding. KO mice lacking the gene for preproenkephalin showed no changes in baseline feeding behavior. However, administration of the opioid antagonist naltrexone (NTX) suppressed food intake in WT mice, but slightly increased intake in the KO. This was true for caloric and noncaloric tastants, indicating that NTX effects were independent of postingestive effects. Quantitative analysis of patterns of lick microstructure and taste reactivity indicated that NTX decreased measures associated with palatability exclusively in WT animals. We analyzed cfos immunohistochemistry to pinpoint anatomical loci where NTX-induced changes in expression diverged between WTs and KOs. Expression in the central nucleus of the amygdala (CeA), a direct recipient of brainstem taste inputs, was decreased in WT mice but unaltered in KOs. NTX infusion into the CeA decreased intake in WT animals, but had no effect in KOs. Finally, we used electrophysiological methods to investigate taste encoding in the amygdala in behaving mice. Preliminary results suggest that NTX acts to attenuate the magnitude of palatable taste-evoked responses in WT but not KO mice. Our results suggest enkephalinergic signaling in the amygdala importantly modulates palatability processing.

#33 Polak Young Investigator Award Winners

Taste cortex contributes to odor quality coding
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Despite distinct peripheral and central pathways, stimulation of both the olfactory and gustatory systems gives rise to the perception of sweetness. It is known that odor quality is coded in piriform primary olfactory cortex, but it is unknown whether the taste-like quality of odors is also coded here. An alternative possibility is that odors acquire the ability to activate taste neurons through learning, since odors come to be perceived as having taste-like qualities by virtue of having been experienced with taste[1]. In this case, the intensity of the taste-like property of the odor should be coded in the insular taste cortex. To determine whether odor sweetness is represented in sweet taste responsive cortex we performed an fMRI experiment (3T Siemens scanner) with 15 subjects smelling sweet food and nonfood odors and tasting two concentrations of sucrose solution. Subjects rated the sweetness of the odors prior to scanning. Comparison of strong vs. weak sucrose produced activation in the mid insula, indicating that this region is sensitive to sweet taste. This same region showed a graded response to food but not nonfood odors depending upon odor sweetness rating. No correlations were observed between odor sweetness and neural response in piriform cortex. These results demonstrate that the sweetness of food, but not nonfood odors is represented in regions of insular cortex that encode sweet taste and not in piriform primary olfactory cortex, which encodes other aspects of odor quality. These findings show that gustatory cortex contributes to odor quality coding.
[1] Richard J. Stevenson et al., *Learn Motiv* **26** (4), 433 (1995).

IFF Special Lecture
Keiko Abe, The University of Tokyo

The sense of taste is indispensable for animals to find out a proper way of living by selection of foods at their discretion. Taste has also been a mainstay for construction of historical human cultures and modern food industries. However, no systematic information has been available regarding the molecular logic of intracellular taste signaling and associated chemical entities.

My study on the molecular biology of sweet, bitter, sour, salty and umami tastes had humble beginnings 20 years ago and then traced a unique path of development to reveal important parts of the signaling pathways and a series of responsible molecules. The process of the study has the following lineup: 1, identification of taste cells in taste buds and analysis of signal transduction; 2, construction of primarily cultured taste bud cells; 3, comparative analysis of taste signaling mechanisms in model fish (medaka) and mammals; 4, genomics of signal transduction from taste nerves to the brain and verification of the long hypothesized "labeled line theory"; and 5, application of these results. The last item (5) was investigated by the use of neoculin, an enigmatic sweet protein occurring in a tropical fruit that has sensory activity to convert sourness to stronger sweetness. Our X-ray crystallography and molecular dynamics simulation of neoculin as well as its mode of binding to hT1R2-hT1R3 provided new insights into the interface between taste molecular biology and new food chemistry exploring a taste (sourness)-taste (sweetness) interaction. The elucidation of this event would contribute to our increased understanding of the sense of taste as a scientifically and industrially interesting modality of life.

#34 **Reciprocal interactions between primary taste and olfactory processing networks and higher cognition**

Top-down Modulatory Influences on Central Encoding of Taste and Flavor in Humans

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Perception of taste, smell and flavor depends upon a multitude of factors other than the physiochemical properties of the stimulus. Recent work from our, and other laboratories, has demonstrated that top-down processes related to selective and directed attention, expectation, and belief modulate BOLD response to taste throughout the gustatory network and beyond. For example, trying to taste in the absence of taste activates insular taste cortex and this same region responds preferentially to the same taste when it is unexpected compared to when it is expected. The insular taste cortex also shows preferential connectivity with the amygdala when subjects taste passively compared to when they are asked to perform a task. This suggests that information transfer between the insula and amygdala is maximal during implicit processing of taste. In contrast, when subjects attend to and make judgements about taste pleasantness the insula is preferentially connected to the lateral orbitofrontal cortex. This finding highlights the importance of information transfer between the insula and the orbitofrontal cortex during explicit hedonic evaluations. Beyond the canonical gustatory network, more recent work in our lab has demonstrated that information about marketing labels influences activity in brain regions regulating energy balance, and that such influence strongly and secifically depends on individual variations in sensitivity to reward. Taken together these data highlight the importanceof top-down modulatory influences on central encoding of taste and flavor in humans.

#35 **Reciprocal interactions between primary taste and olfactory processing networks and higher cognition**

Roles of cognition and attention in the neural processing of taste and odor

Edmund Rolls
Oxford Centre for Computational Neuroscience

How cognition and attention influence the affective brain representations of the taste, flavor, and smell is important not only for understanding top-down influences on multisensory representations in the brain, but also for understanding how taste and flavor can be influenced by these top-down signals. We found using functional magnetic resonance imaging that activations related to the affective value of umami taste and flavor (as shown by correlations with pleasantness ratings) in the orbitofrontal cortex were modulated by word-level descriptors, such as "rich delicious flavor". Affect-related activations to taste were modulated in a region that receives from the orbitofrontal cortex, the pregenual cingulate cortex, and to taste and flavor in another region that receives from the orbitofrontal cortex, the ventral striatum. Affect-related cognitive modulations were not found in the insular taste cortex, where the intensity but not the pleasantness of the taste was represented. Moreover, in a different investigation, paying attention to affective value (pleasantness) increased activations to taste in the orbitofrontal and pregenual cingulate cortex, and to intensity in the insular taste cortex. We conclude that top-down language-level cognitive effects reach far down into the earliest cortical areas that represent the appetitive value of taste and flavor. This is an important way in which cognition influences the neural mechanisms of taste, flavor, and smell, and that control appetite.

#36 **Reciprocal interactions between primary taste and olfactory processing networks and higher cognition**

Cognitive influences on taste processing in the gustatory cortex, orbitofrontal cortex and amygdala

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Taste, often thought of as the start of an ingestive process, is in fact the outcome of a sequence of anticipatory behaviors naturally

guided by cues from other sensory modalities. These cues, besides guiding action, promote expectations which can influence the way gustatory stimuli are perceived. Recent experiments show that when human subjects adjust their perception according to expectations the patterns of activation of their gustatory brain vary accordingly. However, it is not known how single gustatory cortical neurons change their dynamics and what the source of this modulatory input is. Anatomical evidence of a strong interconnectivity with gustatory cortex suggests a possible involvement of regions known to process anticipatory cues, like amygdala and orbitofrontal cortex. In this seminar I will talk about how the expectation of incoming taste stimuli modulates neural activity in simultaneously recorded sensory and high order brain areas of rats. I will present data recorded simultaneously from taste cortex, orbitofrontal cortex and the amygdala, and I will compare single neuron responses to taste stimuli that were either presented unexpectedly or were self-administered following a tone cue (and were therefore expected). The differences between these two conditions will be discussed with a particular emphasis on the emergence in all recorded areas – including the gustatory cortex — of tone responses. In lights of these results, I will discuss the relationship between expectation and the processing of tastes and highlight evidence for the role of high order areas in modulating gustatory cortical activity.

#37 **Reciprocal interactions between primary taste and olfactory processing networks and higher cognition**

The piriform cortex plays an active role in olfactory-guided decision making

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The discreet nature of sniffing provides advantages for investigating the roles of sensory and motor cortices in higher cognitive processes such as decision making. Several recent experiments have called into question the long-held assumption that decision-making occurs as a tripartite, hierarchical process, with sensory recognition followed by prefrontal-based decision-making, and then when appropriate, motor output, with the

conclusions limited, however, by the type of task used, insufficiently high spatial or temporal resolution, or the lack of more advanced signal analysis. Here I will present a line of work indicating that the olfactory cortex directly influences a higher cognitive process, decision-making, using a more naturalistic GO/NO go task in which rats take 2-4 sniffs of a scented food target before "deciding" whether to reach for it. In the first study, we simultaneously recorded local field potentials and spikes from the piriform and motor cortices as rats performed the task, and analyzed the data with two forms of directed coherence technique (primarily Granger causality). Our main finding was that frequency- and time-specific olfactory-to-motor signaling developed as rats learned to discriminate the GO from the NO-GO cues, in response to apparent motor-to-olfactory "queries" about it. In follow-up studies, we have been recording simultaneously from the piriform, orbitofrontal, prelimbic and motor cortices as rats learn and perform this task as well as a more automated and controlled version of it, and analyzing the data with conditional Granger causality methods. We are finding that this olfactory-motor signaling occurs at least partly via the prefrontal cortex, further suggesting that the piriform and motor cortices actively participate in the rat's decision making process.

#38 Functional evolution of chemosensory receptors

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

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Odorant receptors are among the fastest evolving genes in animals. Positive Darwinian selection was implicated in shaping odorant receptor repertoires in different species. However, little is known about the selection pressure that has shaped the functions of individual odorant receptors during evolution. We have recently demonstrated a link between the in vitro function of a human odorant receptor, OR7D4, and in vivo olfactory perception of two steroidal ligands, androstenone and androstadienone, chemicals that are shown to affect physiological responses in humans. In this study, we analyzed what selection pressure might affect the function of OR7D4 in primate evolution. Orthologs of OR7D4 and another closely related receptor, OR7D1, were cloned from different primate species. Ancestral reconstruction allowed us to reconstitute additional putative OR7D4 orthologs in hypothetical ancestral species. Functional analysis of these orthologs showed an extremely diverse range of OR7D4 function in various primate species. We detected evidence for positive Darwinian selection acting on various amino acid residues of OR7D4 throughout primate evolution. Functional analysis of the nonsynonymous changes in the subset of Great Ape lineage revealed that positively selected sites caused changes in receptor function. Our results support the idea that positive selection has exerted influences on the dynamic functional evolution of OR7D4 in primates.

#39 Functional evolution of chemosensory receptors

Copy-number variation map obtained by high-resolution genomics reflects human olfactory receptor diversity and evolution

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Our current research combines large-scale experimental and computational data-mining approaches for studying the extent and the mutational formation-mechanisms of genomic structural variants. Structural variants, frequently referred to as copy-number variants (CNVs), are >1kb deletions, duplications, insertions, and inversions responsible for most genetic variation in humans. We recently developed paired-end mapping, an approach involving massive sequencing of the end-stretches of 3kb genomic DNA fragments and aligning them against a reference genome to identify CNVs at high resolution. Applying this approach to two human genomes allowed us to obtain insights into the extent at which CNVs affect genes. Specifically, we observed a striking enrichment of CNVs among the olfactory receptor (OR) gene family, and thus hypothesized that CNVs may play an important role in the evolution of the human OR repertoire. To enable investigating the impact of CNVs on ORs in a larger population sample, we designed subkilobase-resolution tiling arrays enabling us to detect CNVs in all genomic OR loci across 25 individuals. We identified 93 OR gene loci and 151 pseudogene loci affected by CNVs. Specifically, our results show an enrichment of CNVs among ORs having a close human paralog or such lacking a one-to-one ortholog in chimpanzee. Interestingly, among the latter we observed an enrichment in CNV losses over gains, a finding potentially related to the diminution of the human OR repertoire. Extensively mining data from personal genomics projects enabled us to detect, at basepair-resolution, various novel CNVs, some of which likely represent common variants contributing to inter-individual differences in the human OR gene content.

#40 Functional evolution of chemosensory receptors

Sequencing the entire OR gene repertoire (and other GPCRs) in a large number of individuals

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We developed a multiplexed protocol to sequence the entire repertoire of intact OR genes in humans (as well as hundreds of other GPCRs) using ultra-high throughput sequencing technology. We demonstrate that by using multiplex PCRs in one 96-well plate, and by sequencing the products in one Solexa lane per individual, we are able to fully sequence an individual's OR gene repertoire with enough coverage to call heterozygote sites. Our approach can be used to conduct association studies and population genetic analyses using the entire repertoire of OR genes – while to date, such studies typically focus only on a small subset of OR genes.

#41 Functional evolution of chemosensory receptors

Rapid evolution of two odorant-binding protein genes, *Obp57d* and *Obp57e*, in *Drosophila*

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Odorant-binding proteins (OBPs) are secreted molecules found in insect chemosensory organ, where they function together with odorant and taste receptors. Two OBP genes, *Obp57d* and *Obp57e*, are involved in the evolution of unique host-plant preference in *Drosophila sechellia*. *D. sechellia* exclusively reproduces on the ripe fruit of *Morinda citrifolia* (Tahitian Noni), which contains octanoic acid that is toxic to other *Drosophila* species. Behavioral analysis of *D. melanogaster* knockout strains suggested that *Obp57d* and *Obp57e* participate in the taste perception of octanoic acid. Comparisons of genomic sequences at the *Obp57d/e* locus from 27 *Drosophila* species revealed that the OBP gene number at this locus is different between species. Phylogenetic analysis suggested that *Obp57d* and *Obp57e* arose by gene duplication at the early stage of the *melanogaster* species group evolution, followed by differentiation of the ORF sequences from each other. While most species in the *melanogaster* species group maintain both *Obp57d* and *Obp57e*, some species have lost either gene. Expression pattern of *Obp57d* and *Obp57e* is also different between species. The two OBPs are expressed only in the gustatory sensilla on the legs in the species that have both *Obp57d* and *Obp57e*, whereas the additional expression in the gustatory sensilla on the mouthparts was observed in the other species. Behavioral analysis of various species revealed that the feeding preference for octanoic acid negatively correlates with the OBP transcripts level in the mouthparts. Gene duplication and the subsequent ORF differentiation, as well as changes in the expression pattern, could be important evolutionary mechanisms by which OBP genes develop their functional diversity, promoting the behavioral evolution among species.

#42 Functional evolution of chemosensory receptors

Bimodal Function of *Drosophila* Odorant Receptors

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Odorant signals are detected by binding of odor molecules to odorant receptors (ORs) that belong to the G-protein-coupled receptor (GPCR) family. The receptors couple to G-proteins, most of which stimulate cAMP production. This opens cyclic nucleotide-gated ion channels and depolarises the olfactory sensory neuron. Insect OR proteins lack any sequence similarity to other GPCRs and show an inverted orientation in the plasma membrane. The ORs are dimers composed of an odor-specific OR protein (e.g., *Drosophila* Or22a) and a ubiquitously expressed chaperone protein (as *Drosophila* Or83b). As G-proteins are expressed in insect OR neurons, and olfactory perception is modified by mutations affecting the cAMP transduction pathway this study was aimed at finding out the function of insect ORs. For this sake we expressed Or22a and Or83b in HEK293 cells and performed calcium imaging and patch clamp experiments. Application of odorants produced nonselective cation currents activated via both an ionotropic and a metabotropic pathway, and a subsequent increase in the intracellular Ca^{2+} concentration. Expression of Or83b alone provided functional ion channels insensitive to odorants, but directly activated by intracellular cAMP or cGMP. Furthermore, application of only 1 nM cAMP to excised inside-out patches caused a half-maximal current response. However, as current production developed slowly the activation mechanism is different from the gating known from ionotropic receptors. Insect ORs thus form ligand-gated channels as well as complexes of odorant sensing units and cyclic nucleotide-activated nonselective cation channels.

#43 Making sense of fat taste

Making Sense of Fat Taste

Timothy A. Gilbertson
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The importance of understanding the sensory cues for dietary fat has been underscored by the recent surge in dietary-induced obesity. This epidemic of obesity has been attributed to the increased intake of high fat, high carbohydrate, and calorically-dense diets. While the textural properties of fats have long been accepted to be its most salient sensory cue, emerging data from a number of laboratories has begun to point to their being a "taste of fat" as well. This symposium will provide a multidisciplinary overview of the evidence that supports the idea that the gustatory system is capable of responding to the chemical cues contained in dietary fat and will look at the ability of free fatty acids to act both as a taste primer and taste modulator. The implications of the presence of a fat detection system will be discussed. *Discussants:* J.-P. Montmayeur; R. Contreras.

#44 Making sense of fat taste

Ligand specificities to putative fat receptor candidates CD36 and GPR120 and licking behavior corresponding to the ligands in mice

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Fatty foods are palatable to animals including humans. Several studies have indicated that animals recognize the presence of fat in foods not only by the texture of the food but also chemically in the mouth: this suggests that the chemical perception of fat is involved in the acquisition of a strong avidity for fat. CD36 is known as a fatty acid transporter in the muscle or adipose tissue. Previously we have reported that CD36 is expressed in the taste bud cells of the posterior tongue. CD36-deficient mice showed lower avidity for long-chain fatty acid-enriched solutions. In addition to CD36, we have found that GPR120, a G-protein coupled receptor that functions as a specific unsaturated long-chain fatty acid receptor in the gastrointestinal tract, is expressed in the taste bud cells of anterior and posterior tongue. A wide variety of molecules are known to be a ligand for CD36, including saturated or unsaturated fatty acid, some lipoproteins, cholesterol, thrombospondin and so on. However, HEK293 cells overexpressing GPR120 revealed that only long-chain unsaturated fatty acids are potent ligand for GPR120. In addition, the palatability of fatty acids for mice assessed by the short term licking behavior is very similar to the ligand specificity for GPR120. These results raise the possibility that GPR120

expressed in the taste cells may also be involved in the chemical reception and palatability of dietary fat. CD36 and GPR120, however, are different in the location of expression, ligand specificity and possibly, intracellular signaling pathway. These facts indicate that these two fatty acid receptors expressed in the tongue may construct independent pathways for the sensing of fat.

#45 **Making sense of fat taste**

Fatty Acid Transduction in Chemosensory Cells
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Given the dramatic rise in obesity that appears to be correlated with an increase in dietary fat intake, there has been increasing interest in understanding the cellular and molecular mechanisms the body uses to recognize dietary fatty acids (FA). We have focused on three fat responsive cell types in our attempt to understand these mechanisms. Preingestively, we have attempted to elucidate mechanisms underlying the taste and texture of fat by using molecular and cell-based assays on taste receptor cells (TRCs) and trigeminal neurons (TGNs), respectively. In the gut, we have explored these pathways in the enteroendocrine cells (EECs) of the small intestine. Molecular data have shown that all three cell types express a variety of putative FA receptors, including CD36, FA-activated GPCRs and FA-sensitive DRK channels. Cell-based assays (patch clamp recording; calcium imaging) combined with pharmacological and molecular (RNA interference) approaches have been used by our laboratory to unravel the FA transduction pathway(s) within these chemosensory cells. While differences exist in the transduction pathways in these cell types, commonalities include a dependence upon G protein activation, a role for the phospholipase C, activation of TRP-like channels, and a role for voltage-activated calcium channels in the FA signaling pathway. We will discuss our current data and present a model for the transduction of fatty acids in chemosensory cells.

#46 **Making sense of fat taste**

Oral Detection of Fatty Acids by Rats
David W. Pittman
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Evidence is accumulating to support a role for the gustatory system in providing an immediate sensory signal allowing detection of the fatty acid components of dietary fat during consumption by rats. Early research showed innate preferences for dietary fat in humans and rats. Putative fatty acid receptors have been identified in rat taste receptor cells. Consistent with the action of fatty acids on taste receptor cells, we have shown that fatty acids increase licking to appetitive tastants during brief-access behavioral assays, while licking to aversive stimuli decreased in a manner reflective of an increase in the perceived intensity of the tastants. Conditioned taste aversion studies in our laboratory have demonstrated fatty acid detection thresholds at physiologically-relevant concentrations (2.5-66 M) which are much lower than fatty acid concentrations likely produced by lingual lipase during dietary fat consumption. Additionally, we have demonstrated genetic influences on the sensitivity of fatty acid detection between male / female rats and obesity-prone / – resistant strains of rat as well as an environmental influence of a high-fat diet on the sensitivity of fatty acid detection. New data suggests that olfactory cues are most likely not sufficient to allow detection and avoidance of fatty acids following a conditioned taste aversion and that orosensory signals generated by fatty acids are likely unique from sensations associated with the prototypical tastes of sweet, sour, salty, and bitter chemicals. Finally, the most compelling evidence of the gustatory detection of fatty acids by rats arises from studies in which gustatory nerve transections and genetic knockouts of specific fatty acid receptors in the gustatory system produce impairments in the behavioral detection of fatty acids.

#47 **Making sense of fat taste**

Oral Detection of Free Fatty Acids in Humans
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There is increasing recognition that free fatty acids (FFA) are important signaling molecules. Increasing evidence suggests they may play such a role in the oral cavity of humans. Psychophysical studies that attempt to control non-gustatory cues (e.g., visual, odor, irritancy, tactile) indicate humans can detect FFA varying in chain length (C:6 – C:18) and saturation (saturated, mono-unsaturated, poly-unsaturated). Spatial testing reveals that FFA (C:6 to C:18) can be detected on the dorsal, anterior; lateral posterior, and posterior tongue. All stimuli could be assigned graded intensity ratings from each site. Given the ligand specificity of currently identified putative FFA receptors, the data indicate that humans either have multiple transduction mechanisms in the different tongue regions and/or that there is a non-specific mechanism (e.g., diffusion) responsible. Non-normal threshold distributions are suggestive of a genetic basis for fat detection. Oral exposure to FFA also elicits a biphasic rise in plasma triacylglycerol (TG). Recent findings indicate there is a non-specific component to this response, but that it is most robust to oral exposure to dietary fat. It occurs after a single 10 second exposure, but the second phase shows greater quality specificity with longer stimulus exposures (e.g., 20 minutes). About half of the TG in the acute peak is derived from lipid consumed at the prior eating event indicating there is substantial lipid storage in the GI tract, presumably in jejunal enterocytes. While TG is likely tasteless, the other sensory properties it contributes to foods are generally viewed as positive. In contrast, FFA are aversive, so may serve as a warning to avoid foods with high levels. The full range of implications of fat detection remains to be characterized.

#48 **Workshop: Computational problems in sequential stages of odor processing**

Exploring the Interaction between Odorants and Odorant Receptors using Functional and Computational Methods
Charles W. Luetje, Sarah E. Repicky, Tatjana Abaffy
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The mammalian olfactory system uses a vast array of odorant receptors to detect and distinguish among an enormous number of odorant molecules. However, odorant ligand specificity has been determined for only a handful of odorant receptors and in only a few cases have the details of ligand recognition been examined at the molecular level. Expanding our knowledge of the molecular basis for ligand recognition among odorant receptors is challenging task. We are using functional analysis and site-directed mutagenesis, in combination with computational homology modeling and virtual ligand docking, to examine the interaction between odorant ligands and mammalian odorant receptors. We have developed a functional assay for ORs using the Xenopus oocyte expression system and robotic electrophysiology. This assay allows us to use both conventional mutagenesis and the Substituted Cysteine Accessibility Method to test and refine our initial receptor models. The assay also allows functional testing of ligand predictions derived from virtual docking. By iterating between computational and functional approaches, we hope to improve our understanding of the interaction between odorants and odorant receptors, with a particular emphasis on understanding ligand selectivity.

#49 **Workshop: Computational problems in sequential stages of odor processing**

Modeling Diversity in the Signal Transduction of the Mouse Olfactory Receptor Neuron
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We use various statistical parameterization techniques to drive the development of a physiologically-based computational model of the slow (transduction) and fast (action potential) currents of the olfactory receptor neuron in mouse. The response to an odorant plume can elicit a diversity of responses across a population of ORN. We hope that by bridging various statistical and computational approaches an improved understanding of the molecular basis of this diversity can be obtained.

#50 **Workshop: Computational problems in sequential stages of odor processing**

Odorant mixture interactions in rat olfactory receptor neurons: models and experiments
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An odor perception is the brain's translation of the activation by odorant molecules of olfactory receptor neurons (ORNs) located in the nasal cavity. Most ORNs express a single type of olfactory receptor that is differentially sensitive to a wide variety of odorant molecules. Many kinds of odorant-receptor interactions can take place, especially with mixtures of many odorants which make up the vast majority of real world odors. The simplest kind of interaction is pure competition when two or more agonists can bind to the receptor site which triggers receptor activation, although only one can be bound at a time. Noncompetitive effects may result from various mechanisms, as for example agonist binding to another site of the receptor (allostery). Then two molecules can bind at the same time and binding at the second site may modify the properties of the main binding site. We developed a quantitative model based on odorant-receptor interaction, relating the stimulus concentration to the ORN firing frequency. We extended this model to predict the ORN response in the simple case of pure competitive interaction between odorants. To test the model we recorded the electrophysiological responses of rat ORNs in vivo to odorant agonists and their binary mixtures at various concentrations. We interpreted the resulting concentration-response curves in the framework of our model. We found that it accounts for all curves obtained with single odorants and for about half of those obtained with binary mixtures. In the other half, the experimental curves were significantly different from those predicted by the model, indicating the occurrence of noncompetitive interactions. These noncompetitive interactions raise challenging problems for predicting the perception elicited by most odors, which like perfumes, wine bouquet, food aroma, etc. are mixtures of dozens, even hundreds, of odorants because they imply that the brain's code for mixtures of odorants will not be easily predicted from the known codes of their individual components.

#51 **Workshop: Computational problems in sequential stages of odor processing**

Odor Maps in the Mouse Olfactory Bulb
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Mice and rats use ~1,000 olfactory receptor types to probe chemical space. Each glomerulus in the olfactory bulb receives input from a single receptor type, whose ligand binding properties determine the spectrum of odor responses. In this way, the layout of glomeruli on the bulb forms a two-dimensional map of odors. We recorded the odor responses of dorsal glomeruli in mice expressing synaptopHluorin in sensory nerve terminals. By using a chemically-diverse battery of several hundred odors, we could assign unique functional identities to several dozen glomeruli. This allowed us to determine that the position of a given

glomerulus varies across individuals by only 1 glomerular spacing, corresponding to a precision of 1 part in 1000. We also asked whether the layout of glomeruli is systematically related to their odor sensitivities. The odor response spectra of two neighboring glomeruli were as dissimilar as those of distant glomeruli. This local diversity of odor responses will have important consequences for how mitral cells sample odor space. We have begun to examine this next stage of processing in the bulb by imaging postsynaptic signals in the dendrites of mitral cells in mice expressing the calcium indicator GCaMP2 and multiphoton microscopy. Initial results indicate that the population responses of mitral cell apical tufts are similar to the presynaptic maps constructed from synaptopHluorin mice. We were also able to record robust odor-evoked responses from a large population of individual lateral dendrites, and are currently analyzing their odor tuning.

#52 **Workshop: Computational problems in sequential stages of odor processing**

Distributed Lateral Inhibition in the Olfactory Bulb: Anatomical Evidence and Functional Implications of Long-range Interactions of Mitral and Tufted Cells

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Detecting environmental chemical information is thought to be a high dimensional sensory task. In mammalian olfaction, a complex system involving many proteins targets axons of sensory neurons expressing the same receptor to specific glomeruli organized in roughly a two dimensional sheet. Adjacent glomeruli in this sheet, however, may or may not detect similar odor information. Decorrelation of input through lateral inhibition therefore may need to act over longer distances and more selectively than a simple center-surround as found in the retina. Recent anatomical and physiological data suggest that lateral interactions between mitral and tufted cells mediated by granule cells are sparse and act over relatively long distances. I discuss new evidence of the distribution of synapses on the lateral dendrites, and hypothesize that function of selective lateral interaction is signal decorrelation.

#53 **Workshop: Computational problems in sequential stages of odor processing**

A two-stage model of odor representation and processing in the olfactory bulb

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Much is known about the neurobiology and psychophysics of olfaction, but this knowledge often lacks a common theoretical footing by which it can be integrated into a single framework of odor representation and processing. I here present a general theory of olfactory bulb operations, with emphasis on the architectural and functional differences between the two discrete layers of intrabulbar computation: the glomerular layer and the external plexiform layer. The olfactory system's high sensitivity and broad dose-response functions are consequences of established pharmacological and physiological mechanisms and do not reflect special properties of odorant receptors. Multiple negative feedback circuits normalize odor-evoked activity and facilitate the concentration-independent recognition of odors. Decorrelation (contrast enhancement) among physically similar odorants arises from location-independent synaptic mechanisms within the glomerular layer and can be dynamically regulated by descending neuromodulatory projections. Analogous decorrelation processes proposed to operate in the external plexiform layer are better-suited to learning functional decorrelations based upon experience. In contrast to the temporally unsophisticated spike trains of olfactory sensory neurons, the secondary olfactory representations mediated by mitral cells are sparser and suggest a dynamical, spike timing-sensitive precedence code. These principles underlie a theory of olfactory generalization that governs the perception of similarity among related odorants, including the plasticity of this perception and the observation that experimental omission of components of complex odors can have negligible effects on the results of olfactory perceptual tasks.

#54 **Follow the head, not only the nose: Top-down influences on olfactory perception**

The Nose is Just the Beginning: Patterns, Objects and Experience in Olfaction

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Patterns of sensory receptor neuron activity place constraints on ultimate perceptions, but do not necessarily allow prediction of the perception. Perception derives from computations performed by central circuits, which combine sensory afferent input with past experience, expectation, context and internal state. In this regard, olfaction is no different from other sensory systems. I will briefly argue that primary olfactory cortex is an associative network, merging complex spatiotemporal patterns of olfactory receptor input with descending information about past experience, expectation, context and internal state. In doing so, multi-modal and emotion-laden memories of odor objects are built, shaping subsequent olfactory perception.

#55 **Follow the head, not only the nose: Top-down influences on olfactory perception**

Learning to smell: Olfactory perceptual learning and its ecological impact

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Increasing evidence suggests that human olfaction is not dictated by the odorant chemical structure, but rather, reflects higher-order cognitive influences. In this talk, I will discuss recent work demonstrating that human odor quality coding alters and olfactory discrimination improves with experiences from mere odor exposure to olfactory aversive learning. Collected using psychophysics, autonomic physiology and functional magnetic resonance imaging, these data will indicate that 1) simple odor exposure enhances perceptual and neural discrimination of within-category odors such that, for instance, following passive

experience of a minty odor, one can become a mint expert capable of differentiating various minty scents, and 2) human olfaction possesses such great plasticity such that initially indistinguishable odor enantiomers can be reliably discriminated (behaviorally and neurally) after one enantiomer is associated with electric shock. Importantly, the ability of aptly adjusting odor perception via learning and emotional associations bears ecological significance. New data from my lab suggest that improvement in odor discrimination through aversive learning correlates with anxiety to the extent that failure to distinguish a predictive cue from a perceptually similar inconsequential cue may underlie excessive threat sensitivity and psychophysiological hypervigilance, landmark symptoms in anxiety disorders.

#56 **Follow the head, not only the nose: Top-down influences on olfactory perception**

Expectations About Health Effects Alter Odor Perception
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Recent developments in understanding top-down influences of experience on olfactory perception can be well applied to the problem of medically unexplained symptoms from exposures to low level chemicals as seen in Multiple Chemical Sensitivities (MCS). One of the speakers at this symposium, Dalton, proposed that effects of beliefs about odor-related health effects on health symptom reporting are modulated by changes in perceptions of those odors. We investigated directly the effects of health-related expectations on odor perception using the Event Related Potential (ERP) paradigm, to demonstrate that these effects occur relatively early in the perceptual process. Traditionally, a distinction is made between "exogenous" ERPs, which occur earlier during the signal and are determined by the characteristics of the eliciting odor, and "endogenous" ERPs, which occur later during the signal and are determined by meaning and individual variables. If expectations affect mostly interpretational stages of information processing, we would expect effects only on later ERPs. However, following the basic tenet of this symposium, expectation might also affect earlier perceptual processes, to be reflected by effects on earlier ERPs such as N1. We conducted three experiments using a within-subject design in which the EEG signals related to the perception of an identical odor were compared across conditions in which subjects expected adverse health effects or not. In all three experiments we indeed found effects of expectations of pain from the trigeminal CO_2 on the early N1 peak – and in only one experiment the P2 component. We will argue that high level processes have deep and pervasive effects on odor perception, which in turn may affect reactivity to the odor. This may play a role in conditions such as MCS.

#57 **Follow the head, not only the nose: Top-down influences on olfactory perception**

Implications for Remediation of Health Effects from Odor Exposure
Pamela Dalton
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Unexplained symptoms or illnesses characterized by nonspecific, multisystem complaints are often attributed to occupational or environmental chemical exposures, in which perception of odor is frequently the triggering event. These situations raise difficulties for clinicians as well as regulatory authorities, who are frequently unable to agree on the existence, nature, or source of such illnesses. Many of these difficulties likely derive from an adherence to a traditional stimulus-response model of symptoms, where the application of an ecological or biopsychosocial approach, incorporating top-down influences on the perception and interpretation of sensations, would be more effective. This presentation will discuss evidence from community and occupational settings in which the application of an ecological model would facilitate both the understanding of adverse responses and their remediation.

#58 **Follow the head, not only the nose: Top-down influences on olfactory perception**

Olfaction and cognitive information processing
Denise Chen
Rice University Houston, TX, USA

Olfaction has long been recognized as part of the social motivational system. In humans, however, the specific connections between olfaction and cognition in the context of social behavior remain largely unexplored. In this talk, I will present recent behavioral and neuroimaging work from my lab showing that the human olfactory system actively interacts with emotion and other senses in navigating and interpreting social and sensory terrains. At the behavioral level, we demonstrate that competency at social chemosensory processing reflects one's emotional competency, and that chemosensory emotional cues facilitate the perception of visual emotional cues. At the neural level, we find that the brain encodes emotional chemosensory cues in a holistic fashion.

#59 **GABA in the developing olfactory system: From generation to differentiation**

GABA in the developing olfactory system: from generation to differentiation
Harriet Baker[1,2]
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Gamma aminobutyric acid (GABA) was recognized as a major CNS inhibitory neurotransmitter more than 40 years ago. A role for this neurotransmitter in neuronal development was recognized and documented much more recently, i.e. during the last 20 years. Since the developmental function of GABA was first proposed, evidence has accumulated to indicate that GABA plays a large role in the generation, migration and differentiation of neurons in

several brain regions. This symposium will focus on recent studies that have shown the influence of GABA on virtually all aspects of olfactory bulb interneuron development, from proliferation and migration of progenitors, to maturation and functional synaptogenesis resulting in integration and phenotypic differentiation. Specifically, Angelique Bordey will speak about the interplay between glutamate and GABA on the rate of both generation and migration of olfactory bulb interneuron progenitors. Adam Puche will address issues related to expression of the GABAergic phenotype during migration of these interneuron progenitors. John Cave will present data suggesting that GABA modulates differentiation of GABA/dopamine-expressing periglomerular cells. Pierre-Marie Lledo will discuss how centrifugal innervation of olfactory bulb interneuron progenitors modulates their differentiation. David Willhite will describe GABA-related electrophysiological and projection parameters of olfactory bulb granule cells in relationship to odor processing. The work presented in this symposium highlights the continually expanding knowledge of the pleiotropic functions performed by GABA in the nervous system.

#60 **GABA in the developing olfactory system: From generation to differentiation**

GABA differentially modulations migration of SVZ progenitor subpopulations

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Olfactory bulb interneurons are continuously generated throughout development and in adult. These neurons are born in the subventricular zone (SVZ) and migrate along the rostral migratory stream (RMS) into the olfactory bulb (OB) where the majority become local GABAergic interneurons. While the postnatal SVZ is best known as a source of OB interneurons, it also gives rise to other interneuron populations. We have previously shown neurons also migrate ventrally from the postnatal SVZ to form the islands of Calleja and contribute neurons to the olfactory tubercle. Since neuronal migration from the ganglionic eminence to cortex, and from the SVZ to the OB, can be modulated by neurotransmitters we hypothesized that SVZ derived ventral migration would also be modulated by neurotransmitters. GABA is a potent cue reducing the migratory rate of rostral directed SVZ progenitors. However, unlike rostral migrating progenitors, those heading ventral respond to GABA with enhanced migration. In addition to altering migration rate, progenitor cells migrate toward a point source of GABA suggesting GABA acts as a guidance factor. Indeed, disruption of GABA signaling in vitro disrupts interneuron organization in the basal forebrain. These data suggest that GABA can modulates both rostral and ventral SVZ progenitor migration, but subpopulations respond differentially, and may be an important cue for organizing ventral forebrain interneurons.

#61 **GABA in the developing olfactory system: From generation to differentiation**

GABA and glutamate interplay on subventricular zone cell production

Angelique Bordey
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The production of adult-born neuron is an ongoing process accounting 10,000 to 30,000 immature neurons every day. This high turnover rate necessitates profound control mechanisms converging onto neural stem cells and neuroblasts. These mechanisms provide a balance between proliferation, migration and survival of stem cells and their daughter cells in the neurogenic microenvironment to achieve adequate adult born neuron production. We will elaborate on a novel epigenetic control of adult neurogenesis via highly coordinated nonsynaptic cell-cell signaling. This communication is based on neurotransmitters, whose extracellular concentration depends on cell number. In the adult subventricular zone, glial fibrillary acidic protein (GFAP)-expressing progenitors (called GFAP-progenitors) generate neuroblasts migrating to the olfactory bulb. Recent findings from our lab show that neuroblasts release the neurotransmitter GABA providing a negative feedback control of their own migration and stem cell proliferation through unknown intracellular pathways. Neuroblasts display coordinated calcium activity that is expected to lead to calcium-dependent GABA release. Upon stimulation by GABA, stem cells are depolarized and release glutamate via a vesicular process. Glutamate then activates Glu_{K5} kainate receptors and NMDA receptors controlling neuroblast migration and survival, respectively. The timing of neurotransmitter release and their spatial diffusion will determine the convergent co-activation of neuroblasts and stem cells, and scale their development.

#62 **GABA in the developing olfactory system: From generation to differentiation**

GABA-enhanced differentiation of the olfactory bulb dopaminergic phenotype

John W Cave[1,2], Yosuke Akiba[2], Harriet Baker[1,2]
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GABA regulates the proliferation and migration rates as well as dendritic elongation and stabilization of olfactory bulb (OB) interneuron progenitors. Given these developmental roles, an interesting, but largely unexplored, question is whether GABA can also modulate differentiation of specific interneuron phenotypes. To determine whether GABA modulates activity-dependent expression of tyrosine hydroxylase (TH), a marker of OB dopaminergic neurons differentiation, neonatal forebrain slice cultures were prepared from transgenic mice expressing GFP under the control of the 9kb TH upstream gene regulatory region. These studies revealed that the induction of TH/GFP expression under depolarizing conditions (25mM KCl) is completely inhibited by nifedipine, an L-type Ca+2 channel blocker, and partially inhibited by ∞-agatoxin, a P/Q Ca+2 channel blocker. This combined action of both L and P/Q-type Ca+2 channels is similar to the established mechanism for synaptic release of GABA from periglomerular interneurons. Our studies also revealed that exogenous application of GABA further increased

TH/GFP expression levels in depolarized slice cultures. This GABA-mediated increase of TH/GFP expression was blocked by inhibitors of either GABA-A or GABA-B receptors as well as inhibitors of metabotropic and ionotropic glutamate receptors. Although GABA is sufficient to both depolarize OB interneuron progenitors and activate L-type Ca+2 channels, GABA, by itself, was not sufficient to induce TH/GFP expression in our studies. Instead, the data indicate that induction of TH/GFP expression specifically required glutamate-mediated depolarization and activation of L-type Ca+2 channels, and that differentiation of the OB dopaminergic phenotype is coupled to its co-expressed GABAergic phenotype.

#63 **GABA in the developing olfactory system: From generation to differentiation**

Dynamic development of synaptic inputs on maturing interneurons in the adult OB

Pierre-Marie Lledo, Antoine Nissant, Cedric Bardy, Hiro Katagiri, Kerren Murray, Institut Pasteur Paris, France

In addition to relaying sensory information from the primary sensory epithelium to the cortex, the olfactory bulb (OB) receives massive inputs from several centers. Prime among the centrifugal inputs are the dense innervation that preferentially targets granule cells (GC) of the OB. Since newborn inhibitory neurons, including GC, continuously migrate into the OB throughout life, it is still uncertain whether their first synaptic contact originates from ascending (i.e., sensory inputs) or descending (i.e., centrifugal fibers) pathways. The present study demonstrates that glutamatergic centers, extrinsic from the OB circuit, contact the newcomers on their proximal section of the apical dendrite, much before receiving synaptic inputs from intrinsic OB circuit. This finding indicates that before feed-forward sensory inputs target the distal portion of the new GC dendrite, the animal's behavioral state may shape integration of new OB neurons by establishing early synaptic contacts. To explore the functional consequences of these early glutamatergic synaptic contacts, we investigated plasticity at glutamatergic synapses onto the newly-generated GC. We find that, shortly after their arrival in the bulb, a subset of excitatory synapses exhibits long-term potentiation. This property fades with maturation of the new neurons, and disappears in mature GC. These results demonstrate that recently-generated adult-born OB interneurons undergo different experience-dependent synaptic modifications compared with their pre-existing mature neighbors, and provide a possible substrate for adult neurogenesis-dependent olfactory learning. Understanding how centrifugal inputs develop on adult-generated GC is therefore a critical step necessary to decipher the unexpected impact of behavioral states on adult neurogenesis.

#64 **GABA in the developing olfactory system: From generation to differentiation**

Transsynaptic Tracing Studies Suggest Combinatorial Gating of Olfactory Information is Mediated by GABAergic Interneurons in the Granule Cell Layer

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Subsets of newly-generated GABAergic interneurons integrate into a complex network of synaptic connections in the granule cell layer of the olfactory bulb. Viral transsynaptic tracing studies have shown that there is a tendency toward a columnar relationship of granule cells with M/T cells. We interpreted the columns to arise from clusters of granule cell synapses along the lateral dendrites of M/T cells. Therefore, dual injections of tracing strains expressing either red or green fluorescent proteins at different sites in the medial glomerular layer was expected to produce red, green and yellow granule cell columns. However, the granule cell columns that are connected to both red and green M/T cells tend to show red and green non-colocalization (segregation) rather than yellow colocalization (convergence) within the same spatial columnar unit. This shows that the M/T lateral dendrites from widely separated glomeruli that overlap at a specific location in the EPL are generally connected to different granule cells, rather than the same population. These results suggest that further subdivisions of granule cells within a columnar unit may serve specific functions. To form hypotheses of how this network architecture influences odor information processing, computational models incorporating assumptions of segregated or convergent granule to mitral cell connectivity were created. Results indicate that segregated connections are sufficient under the tested conditions to allow granule cells to modulate M/T cell firing using information from multiple lateral sources, while convergent connections place constraints on lateral influence. I hypothesize that sparse, segregated connectivity allows combinatorial logic gating, a function that is one of the roles of GABAergic interneurons in the olfactory circuit.

#65 **Olfactory and Vomeronasal Systems**

Repertoire of chemosensory receptors from the genome of the jawless vertebrate *Petromyzon marinus*

Scot V. Libants[1], Kevin Carr[2], John H. Teeter[3], Yu-wen Chung-Davidson[1], Curt Wilkerson[2], Weiming Li[1]
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In gnathostomes, chemosensory receptors (CR) expressed in olfactory epithelia are encoded by evolutionarily dynamic gene families encoding odorant receptors (OR), trace amine-associated receptors (TAAR), and two types of vomeronasal receptors (V1R and V2R). Whether these gene families arose in basal or advanced vertebrates has not been resolved because these families have not been examined systematically in agnathan genomes. The sea lamprey *Petromyzon marinus* is the only extant jawless vertebrate whose genome has been sequenced, and has been shown to detect

fewer amino acids and steroids than teleosts. We have identified a repertoire of 59 intact single-exon CR genes, including 27 OR, 28 TAAR, and four V1R-like genes. Expression among these CR families was detected in the olfactory organs of parasitic- and reproductive-stage adults using Roche 454 GS20 sequencing. We failed to identify orthologs or pseudogenes of the multi-exon V2R family that has expanded in teleost genomes, but did identify two intact calcium-sensing receptors (CASR) and several metabotropic glutamate receptors (MGR). We conclude that OR, TAAR and V1R arose in chordates after the cephalochordate-urochordate split, but before the diversification of jawed and jawless vertebrates. The advent and diversification of V2R genes from glutamate receptor-family G protein-coupled receptors, most likely the CASR, appears to have occurred after the divergence of jawed and jawless vertebrates.

#66 **Olfactory and Vomeronasal Systems**

Emx2 Stimulates Odorant Receptor Gene Expression and Controls OSN Axon Growth

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The expression of a single odorant receptor (OR) gene by each olfactory sensory neuron (OSN) is critical for odor discrimination, yet the mechanism of OR gene choice is largely unknown. Putative OR promoters contain homeodomain-like sites, implicating homeobox transcription factors, like Emx2, in OR gene expression. We investigated whether OR gene expression was altered in Emx2$^{-/-}$ mice. The olfactory epithelium of Emx2$^{-/-}$ mice at embryonic day 18.5 had normal pseudostratification and was morphologically normal except for a decrease in thickness due to a 42% reduction in OMP^{+} mature OSNs. This effect was minor compared to changes in OR gene expression. 50% – 80% of Class I and Class II OR mRNAs were disproportionately reduced in abundance compared to the small decreases observed for mature OSN-specific mRNAs. The intensity of in situ hybridization was normal for these ORs, but fewer OSNs expressed them (many fewer than the reduction in mature OSNs). A few Class II ORs (<10%), however, were expressed by significantly more OSNs, consistent with the interpretation that those OR genes least dependent on Emx2 were most frequently chosen for expression. Emx2 stimulates transcription of a majority of ORs, but does not appear to be the dominant factor in deciding which OR is expressed or the zonality of OR expression. In addition, Emx2$^{-/-}$ mice have a previously identified OSN axon defect. The axons cross the cribriform plate but fail to innervate the olfactory bulb, a defect apparently autonomous to OSNs because the bulb does not express Emx2. We have identified 23 axon growth and guidance related mRNAs whose abundance in the olfactory epithelium was decreased in Emx2$^{-/-}$ mice. These genes are candidates for controlling the ability of OSN axons to innervate the olfactory bulb.

#67 **Olfactory and Vomeronasal Systems**

Investigations of Olfactory Receptor Internalizations

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G-protein-coupled receptors comprise the biggest protein superfamily in the mammalian genome. Although odorant receptors represent the biggest subfamily of G-protein-coupled receptors, nearly nothing is known about the intracellular trafficking of these receptors. We investigated the endocytic pathway of mammalian odorant receptors and found that the receptors bind β-arrestin2 with high affinity and are internalized via a clathrin-dependent mechanism. Moreover, β-arrestin2 is redistributed into the dendritic knobs of mouse olfactory receptor neurons after treatment with a complex odorant mixture. To study receptor localization in the olfactory sensory neurons we generated an antibody against the mouse olfactory receptor for eugenol (MOR-EG). Specificity of the antibody could be shown by immunostainings using MOR-EG transgenic mice. After 15 minutes of odorant exposure the receptor protein translocates to the soma of the olfactory sensory neurons. Surprisingly, olfactory receptor density is increased in the dendritic knobs of olfactory receptor neurons when odorant exposure is prolonged to several hours. These are first insights into the mechanisms of receptor adaptation and sensitization in the olfactory epithelium. To gain more insight into these mechanisms we will take a further look at the odorant receptor trafficking and the interactions of odorant receptors with β-arrestin2 and other trafficking proteins in olfactory cells.

#68 **Olfactory and Vomeronasal Systems**

OMP Deletion Alters Functional Maturation of Single Olfactory Sensory Neurons

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Olfactory sensory neuron (OSN) maturation is marked by the expression of olfactory marker protein (OMP). To test if OMP is critical for functional maturation of OSNs, we used patch clamp recording on single cells in intact epithelia. Odorant-induced inward transduction currents were measured in MOR23 OSNs from MOR23-ires-tauGFP mice. We first characterized response kinetics, sensitivity, and selectivity of MOR23 cells during the first month after birth. From P0 to P30, OSNs had improved odorant responses, with faster kinetics (90% rise time: 0.7±0.2 (n=26) vs. 0.2±0.1 s (n=19); 90% decay time: 9.3±0.7 vs. 7.1±0.8 s) and higher sensitivity to lyral (k1/2: 6.0±2.2 (n=13) to 1.4±0.8 μM (n=7)). Remarkably, all MOR23 cells recorded at P0 (n=8) responded non-selectively to odors with diverse structures, and responded specifically to lyral by P30 (n=10). Next, in OMP-KO/MOR23-GFP double-mutant mice, we observed that MOR23 OSNs fail to improve response parameters by P30, responses matching those of wt P0 neurons. OMP-KO cells at P30 showed slower response kinetics than wt cells at P30 (90% rise time: 0.6±0.2 (n=12) vs. 0.2±0.1 s; 90% decay time: 9.9±0.7 vs 7.1±0.8 s) and lower sensitivity (k1/2: 7.4±3.3 (n=4) vs. 1.4±0.8 μM). OMP deleted MOR23 cells responded non-selectively at P0 (n=18), and failed to become specific lyral detectors by P30 (n=26). Interestingly, unilateral odor deprivation shows that postnatal changes in decay kinetics are activity dependent, but

not activation and sensitivity. Furthermore, altered adenylate cyclase III (ACIII) signaling may underlie the immature OMP-KO phenotype, as blocking ACIII phosphorylation in P30 wt cells mimics the KO phenotype. We conclude that OMP is critical for functional maturation of OSNs.

#69 **Olfactory and Vomeronasal Systems**

Sensory Adaptation in the Vomeronasal Organ

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The mammalian vomeronasal organ (VNO) mediates the regulation of social behaviors by complex chemical signals. These cues trigger transient elevations of intracellular Ca^{2+} in vomeronasal sensory neurons (VSNs), but the functional role of such Ca^{2+} elevations is unknown. We show (1) that VSNs undergo sensory adaptation; (2) that VSN adaptation depends on negative feedback signaling by Ca^{2+} which enters the cell through transduction channels but not voltage-activated Ca^{2+} channels; (3) that adaptation is required for sensitivity modulation of stimulus-evoked VSN responses and alters the information transmitted to the brain; (4) that activated CaM is required for these effects; (5) that pheromone-sensitive TRPC2-dependent cation channels are inhibited by Ca^{2+}-CaM and thus provide a substantial target for a Ca^{2+}-CaM-mediated negative feedback loop; and (6) that mouse VSN dendritic knobs express Ca^{2+}-activated cation channels that could be part of an additional, positive feedback mechanism. These findings identify Ca^{2+}-CaM as a previously unrecognized negative feedback signal that tightly adjusts gain and amplification of the chemotransduction process in VSNs. Future studies should aim at understanding how the interaction of negative and positive feedback loops in VSN signal transduction shapes the processing of pheromonal information in the vomeronasal system.

#70 **Olfactory and Vomeronasal Systems**

First Order Blend Processing in the Moth Antennal Lobe

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Animals typically perceive their olfactory environment as a complex mixture of many different compounds. Despite this fact, most studies concerning odor detection and coding in insects have only included single components. In insects, the initial representation of odors occurs in the first olfactory neuropil, the antennal lobe (AL), where three classes of neurons form synapses: olfactory receptor neurons (ORNs), projection neurons (PNs) and local interneurons (LNs). The resultant neural representation of odor mixtures in the AL may be simply a linear summation of blend components or a non-linear interaction due to odor information processing in the AL. The modified representation in the assembly code is carried by PNs to higher order brain centers. Our goal is to reveal mechanisms of host odor information processing at different levels in the AL (PNs, LNs) of the hawk moth, *Manduca sexta.* Using a novel multicomponent stimulus system and intracellular recording, we analyze the fine representation of blends vs. single components in individual antennal lobe neurons responding to combinations of up to seven host volatiles. We examined different temporal characteristics (e.g. spike frequency, interstimulus interval, latency) and among those a variety of blend interactions were observed, including synergism and suppression. Moreover, we found that a single morphological type of neuron can exhibit diverse blend interactions as well as inhibitory and excitatory response characteristics.

#71 **Olfactory and Vomeronasal Systems**

Translation of Olfactory Input into Behavioral Output in the *Drosophila* Larva

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We have investigated principles of odor coding by testing the responses of the *Drosophila* larval olfactory receptors to a panel of chemically similar odorants. The anatomy of the larval olfactory system is similar to that of the adult while being numerically simpler. It contains 21 olfactory receptor neurons that project to 21 glomeruli in the larval antennal lobe. Of 60 odor receptor genes, 21 encode functional larval receptors in our laboratory strain. Using an *in vivo* expression system, the "empty neuron" system, we tested the responses of the larval receptor repertoire to a panel of 24 pyrazines. Six receptors showed strong excitatory responses to one or more pyrazines. Two of these six receptors, Or33b and Or59a, exhibited some excitatory responses that showed surprising temporal dynamics; they took up to 15 minutes to return to the baseline firing rate. By contrast, most strong excitatory responses returned to baseline in less than 3 seconds. We have named these extremely long responses "supersustained" responses. Pyrazines elicited a range of behavioral responses from strong attraction to little, if any, attraction when tested using a two-choice assay. Surprisingly, two pyrazines that have remarkably similar receptor response profiles, 2-ethylpyrazine and 2-methylpyrazine, elicited dramatically different behavioral responses. We are currently investigating mechanisms that may underlie this difference in behavioral response.

Evidence for a chemosignal in human tears

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Emotional tearing is a uniquely human behavior who's evolutionary origin and present-day function remain unclear. We hypothesized that emotional tears may act as a chemosignal. Emotional tears were obtained from a female "donor" who viewed sad films in isolation. In a within-subjects repeated-study design, 42 healthy male subjects (age 19-40) smelled (10 sniffs) either fresh tears or saline, counter-balanced for order, and then watched two video clips, one emotionally neutral and one sad. We measured mood (17-qestion Ekman questionnaire), psychophysiology (skin conductance, electrocardiogram, ear pulse, finger pulse, abdominal respiration, thoracic respiration, skin temperature, and body movement), and endocrine state (salivary testosterone). Within compound analysis revealed that in the presence of TEARS the emotional movie decreased positive mood ($F(1,41)= 8.53$, $p<0.0056$), increased high negative arousal mood ($F(1,41)= 7.2$, $p<0.01$), and increased low negative arousal mood ($F(1,41)=12$, $p<0.001$). In turn, in the presence of SALINE the emotional movie increased only high negative arousal ($F(1,41)=7.7$, $p<0.0083$). Similarly, in the presence of TEARS the emotional movie decreased body temperature ($F(1,37)=4.95$, $p<0.03$), decreased movement ($F(1,40)= 8.55$, $p<0.005$), and increased respiration rate ($F(1,40)=8$, $p<0.007$), yet in the presence of SALINE the emotional movie increased respiration rate alone ($F(1,40)=8.52$, $p<0.006$). Finally, separate ANOVAs revealed that in the presence of TEARS the emotional movie induced a decrease in testosterone compared to baseline, yet SALINE did not (F tears $(3,105)=3.948211$, $p<0.010325$; F controls $(3,105)=0.79$, $p<0.501$). These results indicate that tears may act as a human chemosignal.

POSTER PRESENTATIONS

#P1 **Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits**

Taste disturbances after tonsillectomy – results of a prospective study

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Introduction: Persistent taste disturbance is a rare complication after tonsillectomy. Postoperative dysgeusia has been described in case reports; the number of prospective trials however is limited. The aim of the study was to investigate the frequency, the time course, the extent of subjective postoperative taste disorders after tonsillectomy in adults in a prospective clinical trial. M&M: Since 2007, adult patients undergoing tonsillectomy were asked to take part in the trial. At present, 102 patients (32±12 years, 54% w, 46% m) were included. At the day prior to surgery as well as 2 weeks, 6 months after tonsillectomy a standardized questionnaire was completed by the patients. The questionnaire addressed a self assessment of the ability to taste (visual analogue scale) and potential taste disorders in terms of their quantity, quality, onset, and persistence. Results: To date the results of 2 weeks questionnaire are available from 71 patients, the 6 months questionnaire from 31. 23 patients (32%) reported taste disorders after tonsillectomy 2 weeks (postop.). 13 (18%) described a taste disorder lasting longer than 2 weeks. 5 (16%) experienced taste disorders that were still present at the 6 month follow up (bitter taste). The subjective assessment of the ability to taste was significantly reduced 2 weeks (postop.) (VAS:7,8±1,6to5,7±2,2). Discussion: Subjective taste disorders after tonsillectomy seem to be more frequent than expected, consisting of subjective hypo- and parageusia. A considerable number of patients experience subjective taste disorders lasting for more than 6 months. A bitter taste was most frequently reported and the overall sense of taste seems to be reduced. The trial will continue to gain a longer follow up and a higher number of patients to verify these results

#P2 **Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits**

Long-term olfactory outcome in patients with mild traumatic brain injuries (mTBI)

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The incidence of olfactory deficits in patients with TBI has been related to injury severity suggesting that they are uncommon in mild traumatic brain injury (mTBI). Consequently, there are no studies that have examined the long-term effect of mTBI on olfactory function. We examined olfactory function in a group of patients who sustained a concussion at least one year prior to testing and who complained of persistent post-concussion symptoms (headaches, dizziness, nausea, fatigue, irritability). Their performance was compared to that of an age- and gender-matched healthy control group. None of the subjects with mTBI exhibited olfactory deficits when tested with conventional olfactory tests (Sniffin' Sticks Threshold, Discrimination, and Identification test), but their perception of odor intensity was impaired. In general, odors were perceived as less intense compared to the healthy controls. In contrast, their perception of odor pleasantness was unremarkable. These preliminary findings suggest that perceived intensity of suprathreshold odors may be impaired following mTBI. To our knowledge, we are the first to demonstrate long-standing, albeit subtle, olfactory deficits following mTBI. Grant Acknowledgement: Supported by RGPIN 335938-08 awarded to JD by NSERC.

#P3 **Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits**

Volatile biomarkers for human melanoma cells

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Dogs can be trained to localize not only melanoma samples buried on the skin of healthy subjects, but also the areas with melanoma from cancer patients. The skin lesions of melanoma and nevi can be differentiated instrumentally (e.g. by a gas sensor array). These studies suggest that skin tumors produce a different profile of volatile organic compounds (VOCs) than normal skin. However, the identity of the compounds distinguishing normal skin from melanoma is not currently known. We analyzed the VOCs released from human melanoma and normal pigmented skin cells (melanocytes), cultured *in vitro*, by employing solid phase microextraction (SPME) and gas chromatography/mass spectrometry (GC/MS). There are significant, quantitative differences in the relative amounts of several compounds that distinguish normal melanocytes from melanoma cells as well as each type of melanoma cells from one other. Thus, monitoring these VOCs has the potential to be a useful screening tool for melanoma. This is currently being tested in melanoma patients.

#P4 **Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits**

Olfactory function in childhood maltreatment and post-traumatic stress disorder

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Background: Childhood maltreatment (cm) has often been hypothesized to result in functional changes of amygdalae and orbitofrontal cortex. If such changes exist, we would expect an effect of these changes on olfactory function in adults with a history of cm, because amygdalae and orbitofrontal cortex have outstanding importance for olfactory processing. *Methods:* We compared 31 depressive women with a history of cm, 28 depressive women without cm, and 27 healthy women. For

comprehensive assessment of olfactory function we used the "Sniffin´ Sticks" odor threshold and odor identification test. Furthermore we analyzed chemosensory event related potentials in response to pleasant and unpleasant olfactory stimuli and trigeminal activation. Additionally participants answered a questionnaire for current symptoms of posttraumatic stress disorder (PTSD). *Results:* Contrary to our hypothesis we found no significant difference between the cm-group compared to the two control groups. However, there was a significant correlation between current symptoms of PTSD and olfactory function. Following statistical adjustment for depressive symptoms a significant correlation remained between PTSD symptoms and odor identification, N1 latencies in response to the unpleasant trigeminal (CO_2) and olfactory (H_2S) stimuli, and N1 amplitudes in response to the same unpleasant stimuli. *Conclusions:* The results indicate a preferred processing of unpleasant stimuli in patients with PTSD - irrespective of the childhood history - and fit the information dissociation theory in PTSD. Because we did not find changes in olfactory function depending on cm, the influence of current psychopathology appears to be of higher significance to the amygdalae and orbitofrontal function than the childhood history.

#P5 Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits

"Anosmic smell": Residual olfactory function following hemispherectomy

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Hemispherectomy is a relatively rare neurosurgical procedure, done to treat intractable epileptic seizures, involving complete removal or deafferentation of a whole cerebral hemisphere. We examined olfactory function in one subject who underwent hemispherectomy, and compared her performance to that of a healthy control volunteer, matched for age and gender. DR is a 31-year-old right-handed woman whose level of intellectual function is in the average range. At age 5, at onset of epileptic seizures, she was diagnosed with Rasmussen's chronic encephalitis. At age 17, she underwent a right functional hemispherectomy. We tested olfactory function separately in each nostril, examining her detection thresholds, quality discrimination, and identification, with Sniffin' Sticks. In addition, we requested a confidence rating after each response, in order to compare confidence levels associated with correct versus incorrect responses. As expected, her olfactory performance was preserved with the nostril contralateral to the removed hemisphere. In contrast, she was considerably impaired when tested ipsilaterally. Notably, while her threshold and discrimination results were at chance, she was still able to identify stimuli, albeit with difficulty. Furthermore, she showed a normal divergence of confidence within each task : higher confidence was associated with correct responses and lower confidence with incorrect ones, and this was the case both when tested ipsi- and contralaterally to the lesion. These results demonstrate residual rudimentary olfactory function ipsilaterally to the removed hemisphere, which we call "anosmic smell" and they parallel the "blindsight" abilities previously documented in this subject. Results are discussed in light of her postoperative MRI findings.

#P6 Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits

The perception of malodors: An fMRI study of age and gender related differences between pre and post puberty subjects

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Although learning, experience and socialisation strongly influence the individual perception of odors, central nervous processing of odorous stimuli in different stages of adolescence has to date only rarely been studied. In spite of frequent reports about age and gender modulating odor perception, fMRI has scarcely been applied to substantiate these differences. In this study, 20 right handed subjects were grouped according to gender and age (pre puberty: 9 - 12 yrs. and post puberty: 17 20 yrs.) to build four equally sized samples. By means of fMRI, patterns of cerebral activation in pre and post puberty girls and boys after nasal stimulation with three malodors were compared (Androstadienone, 2-Methyl-3-Mercaptobutanol and H_2S). Data analysis did not reveal significant gender differences, but activation patterns were found to differ between age groups. While pre puberty subjects mainly showed activation of earlier projection stages of odor processing, namely piriform cortex and amygdala, in post puberty participants, enhanced activation was revealed in neocortical areas (insula and medial and inferior frontal gyri). This finding may be interpreted in terms of integrative aspects of odor processing playing an important role in the post puberty group. The pattern of enhanced neocortical activation could reflect a more advanced stage of social and cognitive development.

#P7 Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits

Odor judgments in first episode and chronic schizophrenia patients

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There is consistent evidence that schizophrenia patients have olfactory dysfunction. Impairments in olfactory identification, quality discrimination as well as sensitivity are well described. There has been little investigation of olfactory judgments in schizophrenia, and the few findings are controversial. The aim of this study was to determine whether patients experiencing a first-episode schizophrenia and patients with chronic schizophrenia differ in odor judgments. Olfactory judgment measures included intensity, edibility, familiarity and hedonic ratings. Subjects were asked to rate edibility, familiarity and hedonic about 16 everyday odors (real-world items) on visual 7-point rating scales. Half of odors were edible, half not. Olfactory intensity judgments were performed using the odors from an identification task (Sniffin' Sticks). Unirhinal performance in olfactory judgments were compared between young first-episode patients, young patients with chronic schizophrenia and healthy controls similar in age

and gender (all male). Our results demonstrate that only patients experiencing their first episode showed bilateral impaired familiarity and edibility judgments of odors by comparison with healthy controls. No group differences were observed in intensity ratings. Chronic schizophrenia patients, however, differed significantly in hedonic odor ratings from both, controls and first episode patients. These group differences remained significant even after controlling for lower sensitivity in chronic patients. These findings indicate intact odor intensity in schizophrenia. Impairments in familiarity and edibility judgments are present early in the course of the illness, whereas disturbances in hedonic valence of odors might be related to effects of medication or illness duration.

#P8 **Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits**

Odor Discrimination in Mouse Models of Schizophrenia

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Schizophrenia is a psychiatric disease characterized by inaccurate perceptions of reality including deficits in odor discrimination. Previous studies have shown polymorphisms in the human alpha7-nicotinic acetylcholine receptor (alpha7) promoter and decreased expression of alpha7 in the hippocampus in schizophrenia. Mouse models of schizophrenia have similar polymorphisms in the alpha7 promoter region and decreases in alpha7 expression in the hippocampus. However, it is not known if olfactory deficits persist in animal models. Here we characterize alpha7 expression in the olfactory bulb (OB) and determine odor discrimination of mouse strains (C57 and C3H) with altered alpha7 expression (wild-type – WT; alpha7 heterozygous knockouts – HET; alpha7 homozygous knockouts – KO). Using [^{125}I] alpha-bungarotoxin binding, mean alpha7 expression in the OB was highest in C57 compared to C3H WT mice. Binding was significantly decreased in HET mice and no binding was found in KO mice (ANOVA, $p < 0.05$). For the discrimination task using an aldehyde odor pair, WT mice discriminated an entire log unit lower (alpha = -3.2) compared to HET (alpha = -2.2) and KO mice (alpha = -2.1; ANOVA, $p<0.05$). Similarly for an acetate odor pair, WT mice discriminated more than half a log unit lower (alpha = -2.9) compared to HET (alpha = -2.2) and KO mice (alpha = -2.2; ANOVA, $p<0.05$). These data suggest that differences in alpha7 expression in the adult mouse OB may contribute to the decreased ability to discriminate similar odorants. Thus, by characterizing the relationship between olfactory function and alpha7 expression in the OB of mice, we may provide a new tool to elucidate the mechanism of olfactory dysfunction in patients with schizophrenia.

#P9 **Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits**

A Timeline for Parkinson's Disease

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Objective To provide a consensus view of the evolution of classic Parkinson's Disease (PD) by combining what is known of clinical, epidemiologic, imaging and neuropathologic findings. **Background** PD has a fairly consistent clinical and neuropathologic profile with a lengthy prodromal phase. The duration of the prodrome is unclear and correlation of Braak staging with clinical features is complex.. Several prodromal features are suggested but many are not substantiated by prospective studies. **Methods**. Studies from imaging, neuropathology, clinical and epidemiologic sources were reviewed to synthesize a model of disease onset and progression, incorporating more robust features that appear before disease onset. **Results** The best established premotor features, based on large prospective studies with pathological verification are: hyposmia, constipation, obesity, sleep and sympathetic disorder. We suggest there is a lengthy prodromal period for PD lasting approximately 20 years, followed by a clinical stage of15- 20 years. There is inevitably considerable variability for both estimates, but an overall 40 year disease course would be a reasonable estimate. **Conclusion.** If PD starts 20 or more years before clinical presentation, this provides a reasonably long period for intervention. We suggest that measurement of olfaction, bowel habit, weight and sleep pattern would be an appropriate way to assess at-risk subjects who may be in the prodromal phase of PD. For neuroprotective measures to be of benefit, patients at this stage need to be identified even though they may be healthy outwardly. Initially this goal may be achieved by identifying those at risk in families with mutations known to be associated with PD. For the vast majority who have no such mutations, population screening will be required.

#P10 **Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits**

Functional MRI (fMRI) in Parkinson's disease patients reveals differences according to the degree of hyposmia

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Objective: Olfactory disorders are very common in patients with idiopathic Parkinson´s disease (IPD). Olfactory event related potentials (ERP) in these patients are usually found to be delayed and sometimes cannot be detected at all even though psychophysically olfactory function is present. Using functional magnetic resonance imaging (fMRI) reduced central activity following olfactory stimulation has been shown in IPD.

Combining both techniques we aimed to gain more insight about regions involved in generation of olfactory ERPs. **Subjects and Methods**: Eighteen hyposmic IPD patients of same age and same stage of disease (: ERP+: 3 patients = 1.5; 5 patients ≥ 2; 1 patient =3, vs. ERP -: 5 patients ≤ 1.5; 2 patients ≥ 2; 2 patient =3) were examined using olfactory ERPs. According to the detectability (+) or non-detectability (-) of ERPs patients were assigned into two groups of 9 subjects each (ERP+: *6* women, 3 men; and ERP- :2 women, 7 men) . Central activation during olfactory stimulation was examined using fMRI. **Results:** Both groups, ERP+ and ERP- patients showed activity in areas relevant to olfactory processing such as amygdala, parahippocampal regions and inferior frontal gyrus. ERP+ patients activated additional frontal and temporal regions. Contrasts between both groups showed higher activity in ERP+ patients, especially in amygdala, parahippocampal cortex, insula, cingulate gyrus, striatum and inferior temporal gyrus. **Conclusion:** The degree of central nervous activation in IPD patients reflects the difference in olfactory function as depicted in the presence or absence of an ERP. Moreover, the limited activation in the ERP- group in primary olfactory areas leads to the assumption that further olfactory processing in IPD is severely disturbed.

#P11 Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits

Scent marking and countermarking behaviors as a measure of olfactory communication in the BTBR T+tf/J inbred strain, a mouse model of autism

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Autism is a neurodevelopmental disorder of unknown cause that is usually diagnosed before 3 years of age. No consistent physiological hallmarks of the disease have been identified so far. Diagnosis is currently based on three behavioral criteria: 1) abnormal reciprocal social interactions, 2) impaired verbal and non-verbal communication, and 3) repetitive, restricted interests. Mouse models of autism provide translational strategies to test hypotheses about the causes and to develop treatments. While assays are available for social interaction and repetitive behaviors in those models, there is a need for relevant methods to assess communication impairments. We are currently developing behavioral tools to address specifically the question of olfactory communication in mice. The first task under development examines scent marking in male mice, evoked by female urine. Ultrasonic vocalizations emitted by the male subject mice while sniffing the female urine spot are simultaneously recorded. For this task, we compare sexually experienced male mice to sexually naïve male mice. The second task focuses on the territorial countermarking behavior of male mice. After a first male has scent marked an arena, he is removed and a second male is then placed. Countermarks from the second male are scored. Data will be shown from experiments using those two assays to compare a standard inbred strain that exhibits high sociability, C57BL/6J, to our best mouse model of autism, BTBR T+tf/J (BTBR). Our laboratory and other previously demonstrated that the BTBR inbred strain exhibits multiple social deficits along with repetitive pattern of behaviors that are analogous to the core symptoms of autism. The present experiments explore the hypothesis that BTBR traits also include olfactory communication deficits.

#P12 Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits

Taste damage following radiation treatment for head and neck cancer

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Patients report taste damage following radiation treatment for head and neck cancer. In addition, we recently found an association between reports of altered taste, elevated oral pain and the presence of taste phantoms among 5-year survivors of head and neck cancer compared to a non-cancer comparison group (Logan et al, 2008). To further understand the extent of taste damage from radiation treatment we used spatial taste tests on 2-year cancer survivors (48 men and 13 women) and an age and sex matched non cancer comparison group (38 Men and 17 women). NaCl, sucrose, citric acid and quinine were swabbed on loci innervated by the chorda tympani (CN VII) and glossopharyngeal (CN IX) nerves; whole mouth taste was tested as well. It was hypothesized that the 2-year survivors would show taste damage at sites associated with the glossopharyngeal nerve because of the location of the tumor and radiation treatment (base of tongue and tonsil tumors). Results of t-tests (alpha levels were adjusted for multiple tests) showed significantly lower ratings from the cancer survivors compared to the control group for quinine, sugar, and citric acid at the circumvallate papillae but not NaCl. There were no significant differences between the two groups for the fungiform papillae or whole mouth. Function related oral pain but not spontaneous oral pain was significantly higher among the 2-year cancer survivors; the survivors also reported more taste phantoms. These findings support the taste nerve dis-inhibition hypothesis by showing that localized damage to the glossopharyngeal taste nerve associated with normal whole mouth taste as well as intensified oral pain and taste phantoms.

#P13 Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits

The use of odours as emotional triggers in the study of dysfunctional brain regions in bipolar disorder –an fMRI study

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An emotional over reactivity is a trait characteristic of bipolar disorder. Exploring brain abnormalities underlying this dysfunction is important for understanding the pathology of bipolar disorder and identifying potential risk factors for developing this disease. In this study we used fMRI to investigate the brain activation of 11 euthymic bipolar patients exposed to 2 hedonically different olfactory stimuli compared to a healthy control group matched for age, sex, and smoking behavior. Much more activated voxels in the anterior limbic network were found in the Patients vs. Control contrast when presenting either the pleasant or the unpleasant odor. Olfaction seems to be an interesting model for investigating abnormal patterns of brain

activation in psychiatric disorders due to the overlap with emotional cerebral processing, resulting in immediate and meaningful emotional experiences.

#P14 Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits

Mind over age – Social priming and olfactory function

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Priming of the elderly social stereotype is known to induce changes in subjects' motor and cognitive function, resulting in reduced walking speed and impoverished memory performance akin to those typically associated with the elderly population. To date, no study has explored whether social priming is also capable of modulating primary sensory functions. We explored the effects of social priming on olfactory function using a between-groups design in which subjects were primed by viewing visual and language stimuli of either a neutral (control; n= 18) or elderly (experimental; n= 18) theme before undergoing a battery of motor, cognitive, and olfactory testing. Measures of walking speed were taken covertly as subjects traversed a standard length of hallway to the restroom, word recall scores were taken as measures of memory function, and sensitivity to n-butanol, odor discrimination, and 40-odor identification as olfactory performance measures. There were significant effects of social priming on both walking speed and memory function with experimental subjects demonstrating a 10.77% decrease in walking speed and 28.28% decrease in recalled words, relative to control subjects. However, there were no significant differences between groups in measures of olfactory sensitivity, discrimination, or identification. These data demonstrate that although social priming affects motor and cognitive function, it has limited effects on primary olfactory function.

#P15 Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits

Faster Cognitive Processing of Olfactory Stimuli in an Active Task, Even in Old Age

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The P300 cognitive event-related potential (ERP) was elicited with a single stimulus paradigm for olfactory stimuli in separate active (attend) and passive (ignore) task response experiments utilizing an inter-stimulus interval (ISI) of 30 seconds. Participants were young, middle age and older, healthy adults, all free of cognitive impairment. Amyl acetate was administered via an olfactometer for a duration of 200 milliseconds over 20 trials for each task condition. The passive condition was always presented prior to the active task. In the passive task subjects were instructed to ignore the stimuli and sit and daydream. In the active task subjects were instructed to press a button as soon as they smelled the odor. Previous studies comparing active and passive tasks in the olfactory modality utilized longer ISIs of 45 or 60 milliseconds and demonstrated no significant latency differences between tasks for the P300. Repeated measures MANOVA was utilized for statistical analysis. The current study demonstrates that a 30 second ISI elicits significantly shorter P300 latencies in an active response condition compared to a passive response condition. Additionally this latency difference is consistent across the lifespan. There were no significant age by response task interaction effects. The current study suggests that actively attending to an olfactory stimulus leads to faster cognitive processing of that stimulus, even in old age.

#P16 Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits

Functional Connectivity of Olfactory Processing During a Hedonic Evaluation Task in Young and Older Adults

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Olfactory function declines with age and research using neuroimaging techniques has illustrated reductions in the activation of brain regions involved in olfactory processing in older compared to young adults. The objective of the present analysis was to examine differences in the recruitment of functional networks involved in processing olfactory hedonics in young (ages 18-30) and healthy older adults (ages 65+). Functional connectivity methods allow for the extraction of associations between various brain regions, illustrating the recruitment of an entire functional network involved in a particular task. Comparisons can then be made between network models among different populations. This analysis was run on the functional Magnetic Resonance Imaging time series during which participants received .3ml of a citral solution and were asked to rate the pleasantness of the odor. The resulting patterns of connectivity suggest age-related disturbances in the recruitment of networks related to olfactory processing during evaluation of pleasantness. Identifying differences in functional circuits involved in hedonic evaluation of food-related stimuli in young and older adults may increase understanding of age-related differences in the experience of food reward as well as various nutritional problems that occur with aging.

#P17 Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits

Functional Connectivity during an olfactory recognition memory paradigm is associated with task performance and the e4 allele of the apolipoprotein E (ApoE) gene

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The e4 allele of the apolipoprotein E (ApoE) gene is the strongest genetic risk factor for Alzheimer's disease (AD). The e4 allele is associated with decrements in memory and in olfactory function.

Previous fMRI studies indicate that these processes engage the medial temporal lobes (MTL) and the frontal lobes. The MTL is the initial site of neuropathology in AD. Thus, we expected a disruption in communication between MLT and frontal lobes in e4+ individuals. Moreover, we hypothesized that a task that engages both regions, i.e., odor recognition memory, would be particularly sensitive to loss of connectivity and thus for distinguishing between e4+ and e4- persons. Functional connectivity methods allow for the investigation of the strength of functional associations between different brain regions with the underlying assumption that functional networks underlie specific cognitive processes. The purpose of this study was to investigate differences in functional connectivity during odor memory processing between e4+ and e4- individuals. Prior to fMRI scanning, participants were presented with 16 odors. During two scans, names of odors presented before scanning (targets) or not presented (foils) were shown. Participants discriminated between targets and foils. The results indicate that there were greater associations between bilateral frontal lobes and MTL during correct rejection of foils and correct identification of targets for e4+ relative to e4- individuals. These findings suggest that processes that recruit additional neuronal populations (e.g., compensation, alternative strategies) may facilitate performance in individuals who are genetically at risk for AD. Differences in functional connectivity that distinguish between e4+ and e4- individuals may be useful in predicting incipient dementia.

#P18 **Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits**

Olfactory, but not Gustatory Function, correlates with BMI and Depressive Symptoms in the Elderly

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The decline of chemosensory function known to occur with advancing age has, in turn, been linked to aging-related anorexia and other associated diseases. Few studies, however, have investigated the interaction between chemosensory function and health in the aged population. The National Social Life, Health and Aging Project explores the interactions between physical health and sensory function using a national probability sample of community-residing men and women, aged 57-85 years. We examined chemosensory function and its relation to body mass index (BMI, kg/m^2) and depressive symptoms in this population. Olfactory function was assessed using a five-item odor identification test. Odor ID was separated into food (peppermint, fish, orange) and non-food (rose, leather) items. Four taste-impregnated strips of filter paper (sweet, sour, bitter, salty) were employed to assess gustatory function. Correlation coefficients were computed to determine the correlation between olfactory or gustatory function and BMI or depressive symptoms (measured by the CES-D). The prevalence of ageusia was 14.8%. 'Sour' was identified correctly least often (39.4%). The prevalence of functional anosmia was 2.7%. Food items were better identified than non-food items (88.2% vs 73.7%, p<.001). Odor ID exhibited a positive, albeit weak, correlation with BMI (p=.005) and a slight negative correlation with CES-D (p<.001). There were no significant correlations between taste ID and either CES-D or BMI. The results of this analysis indicate that depressive symptoms are correlated with olfactory, but not gustatory, function, and that BMI is associated with olfactory function. These data also demonstrate that even brief tests are capable of detecting covariation between chemosensory function and clinically relevant health measures.

#P19 **Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits**

Prevalence of Olfactory Impairment in Adults across the Life Span: The Beaver Dam Offspring Study

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Olfactory impairment is common in older adults but there are limited test data available on younger adults in the general population. The objective of the present study is to determine the prevalence of olfactory impairment in adults across the age span and risk factors associated with impairment. The San Diego Odor Identification Test (SDOIT) was used to assess olfaction in participants aged 21-84 years in the Beaver Dam Offspring Study (BOSS), a study of familial and birth cohort effects on aging senses. The SDOIT uses eight common household odors that are presented in opaque jars. Subjects were asked to identify each odorant using a picture board containing the eight odorants and 12 distracter items. Olfactory impairment was defined as correctly identifying fewer than 6 out of 8 odorants after two trials. In preliminary analyses (n=2838, mean age = 49 yrs), 3.8% had an olfactory impairment and the prevalence was higher in men (5.6%) than women (2.4%). The prevalence of impairment was less than one percent in those aged 21-34 yrs and 13.9% in those aged 65 yrs and older. In a multivariate logistic regression model, age (Odds Ratio (OR)=1.48, 95% Confidence Interval (CI)=1.34, 1.65; every 5 yrs), sex (OR=2.44, 95% CI=1.57, 3.78; men vs. women), education (OR=2.30, 95% CI=1.06, 5.01; <12 yrs vs. 12 or more years), ankle-brachial index <0.9(OR=4.21, 95% CI=1.74, 10.18), history of nasal polyps (OR=2.36, 95% CI=1.04, 5.35) and deviated septum(OR=1.85, 95% CI=1.03, 3.30) were associated with olfactory impairment while a municipal water source as a child was borderline protective (OR=0.65, 95%CI=0.42, 1.01; vs well or other source). These results suggest there are potentially modifiable risk factors associated with olfactory impairment in middle-aged adults.

#P20 **Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits**

ApoE Status and Differences in Olfactory Detection Across the Lifespan

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A gradual decline in olfaction is associated with healthy aging. For people who have the e4 allele of the Apolipoprotein (ApoE) gene, that process begins earlier and declines faster than those without

the e4 allele. The ApoE e4 allele is also associated with increased risk for Alzheimer's disease. When measuring olfactory event-related potentials (OERPs), the third peak (P3) is considered to be a cognitive component. The purpose of this study was to investigate the effects of age and ApoE status on the P3 component of OERPs. Subjects in age groups of young (18-28 years old), middle age (45-56), and older (65 +) were administered an olfactory detection task to elicit the OERP. Participants were instructed to respond as quickly as possible after smelling the odor by pressing a button. The stimulus was amyl acetate presented every 30 seconds by olfactometer. Statistical analyses were performed using a multivariate analysis of variance. No significant effects for P3 amplitude were found. There was no significant effect of ApoE e4 status on latency for the young or middle age groups. In the older group, ApoE e4 positive participants had significantly longer latencies than their negative counterparts. They also had longer latencies than the positive young and middle age participants. The effect size in the current study is larger than previous studies that previously demonstrated increased latencies for this population likely due to the decreased inter-stimulus interval to 30 seconds from 45 seconds. The shorter inter-stimulus interval increased the difficulty of the task by giving the participant less time to recover from each stimulus.

#P21 **Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits**

Influence of Cognitive Status on Olfactory Threshold Variability

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Detection thresholds have been used by a number of investigators interested in the effects of neurodegenerative disorders and normal aging on the olfactory system. Odor thresholds are occasionally compared to performance on more cognitively demanding tests under the assumption that threshold measures are only minimally affected by disease or age-related deficits in memory or other cognitive functions, and therefore provide a better measure of "pure" sensory function. We recently demonstrated that although thresholds are less subject to the influence of cognitive changes as compared to odor identification, thresholds are influenced by cognitive variables such as cognitive processing speed. The current study examined the influence of cognitive status on the olfactory abilities of young and older adults as measured by detection thresholds. The main question of interest was whether the ability to discriminate between the two subject groups would change if the effects of cognitive status were taken into account. A sample of 34 healthy, community-dwelling older adults was compared to a sample of 31 healthy, young adults. Odor detection thresholds for butanol were determined on four occasions for each group and supplemented by neuropsychological testing. The two groups of participants were separated easily using the average of the four thresholds with the difference between the groups accounting for 25% of the variance in thresholds. Using the Montreal Cognitive Assessment (MoCA) score as a covariant sharply reduced the difference between the groups, and lower the variance accounted for by the group effect to 8%. These results provide evidence that mild cognitive deficits among older adults can augment differences in odor threshold measurements obtained from younger and older people.

#P22 **Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits**

Olfactory Perceptual Correlates of β-Amyloid Plaque Burden in Alzheimer's Disease Mouse Models

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Alzheimer's Disease (AD) often results in a substantial loss in olfactory perceptual acuity – the cause of which is unknown. To begin addressing this, we explored odor perception in two AD mouse model lines, which express mutated forms of human amyloid precursor protein (APP) and consequently display - β amyloid (Aβ) plaques characteristic of AD. We found a significant olfactory behavioral phenotype in both lines of AD mice. While all mice showed a robust investigation of novel odors, AD mouse lines investigated novel odors for greater durations than age-matched controls and showed an increased latency to habituate to novel odors. These behavioral results suggest a deficiency in normal olfactory coding similar to that reported clinically in humans with AD. Given this phenotype, we are currently examining whether odor perceptual deficits correlate with plaque load in the olfactory bulb and piriform cortex within the same mice. If so, then these results may be consistent with an important role for Aβ or other Aβ pathogenic factors within olfactory circuits on odor perceptual deficits in AD.

#P23 **Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits**

Toxin-Induced Chemosensory Dysfunction: A Case Series and Review

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Toxic chemical exposures, both acute and chronic, are estimated to account for 1-5% of all olfactory disorders. Likewise, taste buds are susceptible to direct chemical injury due to their vulnerable position in the oral cavity and oropharynx. This area has been a relatively neglected topic in the medical literature, with previous publications limited primarily to single case reports or broad reviews. We describe 8 cases here that illustrate different aspects of the diagnostic and therapeutic approach to patients with this disorder. Cases were selected for inclusion based on a retrospective chart review of patients who presented to a university-based nasal dysfunction clinic with toxin-induced olfactory or gustatory dysfunction between January 1985 and December 2008. Patient ages ranged from 17-67 years (mean 41.6 years). Etiology of chemosensory impairment included exposure to ammonia, isodecanes, permanent wave chemicals, chemotherapy, gasoline and intranasal zinc. Workup included complete history, medical examination, psychophysical testing, and imaging. Symptom ratings, odor threshold, odor identification, and/or University of Pennsylvania Smell Identification Test were used to assess olfactory impairment.

Five out of the 8 patients (62.5%) presented with olfactory dysfunction alone, 1 patient (12.5%) presented with gustatory dysfunction alone, and 1 patient (12.5%) presented with both smell and taste loss. Only 1 patient (12.5%) reported parosmias. Olfactory testing revealed mild-to-moderate hyposmia in 1 patient (12.5%) and severe hyposmia-to-anosmia in 6 patients (75%). Both patients who reported taste loss had hypogeusia upon testing. This case series helps illustrate the wide spectrum of this disorder and provides a framework for the workup and treatment of these patients.

#P24 Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits

Gene-targeted deletion of E2F1 evokes olfactory deficits, memory loss, and increased anxiety

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Adult neurogenesis (ANG) plays a substantial role in the maintenance and function of the central nervous system. Throughout the process of ANG, tight control of cell cycle, proliferation, and apoptosis is maintained via cell cycle proteins. Specifically, the cell cycle protein E2F1 plays an important role during generation and replacement of neurons in the adult brain, as demonstrated by a substantial reduction of ANG in the olfactory bulb and dentate gyrus (DG) of E2F1 knockout mice (KO). The OB and DG are critical in olfaction and memory, hence we hypothesized that E2F1 KO mice may develop deficits in olfactory ability and memory. Wild-type (WT) and KO mice of five age ranges were subjected to general anosmia and object memory behavioral paradigms to assess basic olfactory and memory function. E2F1 KO mice displayed a threefold increase in retrieval time of a scented object versus WT mice, suggesting that age-dependent deficits in olfaction and/or motivation arise. Counts of olfactory sensory neurons were performed to determine if peripheral neuron loss could be involved, however, there were no reduction in numbers nor morphological changes. Additionally, all WT age groups demonstrated adequate short-term memory (1 hour), whereas E2F1 KO mice object memory recognition deteriorated over time, failing tests at postnatal day ages 270 and 365. Interestingly, E2F1 KO mice displayed incessant digging during tests, so marble burying and light/dark box (LDB) behavior were assayed to determine anxiety levels. E2F1 KO mice buried significantly more marbles than WT mice in all age groups, and spent significantly less time in the light chamber of the LDB in 4/5 age groups. Thus, E2F1 KO mice display a sequence pattern of early and continued anxiety, followed by deficits in olfaction and then memory.

#P25 Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits

Social Anxiety and Reduced Recruitment of Orbitofrontal Cortex to Human Social Chemosensory Cues

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Social anxiety refers to the prevalent and debilitating experience of fear and anxiety of being scrutinized in social situations. It arises from both adverse social conditioning and inherent factors like shyness. Previous research focuses on negative emotions in socially anxious patients induced by visual and auditory social cues, and posits a dysfunctional orbitofrontal-amgydala circuitry as a primary etiological mechanism. Here using functional magnetic resonance imaging (fMRI) and a unique type of social stimuli, airborne human social chemosensory cues that are inherently social and ubiquitously present, but not typically conditioned to trigger subjective anxiety, we show that individuals with elevated social anxiety demonstrated a reduced recruitment of the orbitofrontal cortex to social chemosensory cues. No reciprocal activity in the amygdala was observed. Our findings point to an internal neural substrate underlying social anxiety independent of previous adverse social conditioning from experience, thereby providing the first neural evidence for the inherent social aspect of the enigmatic phenomenon.

#P26 Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits

***Toxoplasma gondii* infects olfactory structures in the mouse: a possible mechanism for host maniplulation by influencing olfactory function**

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Toxoplasma gondii (*T. gondii*) is a protozoan parasite that infects most warm-blooded vertebrates including humans. Once in the body *T. gondii* can enter the central nervous system where it forms latent cysts. While generally thought to be benign, *T. gondii* in its latent form has been shown to adversely affect predator avoidance in rodents. This is one of the few examples of a parasite manipulating the behavior of a vertebrate host. It is still unclear how *T. gondii* exerts its effects on its host. Here we explore whether T.gondii affects a host's behavior through a direct manipulation of the olfactory system. Using C57/Blk6 male mice that were chronically infected with *T. gondii* we observed two behavioral abnormalities associated with olfactory function. First, we observed a reduced avoidance to a predator odor (cat urine) in *T. gondii* infected mice compared to sham infected mice. *T. gondii* infected mice spent significantly more time investigating cat urine than sham mice in a two choice preference test (t-test, $P<.014$). Second, cat urine induced anxiogenic responses in sham infected but not *T. gondii* infected mice. Sham infected mice significantly reduced the amount of time they spent in the open arms of an elevated plus maze after being exposed to cat urine. *T. gondii* infected mice did not show this same anxiogenic response to the cat urine ($F_{2, 26}=12.1$; $P<0.0002$). Subsequent histological analysis of the olfactory systems of infected mice revealed that latent cysts are observed in multiple olfactory areas

including the olfactory epithelium, olfactory bulbs and the medial amygdala. These results are the first step towards showing that *T. gondii* cysts directly influence the olfactory system leading to an altered behavioral response to predator odors.

#P27 **Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits**

Sexual dimorphism in olfactory bulb structure

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Sexual dimorphism in mammals is often observed in the accessory olfactory system, however, this system is rudimentary in species such as the American mink. Thus, we were interested, if sex-dependent structural differences exist in the main olfactory system. Olfactory bulbs of adult American minks color-variety "standard" (*Neovison vison var. atratus*) were processed histologically and always the right bulb analyzed with a morphometric system using weight/volume correction factors. The olfactory bulb is significantly bigger in males (152mm^3) compared to females (107mm^3), however, females are much smaller than males and so is their absolute brain weight (m 11.51g; f 8.43g). Thus, the bulb-portion on the whole brain is similar (m 1.37%; f 1.32%). On the other hand, the brain relative to the body mass is much bigger in females (0.85%) compared to males (0.55%), resulting in significantly different bulb/body ratios (m 0.0076%; f 0.0112%). Sex-dependent differences exist also in the proportion of the neuronal layers: In males, the olfactory fila comprise the major portion of all layers (26.9%) but only 17.7% in females, where the majors are granule cell (GCL 28.8%) and external plexiform layer (EPL 22.8%) overwhelming the proportions in males (GCL 21.7%; EPL 19.2%) significantly. The mitral cell layer is significantly thicker in males (4.7%) than females (3.5%).This indicates that the fila layer is related more to overall body size (increase in the olfactory sheet / axon numbers) whereas the information processing GCL and EPL is related to the brain size. This suggests also, that information processing is much more complex in females and to sustain the extended GCL there might be a higher neurogenesis. Therefore, a significant sexual dimorphism in the main olfactory bulb exists, not reported before.

#P28 **Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits**

Heterogeneous Sensory Innervation of Individual Necklace Glomeruli

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The mammalian nose employs several olfactory subsystems to recognize and transduce diverse chemosensory stimuli. These subsystems differ in their anatomical position within the nasal cavity, their targets in the olfactory forebrain, and the transduction mechanisms they employ. Here we report that they can also differ in the strategies they use for stimulus coding. Necklace glomeruli are the sole main olfactory bulb (MOB) targets of an olfactory sensory neuron (OSN) subpopulation distinguished by its expression of the receptor guanylyl cyclase GC-D and the phosphodiesterase PDE2, and by its chemosensitivity to the natriuretic peptides uroguanylin and guanylin and the gas CO_2. In stark contrast to the homogeneous sensory innervation of canonical MOB glomeruli from OSNs expressing the same odorant receptor (OR), we find that each necklace glomerulus of the mouse receives heterogeneous innervation from at least two distinct sensory neuron populations: one expressing GC-D and PDE2, the other expressing olfactory marker protein (OMP). In the main olfactory system it is thought that odor identity is encoded by a combinatorial strategy and represented in the MOB by a pattern of glomerular activation. This combinatorial coding scheme requires functionally homogeneous sensory inputs to individual glomeruli by OSNs expressing the same OR and displaying uniform stimulus selectivity; thus, activity in each glomerulus reflects the stimulation of a single OSN type. The heterogeneous sensory innervation of individual necklace glomeruli by multiple, functionally distinct, OSN subtypes precludes a similar combinatorial coding strategy in this olfactory subsystem. Instead it suggests that the necklace glomeruli could serve as coincidence detectors for multiple chemosensory stimuli.

#P29 **Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits**

Spatial analysis of olfactory bulb activity in the sea lamprey

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The sea lamprey (Petromyzon marinus) uses olfaction to detect a variety of odours that induce movement and searching behaviours. The peripheral olfactory organ of sea lamprey is comprised of the main olfactory epithelium (MOE) and the accessory olfactory organ (AOO). Neural connections from the AOO project solely to the medial region of the olfactory bulb (OB) while projections from the MOE are distributed to all glomerular regions. Our goal is to examine odour input specificity and processing in the OB of sea lamprey as it relates to behaviour. To that effect, we examined the morphological characteristics of the MOE and AOO as well as the organization of odour processing in the OB. The peripheral olfactory organ of metamorphic, parasitic, and migratory adult lamprey was dissected, sectioned, and the relative area of the MOE and AOO was calculated. The proportion of AOO in the peripheral olfactory organ increased from 22% in metamorphic lamprey to upwards of 30% in parasitic and migratory adult sea lamprey indicating an increase in input from the AOO to the OB during adult life stages. Additionally, multi-unit action potentials in response to odours were recorded in the OB of the live ex vivo brain of parasitic and migratory adult sea lampreys. Action potential frequency increased in the medial OB in response to bile acids and the lamprey pheromone 3KPZS. Action potential frequency also increased in the lateral and ventral-lateral OB in response to basic amino acids and 3KPZS, respectively. These results indicate that odour input and processing in the medial OB is important for responding to behaviourally-relevant odours.
Funding provided by the GLFC and NSERC.

#P30 **Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits**

Spatial Representations of Natural Odor Objects Across the Glomerular Layer of the Rat Olfactory Bulb

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We previously have determined glomerular responses to 365 monomolecular odorants, using uptake of 2-deoxyglucose to quantitatively assess activity across the entire rat olfactory bulb. To determine how these response patterns compare to responses evoked by more natural odor stimuli, we now have mapped uptake during exposures to vapors arising from a variety of objects that might be important to rodents in the wild. Sixteen distinct stimuli ranging from possible food sources such as fruits, vegetables, and meats to environmental objects such as grass, herbs, and tree leaves were chopped or homogenized and then their vapors introduced into an air stream in the same manner as we used previously for pure odorant chemicals. The natural odor objects evoked robust and surprisingly focal patterns of 2-deoxyglucose uptake that in some cases were closely related to patterns evoked by known major monomolecular components that are represented in our archive, but that in other cases were more simple than might have been predicted given the multiplicity of components that must have been present in the vapors. These data suggest the possibility of important mixture response interactions and provide a foundation for understanding the neural coding of natural odor stimuli.

#P31 **Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits**

Analysis of responses to musk odorants in olfactory sensory neurons and in the main olfactory bulb

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Musk odorants are widely utilized in various perfumes because of their fascinating aminalic note. They exhibit similar odor characters despite their different chemical structures such as macrocyclic, nitro and polycyclic moieties. To elucidate the mechanism of musk odor perception, we investigated the response patterns of mouse olfactory sensory neurons (OSNs) and the main olfactory bulb (MOB) to musk odorants. In Ca^{2+} imaging, 68 neurons out of ~3000 dissociated OSNs responded to eugenol, whereas only 4 neurons responded to muscone, one of macrocyclic musk odorants. We next examined responses to musk odorants in the MOB using OMP-spH mice. No responsive glomerulus was found in the dorsal and lateral regions of MOB. Then, we performed unilateral bulbectomy to image responses in the medial region of MOB. This surgical technique enabled us to observe a large part of the medial region of MOB, the area that had not been imaged previously. Interestingly, only a few glomeruli in the medial region showed responses to muscone. The muscone-responsive glomeruli did not respond to nitro musks, polycyclic musks and other classes of odorants such as aldehydes, acetates and benzenoid compounds. Using c-Fos expression as a marker for odor-induced neuronal activity in the glomerular layer of the MOB, we analyzed spatial patterns of c-Fos positive glomeruli that were stimulated with musk odorants. c-Fos induction by muscone was observed around only a few glomeruli that were located in a region very similar to those obtained by the bulbar imaging. Our findings indicate that muscone appears to be recognized by a few narrowly-tuned ORs and muscone activates a few glomeruli in the restricted region of the medial MOB in mice.

#P32 **Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits**

Input Driven Synchrony of Oscillating Olfactory Receptor Neurons: A Computational Modeling Study

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Temporally structured activity in bursting and/or oscillating neurons can be utilized to embed the temporal structure of sensory input into an instantaneous population code. We hypothesize that spontaneously bursting olfactory receptor neurons (ORNs) reported earlier by our group can extract temporal features of the odor signal. We showed that, at least in lobster, each such ORN responds to a narrow range of stimulus frequencies based on their spontaneous bursting discharge and phase dependent response to odor stimulation. A heterogeneous population of such ORNs could encode a wide spectrum of stimulus intermittency and also increase the reliability of encoding. Computational model based on a modified renewal process was extrapolated from experimental recordings from single lobster ORNs, and used to generate population responses to a stimulation pattern. Simulation and analytical methods were used to obtain the probability of various temporal patterns of response. A homogeneous population showed synchronization dynamics which changed in a stimulus frequency dependent manner and could be enhanced, maintained, or depressed. In the absence of stimulation, the population quickly approached the asymptotic pattern. To test if the heterogeneous population could actually encode stimulus intermittency, a neural decoder was built using machine learning. We suggest a biologically plausible neural decoder based on a simple integrating neuron.

#P33 **Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits**

Increase in Number of Androgen Receptor Immunoreactive Cells in the Medial Amygdala of Male Hamsters in Response to Chemosensory Input

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In many species, including hamsters, mating behavior is dependent on integration of chemosensory and hormonal cues. Chemosensory stimuli are detected by vomeronasal system, which projects to many regions that contain steroid receptors, including the medial amygdala (Me). Sexual behavior is also facilitated in male hamsters by direct action of testosterone within the medial amygdala. Me can be subdivided into anterior (MeA)

and posterior (MeP), which are differentially activated by different types of chemosignals. Conspecific stimuli activate MeA and MeP, while heterospecific stimuli only activate MeA. Furthermore, chemosensory stimuli with different social significance may differentially activate the dorsal and ventral subdivisions of MeA and MeP. Therefore, it is likely that steroid receptors facilitate stimulation of Me by different types of chemosensory stimuli. We examined activation, using Fos protein expression, of androgen receptor (AR)-containing cells in Me by heterospecific and conspecific stimuli with different social relevance (i.e., reproductive, competitive). Surprisingly, in response to chemosensory stimulus exposure, the number of AR-immunoreactive (IR) cells was significantly different from control and between stimuli with different social significance. Activation of AR-containing cells was also significantly different from control and between the different stimuli. These effects may be due to acute increases in testosterone that occur in response to chemosignal exposure. Thus, castrated males with testosterone replacement treatment are currently being tested in order to eliminate the possible testosterone feedback that occurs in response to chemosensory stimulus exposure.

#P34 **Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits**

Cytokine profiles in nasal lavage fluid of patients with chronic rhinosinusitis

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Chronic rhinosinusitis (CRS) is an inflammatory condition of the nose and the paranasal sinuses characterized by an infiltration of inflammatory cells, with associated edema, pain, and congestion, that significantly impairs quality of life and, in approximately 1/3 of cases, impairs olfactory sensitivity. Inflammation is a necessary aspect of the response to a foreign irritant or infection, and under normal conditions triggers recovery and regeneration. In some individuals, normal anti-inflammatory counter-regulatory pathways fail to activate sufficiently to resolve the inflammatory process, and the reasons for this failure are not understood. Complete understanding of the nasal inflammatory process requires measurement of multiple cytokines simultaneously. In this study, we aimed to examine the level of cytokines in nasal lavage fluid obtained from the initial visit of patients diagnosed with CRS (n=64) and healthy controls (n=26). A multiplex assay (Beadlyte, Millipore) was used for simultaneous quantification of 19 cytokines. Variation was greater among patients, and levels of IL2, IL4, IL13, IL15, IL1beta, MIP1alpha and Rantes were below the limit of detection in too many samples for reliable comparison. Levels of IL8 were significantly elevated in CRS patients relative to controls, and similar trends were observed for EGF, eotaxin, GRO and MCP1. Analyses of these data in relation to tissue degradation and patient outcomes may aid in the prediction of olfactory loss or disease resolution.

#P35 **Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits**

Voltage-sensitive dye imaging of odor evoked activity patterns in the trigeminal ganglion *in vivo*

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Chemosensation from the mammalian nasal cavity is predominantly mediated by two independent neural systems, the olfactory and the somatosensory (trigeminal) system. Optical imaging techniques have thus far added significant knowledge regarding the functional organization of information processing at the level of the olfactory bulb. In contrast, due to the difficulty in accessing trigeminal ganglia somata and nerve fibers experimentally, a direct visualization of evoked population activity in the trigeminal ganglion *in vivo* remained elusive, leaving questions about the spatio-temporal representation of odor related stimuli in the trigeminal ganglion unexplored. We established a preparation that allows high-resolution recording of optical signals arising from a large region of the rat trigeminal ganglion *in vivo*, using voltage-sensitive dye imaging. Stimuli were individually delivered by a specialized custom-made olfactometer. Tested substances include CO_2 as a pure pain activator, odorants known to have a strong trigeminal component, as well as classical olfactory stimuli. Our data indicate a prototypical activation pattern related to a painful CO_2 stimulus. Stimulation with Ethanol, an odorant with a strong trigeminal component produced an activation that showed high similarity to this "pain"-pattern. Moreover the Ethanol map included unique activation spots that might code for odor identity. In contrast classical olfactory stimuli elicited activation patterns clearly distinct from such "pain"-pattern. In conclusion this study provides first evidence that coding odor information might not be a feature unique to the olfactory system, but to some extent also possible via the trigeminal nerve.

#P36 **Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits**

Microglial response in the nucleus of the solitary tract after chorda tympani nerve injury

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Following transection of the chorda tympani (CT), the nerve reinnervates peripheral targets to permit regeneration of taste buds. Despite the peripheral recovery, there remains persistent alterations of gustatory terminal fields in the nucleus of the solitary tract (nTS), the primary gustatory nucleus in the brainstem (Reddaway and Hill, AChemS abstract 2007 #516). In primary sensory nuclei of other systems, peripheral nerve damage provokes changes in microglial populations which may be involved in resultant synaptic remodeling. The gustatory system remains unexplored regarding this possibility. We sought to test whether microglia in the nTS respond to unilateral transection of

the CT. In adult mice, the CT was sectioned in its course via the middle ear. The mice survived for various periods (1-30 days; n=4,5 at each time). Immunohistochemical staining for microglia using Iba1 (cytoplasmic calcium binding protein specific for microglia) reveals a dramatic increase in the number of stained microglia in the ipsilateral nTS at 2 days post lesion. Microglial cell counts on the uninjured side of the nTS are not significantly different from those of uninjured animals. Further, the microglia on the injured side have an altered morphology. In normal animals, Iba1 staining shows microglia with thin, highly ramified processes and smooth nuclei indicative of a resting state. On the injured side, microglia in the nTS have short, thick processes and a more 'ragged' nuclei, a morphology typical of active microglia. In this state, these cells are capable of releasing a variety of secretory molecules and becoming phagocytic. We also tested whether microglia proliferate locally in the nTS or are being recruited from the hematopoetic system in response to the peripheral injury.

#P37 **Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits**

Convergent Innervation Patterns of the Chorda Tympani and Glossopharyngeal Nerves onto Nucleus of the Solitary Tract Projection Neurons

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The innervation patterns of gustatory responsive nerves in the rNTS is relatively unknown. We aimed to identify putative synapses between either chorda tympani (CT) or glossopharyngeal (IX) nerves and individual projection neurons in the rostral nucleus of the solitary tract (rNTS). Biotinylated dextran amine (BDA) was injected into the waist region of the parabrachial nucleus to retrogradely fill projection neurons in the rNTS. Subsequently, CT was labeled with tetramethylrhodamine and IX with Cascade Blue and allowed 48 hours to transport. After perfusion and tissue sectioning, BDA was bound with streptavidin Alexa488, resulting in triple fluorescently-labeled tissue. Sections containing CT and IX terminal fields as well as filled projection neurons were then imaged at 90x on a laser scanning confocal microscope. The images were then deconvolved, the dendritic trees reconstructed from multiple confocal z-stacks, and the cell visualized in 3-dimensions. The dendritic trees were traced and any contacts between the labeled nerves and the cell identified. From these diagrams, innervation patterns were ascertained for neurons that received convergent input from CT and IX nerves. The majority of the imaged neurons received convergent input, often onto the same dendritic tree. Both multipolar and bipolar projection neurons were analyzed and further classified based on their dendritic branching patterns as well as location in the rNTS. Further, innervation patterns for each neuron were also examined, such as the distance of the input from the soma and the clustering of like-inputs. This is the first anatomical demonstration and analysis of convergent input from CT and IX and as such, these results will lead to a greater understanding of information processing in the rNTS.

#P38 **Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits**

Synaptic Profile of Amygdala Terminals in Rodent Brainstem Gustatory Nuclei

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Neurons in the central nucleus of the amygdala (CeA) send projections to the nucleus of the solitary tract (NST) and parabrachial nucleus (PBN) that modulate taste-elicited responses. However, the proportion of forebrain-induced excitatory and inhibitory effects appears to differ when taste cell recording changes from the NST to the PBN. The present study investigated the synaptic targets of CeA terminals in the rat NST and PBN. Following injection of biotinylated dextran amine (BDA) into the CeA, ultra thin sections of NST and PBN tissue containing BDA-labeled terminals were processed for electron microscopy and immunogold labeled for GABA. A total of 46 synapses were analyzed: n=19, NST; n=17, medial/waist PBN; and n=10, lateral PBN. The average pre-synaptic terminal area was 0.768 ± 0.079 μm² for NST, 0.560 ± 0.059 μm² for medial/waist PBN, and 0.547 ± 0.058 μm² for lateral PBN. Post-synaptic profile size was comparable between brainstem regions ranging from 1.177 ± 0.290 μm² to 1.442 ± 0.422 μm². Based on a threshold of 32.334 gold particles/μm², the majority of CeA terminals were GABA positive (NST, 77.825 ± 7.357 Au/μm²; medial/waist PBN, 108.676 ± 9.828 Au/μm²; and lateral PBN, 83.276 ± 14.184 Au/μm²). The post-synaptic targets of CeA terminals were primarily non-GABAergic (NST, 13.991 ± 1.809 Au/μm²; medial/waist PBN, 26.590 ± 8.317 Au/μm²; and lateral PBN, 17.433 ± 8.124 Au/μm²). The present results indicate that differences in descending modulation of taste processing in NST and PBN are not due to terminals of CeA origin differentially targeting post-synaptic GABAergic neural elements.

#P39 **Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits**

Activation of the dorsal motor nucleus of the vagus nerve modulates taste responses of the neurons in the parabrachial nuclei

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The PbN is one of the sites that integrates gustatory and visceral sensory information. It was reported that taste-responsive neurons in the PbN were coactivated by cervical vagus nerve stimulation and that taste responses of PbN neurons were modulated by gastric distension or duodenal lipids in the rat. The dorsal motor nucleus of the vagus nerve (DMX) is one of the areas that visceral afferent fibers terminate. Anatomically, two types of neurons were identified in the DMX; the cells that form efferent fibers of the vagus nerve and cells that do not. The latter type of cells were retrogradely labeled after injection of HRP into the PbN, suggesting that part of visceral afferent information is transmitted to the PbN through the DMX. Here, we investigated whether taste-responsive neurons in the PbN can be coactivated by DMX stimulation and whether activation of the DMX modulates taste responses of the neurons in the PbN. Seventy-eight of 80 PbN neurons were orthodromically activated following ipsilateral and/or contralateral DMX stimulation;

75 and 39 of 80 PbN cells were activated after the ipsilateral and contralateral DMX stimulation, respectively. Among these activated cells, only 7 cells were inhibited. All inhibitory responses were evoked after contralateral DMX stimulation. The effect of activation of the DMX on taste-driven responses of the PN cells was examined on 20 PbN cells that were excited, and all seven cells that were inhibited after the DMX stimulation. The DMX stimulation enhanced or inhibited the taste-evoked activities in all cells tested, parallel to the type of inputs which they received from the DMX. These results suggest that taste-responsive neurons in the PbN receive convergent input from the DMX and that DMX activation modulates taste responses of these cells.

#P40 **Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits**

Taste Responsive Multipolar and Elongated Neurons in Hamster Nucleus of the Solitary Tract (NST) Project Differentially to Targets in the Brainstem: An In-Vivo Intracellular Recording, Labeling, and Tracing Study

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Neurons in the rostral part of the nucleus of the solitary tract (NST) have been described morphologically as multipolar, elongated (fusiform), and ovoid. Attempts, including our own, to relate neuronal structure (cell size, shape, and/or dendritic configuration) to function (taste specific input, firing characteristics) have met with limited success. One avenue, still unexplored is whether classes of NST neurons project to different targets in the brainstem. To address this possibility, we recorded the activity of taste responsive neurons in NST, labeled those neurons with biocytin, and traced their projections to target regions in the brainstem. We recorded responses to lingual stimulation with anodal current and to tastants that included sucrose, NaCl, citric acid, and quinine. Recorded cells were labeled with 2% biocytin, and the animal maintained for a minimum of 2.5 hrs to allow for axonal transport. Hamsters were anesthetized and then perfused with saline followed by 4% paraformaldehyde. Brains were removed, sectioned at 100 µm thickness in a horizontal or sagittal plane, stained with cytochrome oxidase and/or Nissl to visualize brainstem nuclei and labeled cells. Data were taken from ten unambiguously labeled neurons, which were examined for taste input, firing properties, and reconstructed using Neurolucida. Our results suggest that elongated cells (n=3) project exclusively to the hypoglossal nucleus while multipolar (n=7) project to parabrachial nucleus, reticular formation and/or trigeminal sensorimotor nuclei. These results obtained from a limited number of labeled cells suggest that NST cell morphology may relate, in part, to their efferent target(s).

#P41 **Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits**

Analysis of Spike Train Variability in Chemosensory Neurons Within the Rat Geniculate Ganglion

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Sensory neurons encode information by using the number of spikes and /or the precise timing of these spikes. Here, we examined the coefficient of variation (CV) of the inter-spike interval distribution of GG neurons after application of the basic taste stimuli. In anesthetized male rats, we recorded single-cell 5-s responses from 17 narrowly tuned (8 to sucrose; 9 to MSG) and 15 broadly tuned (7 NaCl-best; 8 citric acid-best) neurons. Broadly tuned neurons responded robustly to 10 mM citric acid and 100 mM KCl indicating input from Type III, presynaptic cells in fungiform taste buds, while narrowly tuned neurons were unresponsive to these two stimuli indicating input from Type II, receptor cells. Narrowly tuned neurons had an average spontaneous rate of 0.3± 0.2 spikes/s, response latency of 0.6 ± 0.3s, and response frequency of 8.5 ± 3.2 spikes/s. Their CV dropped gradually from 0.8 before stimulation to 0.5 after the start of the response and remained stable thereafter during the stimulation time. Broadly tuned neurons had an average spontaneous rate of 2.3 ± 0.6 spikes/s, a response latency of 1.5± 0.7s, and response frequency of 12 ± 3.2spikes/s. Their CV decreased from 0.9 before stimulation to an intermediate low and achieving a distinct minimum of 0.4 3-s after stimulus application and lasting a few hundred milliseconds. These temporal discharge patterns were specific to some chemical stimuli or mixtures of them. Analysis of patterns of activity across many neurons will help us understand how GG neurons code taste information.

#P42 **Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits**

Neuron Survival and Central Terminal Field Persistence Despite Limited Peripherial Regeneration of the Injured Chorda Tympani Nerve in Adult Rats

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Unilateral chorda tympani nerve transection (CTX) produces persistent morphological and physiological changes in the peripheral and central taste systems despite functional regeneration of the injured nerve in adult rats. To better understand changes resulting from CTX, we focused experiments on the injured CT nerve. Primary sensory neuron death and transganglionic degeneration are typical consequences of sensory nerve transection and are often attributed to the loss of trophic support from peripheral target organs. However in the taste system where the peripheral targets (taste buds) are constantly turning-over, there is potential for an attenuated peripheral trophic dependence. The current study uses fluorescent retrograde tracers to measure CT cell numbers in the geniculate ganglia and nerve terminal field volumes in the rostral nucleus of the solitary tract (rNTS) following nerve injury. Two different fluorescent labels, the first (DiI) applied at the time of CTX and the second (Micro-Emerald) at 14, 60, 90, or 120 post-CTX, were used to measure 1) the total number of CT neurons surviving CTX

and 2) the number of CT neurons with regenerated peripheral axons. Transgangionically transported fluorescent label allows us to compare the volume of vestigial and functional CT nerve terminal field post-CTX. Our results indicate that CT cells do not die following CTX, despite attenuated regeneration of peripheral axons. In addition, measures of labeled CT terminal fields in the rNTS indicate 1) a lack of degeneration, and 2) a reduction in central axons that are functionally connected to peripheral targets. Such measures will be useful for understanding injury-induced changes seen in experimental animal populations and taste abnormalities seen in human patients after CT nerve injury.

#P43 **Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits**

A Network Model of Taste Processing in the Nucleus of the Solitary Tract

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Though the functional architecture of many primary sensory nuclei has been well characterized, the organization of the nucleus of the solitary tract (NTS), the first central relay for gustation, remains a mystery. Here, we used electrophysiological data recorded from single cells in the NTS to inform a network model of taste processing. Previous studies have revealed that stimulation of the chorda tympani (CT) nerve initiates two forms of inhibitory influences in separate groups of NTS cells. These forms of inhibition differed in time course and were correlated with distinct NTS taste response properties. That is, one inhibitory influence peaked early, decayed rapidly and was associated with short latency responses to CT stimulation and narrow tuning across tastants. Conversely, the second inhibitory influence peaked late, decayed slowly and was seen in cells with long latency responses to CT stimulation and broad tuning. Based on these data, we designed a model of the NTS consisting of discrete cell assemblies with a projection neuron and two different types of inhibitory interneuron. Each cell assembly is reciprocally connected to every other and is characterized by a distinct profile of sensitivity across tastants. Input to the network of integrate-and-fire model neurons was based on recordings from the CT nerve. Responses to taste stimulation as well as paired-pulse CT stimulation were simulated. The network dynamics of the NTS model operated in a "winner-take-all" fashion, where differences in the stimulus sensitivities between assemblies enhanced discrimination between taste qualities. We propose that such dynamics may account for the coherence in across neuron patterns of NTS responses between similar tastants.

#P44 **Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits**

Linoleic acid does not enhance chorda tympani nerve responses to sucrose, citric acid and quinine hydrochloride

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Previous studies suggest that the chorda tympani nerve (CT) is important in carrying fat taste information to the central nervous system, as bilateral transection of the CT (CTX) raises the taste discrimination threshold for the free fatty acid, linoleic acid (LA). Surprisingly, the CT is unresponsive to lingual application of LA alone. However, electrophysiological studies of isolated taste receptors have shown that LA inhibits delayed rectifying potassium channels, presumably broadening action potentials, and augmenting responses to other taste stimuli (Gilbertson et al, 1997). Thus, the contribution of the CT in this process may depend upon the presence of other taste stimuli. In this regard, we previously found that co- application of LA and either monosodium glutamate (MSG) or sodium chloride (NaCl) elicited greater CT responses than did either MSG or NaCl presented alone (Stratford et al, 2008). In the present study, we recorded CT electrophysiological activity in response to taste mixtures of LA and sucrose (SUC), citric acid (CA), or quinine hydrochloride (QHCl) in anesthetized male rats. Unlike the effects observed with MSG and NaCl, we found that the addition of LA did not alter CT responses to SUC, CA and QHCl. However, CT is weakly responsive to SUC, CA and QHCl in rats. Therefore, CT whole nerve recordings may lack the sensitivity to detect small changes in CT responses to these taste stimuli. It is more probable, however, that LA may affect CT responses to MSG and NaCl only, perhaps by specifically modulating gustatory processing of Na+- which is found in both MSG and NaCl. This possibility may be explored in future studies using a pharmacological antagonist of epithelial sodium channels or a non sodium salt, such as potassium chloride.

#P45 **Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits**

Improvement of olfactory function in patients treated for chronic rhinosinositis is related to increasing olfactory bulb volume

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Aim of the present study was to investigate whether the human olfactory bulb (OB) volume increases after short-term treatment of olfactory function. Nineteen patients with chronic rhinosinositis with polyps were investigated. All patients received functional endoscopic sinus surgery. In addition, 18 volunteers without history and endoscopic signs of chronic rhinosinositis were also investigated. Measurements were performed on 2 occasions separated by 3 months in patients and 4 month in controls. Olfactory function was evaluated in great detail separately for each nostril; MR scans of the OB were performed. Volumetric measures of the OB were based on planimetric manual contouring. In healthy controls the OB volume was not significantly different between the two measurements. In contrast, OB volume in patients increased significantly from initial 64.5 ± 13.9mm^3 to 70.0 ± 15.3mm^3

on the left side and from 60.9 ± 15.5mm³ to 72.4 ± 12.4mm³ on the right side. Change of odor thresholds correlated significantly with the change of OB volume. However, changes in odor discrimination and identification did not show such a significant correlation. Results of this study support the idea that olfactory stimulation of olfactory receptor neurons impacts on the cell death in the OB not only in rodents but also in humans. To our knowledge this is the first study that describes an enlargement of the human OB due to improvement of peripheral olfactory function.

#P46 **Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits**

Terminal Field Organization of the Chorda Tympani, Greater Superficial Petrosal, and Glossopharyngeal Nerves in Nucleus of the Solitary Tract in C57BL/6J Mice

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The nucleus of the solitary tract (NST) is the first central relay in the gustatory system, receiving information originating from discrete receptor populations in the oral cavity through multiple nerves. Three of the nerves carrying taste information have distinct and partially overlapping terminal fields in the adult rat NST. The development of each field is unique and each is differentially susceptible to early dietary manipulations. Therefore, the primary afferent terminal fields in rat are "plastic" during normal and experimentally altered development. To complement these findings, we have begun to characterize the terminal field organization in adult mice that are often used as the background strain for genetic manipulations. Through the use of triple-fluorescence labeling and confocal laser microscopy, terminal fields of the greater superficial petrosal (GSP), chorda tympani (CT), and glossopharyngeal (IX) nerves were visualized concurrently in horizontal sections of the NST in adult C57BL/6J mice (> 60 days old). The terminal fields overlapped extensively with each other, with the IX terminal field located slightly more dorsally than the CT and GSP. The CT terminal field was positioned slightly more lateral than the GSP; however, there was significant overlap between them from their dorsal to ventral extent. Compared to rat, the terminal fields in mice overlapped significantly more with a different topography. These findings will be fundamental in designing studies that focus on the underlying cellular and molecular mechanisms responsible for terminal field development and plasticity. Supported by NIH grant R01 DC00407.

#P47 **Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits**

Characteristics of convergent synaptic activity between the caudal brainstem gustatory nucleus and neurons in the chorda tympani terminal field projecting to the parabrachial nucleus

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The rostral nucleus of the solitary tract (rNST) is the first rely in the central taste pathway. The rNST distributes gustatory information derived from stimulating oral structures to either brainstem motor nuclei or to more rostral brain regions. We have focused on the rostrally projecting rNST neurons to the parabrachial nucleus (PBN) identified by retrograde labeling with an iontophoretic injection of DiI into the PBN. Anatomical and *in vivo* electrophysiological studies have reported intra-rNST connection in the rostrocaudal direction suggesting convergence of chemosensory information from anterior and posterior oral receptive fields. The purpose of this study was to characterize this convergence between the glossopharyngeal (IX) terminal field and the PBN-projection neurons located in the chorda tympani (CT) terminal field. We recorded postsynaptic currents (PSC) from PBN-projection neurons in the rostralmost rNST in rat brainstem slices at postnatal ages of 50-60 days. PSCs were evoked by electrical stimulations of the solitary tract in the IX nerve terminal field. Two groups of PSCs were recorded (24 postsynaptic events from 12 neurons). The first group had a long latency with jitter (standard deviation of mean latency) values that exceeded 200 s and had very variable PSC amplitude. The second group had short latencies with low jitter values and constant PSC amplitudes. The results indicate that individual rNST PBN-projection neurons in the CT terminal field receive convergent IX afferent input via both monosynaptic and polysynaptic connections. Supported by NIDCD grant DC000288 to RMB.

#P48 **Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits**

A Murine Model for Induced Allergic Rhinitis

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The aim of this study was to develop a murine model for induction of allergic rhinitis (AR) so as to enable future studies of AR effects on olfactory mucosa, olfactory functional capabilities, and effects of pharmacological treatments thereupon. We report results using a modification of the localized asthma induction protocol of McCusker et al. (J.Allergy Clin. Immunol.110:891, 2002). Balb/C mice were subjected to bilateral nasal infusions of 7.5µl/naris of filter-sterilized (0.2µm pore size) ovalbumin (OVA; 1% in PBS) or PBS. Chronic treatment involved infusions made M-F for 6 or 11 wks, with defined breaks in the regimen to enhance allergic response intensity. Acutely treated mice were sacrificed one day after single bilateral infusions. Untreated animals served as controls. Mice were deeply anesthetized and perfused with 4% PFA following cardiac blood collection. Paraplast serial sections through the nasal cavities were examined for overall histology of nasal epithelia, presence and distribution of eosinophils, and distribution of OMP and NT-III immunoreactivies. Results indicate that significant allergenic responses occurred in all chronically OVA-treated animals: serum ELISAs show large OVA-specific IgE increases and lamina propria eosinophil numbers were strikingly increased immediately subjacent to the RE and to a lesser extent deep to the OE. The OE showed noticeable disruption, especially at 11 wks, including OSN loss, Bowman's gland OE intrusion, and lamina propria neuroma formation. Significant RE cellular edema also occurred. None of these changes was seen in acute PBS or OVA, chronic PBS, or untreated animals. Examination of OSN turnover is planned.

#P49 **Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits**

Objective evaluation of the impact of chronic rhinosinusitis (CRS) on olfactory function

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Chronic rhinosinusitis (CRS) is both one of the most common chronic diseases in the U.S., afflicting over 30 million adults, and one of the most common causes of smell loss. Yet, not all sufferers of CRS experience smell problems. In order to develop targeted therapies for this form of smell loss, it is critical that we identify effective tools to evaluate the functional impact of the disease process on the olfactory system. To this end, the Monell-Jefferson Chemosensory Clinical Research Center has enrolled 55 patients with clearly defined CRS and performed a battery of objective assessments on them, including acoustic rhinometry, rhinomanometry, CT scans and nasal endoscopic evaluations, with each tool indexing different aspects of disease status. Rhinometry primarily reflects the airway cross-sectional area and resistance contributed by the anterior portion of the nasal cavity, endoscopy evaluates the main nasal airway, ostiomeatal complex and olfactory cleft, whereas CT staging scores weight heavily on the surrounding sinuses. Our findings indicate that none of these tools by themselves discriminate degrees of olfactory loss due to CRS. Endoscopy scores and CT scores of the ethmoid sinuses are excellent indices for the most severe olfactory loss, anosmia, yet fail to differentiate hyposmic patients from those with no olfactory loss. The minimum cross sectional area (MCA) measured by acoustic rhinometry correlates significantly with unilateral olfactory thresholds of patients, but only for the high soluble odorant l-carvone, not for the low soluble d-limonene, which may reflect a conductive mechanism. In the future, carefully weighted combinations of multiple objective tools may provide a better evaluation of the aspects of this disease process that impact olfactory function.

#P50 **Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits**

Palinosmia: Olfactory Perseveration

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Sensory perseveration occurs in the auditory (Palinacousis), somesthetic (Palinesthesia), and visual (Palinopsia) spheres. Olfactory perseveration (Palinosmia) has not been described. Four cases are presented. Case 1: 64 year-old male with upper respiratory infection, followed by a smokey, burnt wood phantosmia. Smelling or eating replaced the phantosmia with the ambient or retronasal odor which would persist four hours after inhalation or consumption. Fiberoptic endoscopy, MRI of brain, and sinus CT were negative. UPSIT (27/R, 20/L), PEA Threshold (>-2.0/R, –5.0/L). Case 2: 38 year-old female, 19 years prior, sustained a traumatic subdural hematoma and coincident anosmia with monthly phantosmias of smoke or fish. Three months prior to presentation, she developed a prolongation of perception of smell, even after removed from the vicinity of the odor. UPSIT (24/R, 28/L). Case 3: 32 year-old woman fell off a horse with frontal contusion and basal skull fracture, anosmia and an intermittent unpleasant chemical smell which was precipitated by exposure to strong odors, persisting for days after the ambient odor stimulus had been removed. PEA (> –2.0/R, >-2.0/L) and UPSIT (11/R, 7/L). Case 4: 48 year-old woman with upper respiratory infection-induced anosmia replaced by a "foul, rotten fish" odor in response to any smells or tastes which persisted for hours after the stimulus odor. Fiberoptic endoscopy, CT of sinuses, and MRI of brain were negative, UPSIT (20/R, 18/L), PEA (-4.5/R, -2.0/L). CONCLUSION: Unlike phantosmia,Palinosmia may be viewed as the abnormal perseveration of a true or distorted olfactory stimulus.

#P51 **Poster session I: Chemosensory disorders, models and aging/Central chemosensory circuits**

Bimodal odorant perception in anosmic subject: a fMRi study

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Most odorous compounds stimulate both olfactory and intranasal trigeminal receptors.It is not entirely clear which brain areas specifically relate to within each system and those common to both systems. In order to further investigate the cross-interaction between the two systems, a block design functional magnetic resonance (fMRI) study was set up. For stimulation we chose the bimodal stimulus menthol was presented in two different concentrations to two groups of subjects, an healthy control group and an anosmic group (no sense of smell) (17 subjects in each group). For stimulus presentation computer-controlled air-dilution olfactometer was used. Image acquisition was performed by means of 3T MRI-scanner (Siemens Magnetom Trio Tim System 3T; TR 2s; TE 30ms; FA 90°; 1.72x1.72x2 mm). SPM5 was used for data analysis. Normosmic subjects exhibited activation in the anterior and posterior cingulate cortex, prefrontal cortex, and cerebellum. On the other side, anosmic patients activated the same area inside the anterior cingulate; moreover a cluster of activation was found in the left parahippocampal gyrus. In controls, an effect of stimulus intensity was localized between the anterior cingulate and the medial frontal gyrus; such areas could not be found in anosmic subjects. Among others these results clearly indicate that the olfactory system seems to amplify trigeminally mediated information resulting resulting in more efficient information processing related to differentiation between stimulus intensities.

#P52 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

The Nutritional Significance of Oral Starch Digestion

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The digestion of starches is accomplished by salivary and pancreatic α-amylases, endo-enzymes that catalyze the hydrolysis of α-1,4 glycosidic linkages to produce maltose, maltotriose and larger oligosaccharides. Salivary amylase initiates the digestion of starch in the oral cavity, producing a rapid decrease in glucose polymer chain length and viscosity with the cleavage of relatively few glycosidic bonds. Amylase is the most abundant protein in human saliva, with a significant amount of variation among individuals. However, the selective advantage provided by the breakdown of starch in the oral cavity has never been established, since the majority of ingested starch is digested in the small intestine by pancreatic amylase. Accordingly, the nutritional significance of oral amylase production is unknown. We posit that the presence of salivary amylase facilitates a more efficient and complete breakdown of ingested starch than does pancreatic amylase alone. We address this question by examining how the pre-ingestive breakdown of starch by salivary amylase affects absorption of glucose in the small intestine. Furthermore, individuals who produce more salivary amylase may have faster and larger postprandial blood glucose responses. This question is addressed by measuring changes in blood glucose levels following starch ingestion and quantifying salivary amylase levels and enzymatic activity. The results of this work will help elucidate the nutritional consequences of the production of high salivary amylase levels.

#P53 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Characterization of Off-odor development in human milk during storage at -18 °C

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Objectives. Human milk has been reported to be storable at -18 °C for a maximum of 3 to 6 months, and at +4 °C for 3 to 8 weeks. Subsequent recommendations on storage have been based on stabilities of beneficial milk constituents, as well as on hygiene considerations [1]. However, sensory changes of human milk during storage have hitherto not been investigated. Since chemosensory aspects of human milk have been shown to be of enormous importance for the neonate [2], this study aimed at characterizing potential flavor changes in human milk during storage. **Experimental.** Changes in ortho and retronasal flavor profiles of fresh human milk in comparison to those stored at -18 °C were monitored by descriptive sensory evaluation. Furthermore, identification of predominant odor substances was accomplished by senso-analytical techniques such as gas-chromatography-olfactometry/mass spectrometry (GC-O/MS), comparative dilution assay (cAEDA), and stable isotope dilution assays for quantification of major flavor changes. **Results.** Sensory evaluation of the ortho and retronasal flavor attributes of fresh and stored human milk indicated dramatic differences, most specifically with the formation of fishy, rancid, metallic and cooked-milk-like aroma impressions in the stored samples. The chemo-analytical methodology was successfully applied for identification of more than forty odorants in fresh and stored human milk, and allowed for molecular characterization of the key odor modifiers of the fresh milk smell. **Conclusions**. Conventional storage led to major flavor changes of human milk and should be reconsidered on the basis of the sensory and chemo-analytical data presented here. **References**. [1] Pardou A et al. Biol Neonate 65:302 to 309, 1994. [2] Soussignan R et al. Physiol Behav 1997, 62, 745-758.

#P54 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Chronic otitis media is associated with a marker of taste damage and higher weight status in children

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Otitis media (OM) is the most common illness of young children. Because the chorda tympani nerve passes through the middle ear, it may be particularly vulnerable to OM. Consequently, taste perception and food habits may be altered due to the damage or other unknown factors associated with OM. In support of this hypothesis, Bartoshuk and colleagues report that adults with more severe histories of OM prefer diets higher in sugar and fat and have higher BMIs. In the present study, we obtained retrospective reports of OM history from the mothers of 227 children (age 3 to 10 years). Mothers were also queried about their smoking habits and children's feeding histories. Children were measured and weighed, genotyped for the TAS2R38 gene, and phenotyped for propylthiouracil (PROP) sensitivity and sucrose preferences. Irrespective of genotype, children who reported the 560μmol/L PROP solution tasted metallic ("like a penny"), an indication of taste nerve damage, had experienced more episodes of OM than children who did not report a metallic taste ($P<.01$). In white children only, chronic OM was associated with preference for significantly higher concentrations of sucrose compared to occasional or no OM ($P<.05$). Children who experienced chronic OM had higher BMI z-scores than children who experienced occasional OM or no OM ($P<.05$). Children of mothers who smoked experienced their first OM episode at a younger age than children of non-smoking mothers ($P<.05$); younger age at first OM episode was predictive of significantly more OM episodes in a child's lifetime ($P<.001$). Breastfeeding protected children of smokers from OM. In conclusion, this correlational data supports associations between chronic ear infections, a marker of taste damage, higher sweet preferences and higher weight status. Maternal smoking increased, while breastfeeding moderated, risk for ear infections.

#P55 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Food Liking, Ear Infections and Body Mass Index Among Preschoolers

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National overweight rates among preschoolers have grown from 5% in 1976 to 13.9% in 2004. Otitis media (OM) history has been linked to children's overweight risk from a small study (Tanasescu et al, 2000) and nationally-representative longitudinal study (Hoffman et al, 2006). We examined OM history and dietary behaviors associated with overweight risk among 485 preschoolers (248 males, mean age=45±7 months) in two urban preschool centers. Most of the preschoolers were Black (27%) or Hispanic (56%). From measured weight/height and age/gender-specific BMI percentiles, 4% were underweight, 21% had overweight risk (85-95th) and 21% were overweight (≥95th). From parent-completed surveys, 70% had OM at least once and 31% were food neophobic. Sweet, salty or high-fat foods/beverages were liked most while vegetables were liked least. In analysis of variance, controlling for demographic variables (ANCOVA), food neophobic preschoolers had less liking for a vegetable/fruit group than did non-neophobic preschoolers ($p<.001$); a parallel analysis with liking for a group of high fat/sweet/salty foods showed no effect of neophobia. In a liner dose response, vegetable/fruit liking fell as OM exposure increased from none to ≥6 bouts ($p<.01$). In a 2-way ANCOVA, food neophobia and OM exposure each explained variation in vegetable/fruit liking; the interaction term was non-significant. Preschoolers with exposure to OM and food neophobia had lowest vegetable/fruit liking. Reported liking for fat/sweet/salty foods (but not for vegetables/fruits) showed a small but significant association with BMI. Preschoolers with ≥6 OM bouts had the highest BMI percentile. Food neophobia failed to explain difference in BMI percentile. In summary, significant OM exposure and food neophobia may limit a preschooler's liking of vegetables and fruits, which increases current or future risk of being overweight.

#P56 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Odor Intensity, Diet and Nutrition-Related Health Indices Among Females

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The impact of olfactory dysfunction on health has been of interest. Less studied is the impact of olfactory acuity on health (eg, is a more acute sense of smell an advantage for dietary health?). Here, we studied the association between perceived odor intensity and food liking, dietary behaviors, and nutritional risk among 100 women (mean age 47±7 yrs). The women used the general Labeled Magnitude Scale to rate intensities of eight orthonasally-perceived odorants from a standard olfactory test (Cain et al, 1988), retronasally-perceived coffee jellybean with nose pinched/unpinched, 1000 Hz tones as a cross-modal standard, and degree of liking of foods on a survey. Most of the women (80%) correctly identified at least 6 of 8 odors; 70% denied age-related smell or flavor loss. Nutritional risk was assessed from adiposity (measured height/weight, waist circumference), blood pressure, and fasting serum lipids. In multiple linear regression analysis controlling for age and tone intensity, women who had greater average odor intensity score (OI-score) reported more frequently engaging in healthful dietary behaviors (eg, using low-salt, sugar foods), but no difference in food/cooking interest. A higher OI-score also associated with greater fruit/vegetable liking but no difference in sweet, salty, or high-fat food liking. Although adiposity failed to vary with OI-score, more favorable serum triglycerides were seen among those women who had highest OI-scores. No significant associations were found between OI-score and either blood pressure or other serum lipids. In summary, greater olfactory acuity appeared to confer some benefit for following a healthy diet. These data support the utility of assessing intensity of odor perception for making chemosensory-health connections.

#P57 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Taste loss, retronasal olfaction loss and reduced food liking

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During a 4 year case study of a patient with Alzheimer's, retronasal olfaction was lost later than orthonasal olfaction and at a much faster rate. Also, the gradual decline in orthonasal olfaction was not as devastating to the patient as his more severe retronasal loss. Over a year, food no longer "tasted" right and he lost 40 pounds. Testing showed taste damage to the anterior tongue. Recent work showing that taste anesthesia reduces retronasal olfaction (Snyder, 2007) suggested that this patient's taste loss would leave orthonasal olfaction intact, but would reduce retronasal olfaction thus impairing flavor. The possibility that taste loss could be responsible for a dissociation between orthonasal and retronasal olfaction that could diminish pleasure in eating was examined with a dataset that included orthonasal and retronasal assessments (gLMS) of a strawberry roll-up. Plotting retronasal vs. orthonasal perception of the strawberry sensation showed that some individuals perceive considerably less retronasal intensity for the same orthonasal intensity. Analysis showed that these individuals had reduced taste. Subjects rated their liking for 26 foods (hedonic gLMS). Factor analysis resulted in two reliable groups: sweet foods and fat foods (Chronbach alphas of .73 and .64, respectively). Those individuals experiencing the least retronasal intensity for equivalent orthonasal intensity liked the foods less. Future investigations of patients with neurodegenerative diseases known to reduce olfactory perception will focus on the possibility that taste loss with resulting diminution of retronasal olfaction plays an important role in reduction of the pleasure of eating and thus quality of life for these patients.

#P58 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Bitesize is affected by Food Aroma presented at Sub- or Peri Threshold Concentrations

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Bite sizes for foods typically vary with the food's familiarity and with its hedonic and textural properties. More over, smaller bite sizes tend to be more satiating than larger ones and bite sizes tend to become smaller when the consumer becomes more satiated. These results indicate that bite size control is sensitive to general food properties as well as to the internal state of the consumer. To explore the effect of food aromas on bite size, a semi-solid vanilla custard dessert was delivered into the mouth of subjects using a pump while a cream aroma was presented retro-nasally in the nose. Termination of the pump, which determined bite size, was controlled by the subjects via a pushbutton. Over 30 trials the custard was presented randomly either without an aroma, or with aromas presented below or near detection threshold. Results for 10 subjects, 4 females and 6 males aged between 26 and 50 years, indicated that aroma intensities affected sizes of the corresponding bites as well as subsequent bites. Higher aroma intensities resulted in significantly smaller sizes of the corresponding bite. Higher aroma intensities resulted in a directional similar but smaller and non-significant effect on the subsequent bite, and a reversed and significant effect on the bite thereafter. These results suggest that bite size control during eating is a highly dynamical process affected by sensations elicited by present and preceding bites. The results contribute to our understanding of bite-size and food intake regulations, and may be of relevance for weight-management.

#P59 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Similarities in Food Cravings and Mood States between Obese Women and Women who Smoke Tobacco

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Women who smoke crave starches and fats more frequently than those who never smoked. Here, we determined whether these cravings and some mood disturbances were related to effects of smoking *per se* or were characteristic of women who were likely to smoke. Further, because obese individuals crave fats more frequently than lean counterparts, we explored whether these associations were affected by body weight. We interviewed and grouped 229 women according to smoking history (never smokers, former smokers and current smokers) and body weight category (normal weight, overweight, obese). Each subject completed the Food Craving Inventory to measure cravings for sweets, high fats, starches, and fast food fats and the Profile of Mood States to measure psychological distress. We found that, even after controlling for socioeconomic factors, smoking and obesity were independently associated with specific food cravings and mood states. Current smokers craved fats more frequently than former and never smokers. They also craved starches more frequently, and felt more depressed and angrier, than never smokers, but not former smokers. Similarly, obese women craved fats more frequently than non obese women and depression symptoms were intensified with increasing body weights. In conclusion, whereas cravings for starchy foods and some mood states may be characteristic of women who are likely to smoke, more frequent cravings for fat is probable an effect of smoking *per se*. We hypothesize that the overlapping neuroendocrine alterations associated with obesity and smoking and the remarkable similarities in food cravings and mood states between women who smoke and women who are obese suggest that common biological mechanisms modulate cravings for fat in these women.

#P60 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

PROP Sensitivity and Dietary Intake of Antioxidant-Rich Foods

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Antioxidant-rich foods are known to be an important part of a healthy diet. However, often these foods can have high levels of phytonutrients which impart a bitter taste, making them less acceptable to consumers. Genetic sensitivity to 6-n-propylthiouracil (PROP) may be a marker for the selection of antioxidant-rich foods. Some studies have demonstrated that PROP non-tasters (NT) showed a higher acceptance of bitter fruits and vegetables (sources of Vitamins A, C, and E) and vegetable oils (source of Vitamin E) than medium (MT) and super-tasters (ST). The aim of this study was to relate PROP sensitivity to the dietary intake of these major antioxidant vitamins. Healthy, non-vegetarian females, ages 21-44 yrs, who did not take dietary supplements were classified as NT (n=30), MT (n=33), and ST (n=30) based on the PROP paper disk method. The subjects provided three, 24-hour diet recalls which were collected and analyzed using NDS-R software. Eating attitudes were measured using the Dutch Eating Behavior Questionnaire. There were no differences in the consumption of fruits, vegetables, or vegetable oils across taster groups. However, when the subjects were further divided by restrained eating, NT who were also low in dietary restraint had higher intakes of a-tocopherol (Vitamin E) than the other groups (p=0.05). These data are consistent with Tepper et al, 2008, showing that NT had higher plasma a-tocopherol levels than MT and ST. Taken together, these data suggest that NT consume more a-tocopherol (principally derived from green vegetables and vegetable oils in the U.S. diet), which may be reflected in higher plasma indices for this antioxidant nutrient. The health implications of this relationship should be further examined.

#P61 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Association of a *TAS2R38* Polymorphism and the Eating Behavior Disinhibition in a Female Amish Cohort

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Insensitivity to the bitter-tasting compound 6-n-propylthiouracil (PROP) has been associated with increased adiposity, especially in women. The principal genetic determinants of phenotypic variation in PROP taste sensitivity are haplotypes of the *TAS2R38* gene, which encodes a taste receptor sensitive to thiourea compounds such as PROP and phenylthiocarbamide. As dietary intake can affect adiposity, we asked if variation in *TAS2R38* is associated with any of 3 eating behaviors: restraint (a cognitive avoidance of eating to control body weight), disinhibition (a loss of restraint associated with overeating), and hunger. We had previously genotyped haplotype-tagging, single nucleotide polymorphisms (SNPs) located within the *TAS2R* gene cluster on human chromosome 7 (which includes *TAS2R38*) in 729 nondiabetic individuals (381 females, 348 males) within the Amish Family Diabetes Study. Eating behaviors were assessed in these individuals using the Three-Factor Eating Questionnaire. We performed association analysis of the *TAS2R38* SNP rs1726866 and these three traits. Results of our analysis (adjusted for age and sex) showed a marginally significant association of the minor (C) allele with decreased disinhibition (P=0.03). Stratification of the cohort by sex revealed a strong association in females (P=0.0002) but not in males (P=0.76). Analyses with other haplotype-tagging SNPs in close proximity to rs1726866 suggest that this locus is principally responsible for the association signal. Therefore, our results indicate that a polymorphism in *TAS2R38* is associated with differences in ingestive behavior. Support: DC005786, HL076768, DK072488, DE007309, UMSOM.

#P62 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Modulation of sweet taste sensitivity by glucagon signaling in taste buds

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Hormones that facilitate glucose homeostasis in the gut may also regulate taste sensitivity in taste buds. For example, glucagon-like peptide 1 (GLP-1), a regulator of insulin biosynthesis and release, is expressed in taste receptor cells and appears to modulate sweet taste sensitivity through local paracrine signaling. We previously showed that glucagon is expressed in a subset of taste receptor cells, but its pattern of distribution and effects on taste function are not known. We quantified the expression of glucagon, its receptor (GlucR) and other taste cell markers in mouse circumvallate taste buds using immunohistochemistry and stereological cell counting. In contrast to GLP-1, which is expressed in subsets of both 5HT+ and PLC 2+ cells, glucagon immunoreactivity is largely restricted to a subset of PLC 2+ cells. We saw similar expression patterns in fungiform and foliate taste buds. GlucR expression appears to overlap with that of glucagon, suggesting an autocrine signaling mechanism in taste buds. We next tested the effects of a highly-specific GlucR antagonist L-168,049 using a brief access taste test. Mice that received the drug showed a significant reduction in taste sensitivity to sucrose; there were no significant differences in responses to NaCl or denatonium benzoate. Together, these data suggest a role for glucagon signaling at the level of the taste bud in the modulation of sweet taste sensitivity.

#P63 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Sweet receptor gene (*Tas1r2*) structure and preference for sweet stimuli in species of Carnivora

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The extent to which taste receptor specificity correlates with, or even predicts, diet choice is not known. We recently reported that the insensitivity to sweeteners shown by species of Felidae can be explained by the observation that the gene for their sweet taste receptor is a pseudogene. To broaden our understanding of the relationship between the structure of the sweet receptors and preference for sugars and artificial sweeteners, we measured taste responses to 12 sweeteners in select species of Carnivora using two-bowl preference tests, and sequenced the *Tas1r2* gene in these same (or closely related) species using PCR. Based on behavioral and molecular data, we found that lions showed no preference for any of the 12 sweet compounds tested and that they possess the pseudogene of *Tas1r2*. All other species tested preferred some of the natural sugars, and their *Tas1r2* sequences, having complete open reading frames, predict functional sweet receptors. One species, the lesser panda, in addition to preferring natural sugars, also preferred three (neotame, sucralose and aspartame) of the six artificial sweeteners. Heretofore it had been reported that among vertebrates, only Old World simians could taste aspartame. These data suggest an evolutionary convergence or a serendipitous mutation in the sweet receptor of the lesser panda. Continuing studies will provide insights into the nature and function of taste receptor genes and how their variation affects taste perception, food choice and nutritional status.

#P64 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

GABA-A Receptor Activation Influences Consumption of Appetitive and Aversive Tastants

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Benzodiazepines act through GABA-A receptors to increase the inhibitory effect of GABA in the brain. Previous research has shown that hyperphagia is a common side effect of chlordiazepoxide (CDP) and benzodiazepines in general. Although the majority of previous research has focused primarily on the palatability of sweet stimuli, a taste reactivity test suggested that benzodiazepines influence appetitive responses with little or no effect on responses to sour or bitter taste stimuli. The objective of this study was to examine the effects of CDP on licking responses to not only appetitive stimuli but also aversive stimuli across a range of concentrations. Using counterbalanced methodology, a within-subject design assessed the effect of CDP (10mg/kg) versus saline on the microstructural licking patterns of water-restricted rats (n=16) to one concentration of saccharin, sucrose, NaCl, MSG, citric acid, or quinine (Q-HCl) during 1-hr tests. Licking responses across 3 concentrations of each tastant were compared using a between-subject analysis. Results from the present study show that CDP increased the appetitive qualities across all of the taste categories, primarily through changes in taste-mediated variables. CDP increased the number of meal licks (sucrose, saccharin, MSG, NaCl, Q-HCl) through shorter pause durations (sucrose, saccharin, MSG, NaCl, citric acid), increased lick rates (sucrose, saccharin, MSG, Q-HCl, citric acid), and increased licks in the first minute of testing (saccharin, NaCl, citric acid, Q-HCl). There was no drug effect on variables associated with post-ingestive feedback such as meal duration or number of bursts. This supports previous research that benzodiazepines enhance the taste palatability of sweet stimuli and expands this finding to umami, salt, and bitter tastants.

#P65 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Conditioned preferences for glucose and fructose in T1R3 KO and TRPM5 KO mice

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Deletion of the genes for the sweet taste receptor subunit T1R3 or the signaling protein Trpm5 greatly attenuates sweetener preference in mice. However, knockout (KO) mice missing T1R3 or Trpm5 develop preferences for sucrose solutions in 24-h tests due to the post-oral actions of the sugar. The present study compared the preferences of KO and C57BL/6J wildtype (WT) mice for glucose and fructose in 24-h taste tests with 0.5-32% sugar vs. water. Unlike glucose and sucrose, fructose has minimal post-oral reward effects. T1R3 KO mice were initially indifferent to dilute glucose solutions (0.5-4%) but developed preferences for 8-32% sugar in test 1. They strongly preferred (~90%) all glucose concentrations in test 2. New T1R3 KO mice were indifferent to 0.5-8% fructose but avoided 16-32% fructose in both tests. Trpm5 KO mice were indifferent to 0.5-4% glucose but preferred 8-32% glucose in test 1 and all concentrations in test 2. New Trpm5 KO mice were indifferent to 0.5-32% fructose in test 1 but mildly preferred (~75%) fructose in test 2. WT mice preferred glucose and fructose in both tests. Why T1R3 KO but not Trpm5 KO mice avoided 16-32% fructose is uncertain. Perhaps fructose absorption is impaired in T1R3 KO mice (due to missing gut T1R3 receptors) which inhibits fructose intake. Yet T1R3 KO and Trpm5 KO mice given glucose in tests 1 and 2 subsequently displayed strong (~90%) preferences for 0.5%-32% fructose in a third test. Apparently, the post-oral effects of glucose condition a strong preference for the sugar's T1R3-independent orosensory properties (odor, texture) that generalize to those of fructose. Fructose, rather than glucose or sucrose, can be used with taste-impaired KO mice to evaluate 24-h sugar taste preferences with post-oral reward effects minimized.

#P66 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Nutrient-specific preferences in trpm5 knockout mice

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We are investigating the relative influence of postingestive effects, in contrast to gustatory input, on amino acid vs. carbohydrate intake. Mice knockouts of TRPM5 (KO, [1]), a TRP ion channel, lack behavioral and cranial nerve responses to sweet and L-amino acid tastants [1]. These animals nevertheless retain the ability to develop preferences for sipper locations associated with nutrient availability [2]. We have monitored behavioral, thermogenic and brain dopamine responses following glucose and L-serine intake in both KO mice and their wild-type counterparts (WT). During short-duration two-bottle tests, WT mice displayed overwhelming preferences for glucose vs. isocaloric L-serine and L-histidine solutions. In contrast, KO mice exhibited indifference to the choices as well as significantly lower levels of intake. However, when allowed to form associations between the postingestive effects of the solutions and the respective sipper locations, both KO and WT mice displayed a robust preference for sipper locations previously associated with glucose availability, compared to isocaloric L-serine. Indirect calorimetry revealed a close relationship between respiratory quotient values and intake levels, suggesting an association between fuel utilization and nutrient selection. Furthermore, microdialysis measurements revealed nutrient-specific dopaminergic responses in ventral striatum upon intra-gastric infusions of glucose or L-serine. These preliminary findings suggest that preferences for carbohydrates over other nutrients can develop independently of taste quality or caloric load, an effect potentially associated with the ability of a nutrient to regulate glucose metabolism and stimulate brain dopamine release. Refs. [1] Zhang et al Cell 2003 112:293-301. [2] de Araujo et al Neuron 2008 57:930-41.

#P67 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Taste Receptor T1R3 is Involved in Detection of Ethanol Flavor in Mice

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Previous studies suggested that humans and rodents perceive sweet and bitter components of ethanol flavor, and that in mice genetic differences in ethanol preference depend on allelic variation of the *Tas1r3* gene encoding the sweet-taste receptor protein, T1R3. The aim of the present study was to examine the role of T1R3 in qualitative taste perception of ethanol by mice. We used mice from the alcohol-preferring strain C57BL/6J with either intact *Tas1r3* gene (wild-type) or with a null mutation of this gene (knockouts, *Tas1R3* -/-). In these mice, consumption of ethanol solutions was assessed in the long-term 48-h two-bottle test, and then licking responses to ethanol (1.25-20%), sucrose (1-32%) and quinine (0.01-1 mM) were recorded in brief-access tests using the Davis MS-160 gustometer before and after LiCl-induced conditioned taste aversion (CTA) to 10% ethanol was developed. Compared to wild-type mice, *Tas1r3* -/- mice demonstrated concentration-dependent reduction of consummatory responses to ethanol in both brief access and two-bottle tests, and also had reduced licking rates of sucrose solutions. The wild-type and *Tas1r3* -/-mice had similar licking responses to quinine and did not differ in CTA to ethanol, which generalized to quinine, but not sucrose, in mice of both genotypes. These data support a conception of two-component (sweet and bitter) taste of ethanol and suggest that perception of its sweet component depends on the T1R3 receptor, while perception of its bitter component does not depend on T1R3. Probably, complexity of 10% ethanol flavor and/or salience of its bitter component prevented generalization of CTA from ethanol to sucrose in this experiment.

#P68 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Effect of *kokumi* taste active peptides on amiloride-insensitive salt taste preference in C57BL/6J mice

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Previously, we have shown that *kokumi* taste active peptides (FII) derived from a mature Korean soy sauce modulate salt taste on human and the amiloride-insensitive NaCl chorda tympani (CT) taste nerve responses by interacting with TRPV1 variant salt taste receptor (TRPV1t). To identify the peptides that modulate TRPV1t, FII was further separated into LHa, LHb, LHc, LHd, LHe by column chromatography and their behavioral effects were tested in wild-type C57BL/6J mice using 48h two-bottle preference tests, in which one bottle contained distilled water and the other a test solution made with NaCl + 10 M amiloride. Intake of NaCl expressed as the preference ratio. In solutions 0-300 mM NaCl containing 10 M amiloride, 150 mM NaCl gave a maximum preference and 300 mM decreased the preference to the maximum by 50%. In 100 mM NaCl + 10 M amiloride, varying the concentrations of FII (0-1.0%) produced biphasic effect of NaCl preference. Between 0.1% and 0.5% concentration, FII enhanced NaCl preference, but above 0.5%, FII lowered the preference. The salt taste modulating effect of FII was transited to LHe: LHe (0.25%) significantly lowered preference for 100 mM NaCl as compared with control (p <0.001), but others did not. Between 0.1% and 0.25% concentration, LHe lowered NaCl preference but above 0.25%, LHe gave no significant influence. Resiniferatoxin (1 μM), a specific agonist of TRPV1t, lowered 70% of NaCl preference in 100 mM NaCl + 10 M amiloride, and enhanced the response to 0.25% of LHe by 20%. SB-366791 (5 M) or capsazepine (25 M) did not modulate the NaCl preference in this test. These data further support to the suggestion that the *kokumi* active peptides FII modulate the amiloride-insensitive salt taste by interacting with the TRPV1 cation channel in taste receptor cells.

#P69 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Responses of *Trpv1* Knockout Mice to Trigeminal Irritants in Two Different Behavioral Assays

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The trigeminal nerve is composed of multisensory neurons which innervate the nasal cavity, nasopharynx, oral cavity and cornea. Trigeminal nociceptive fibers express a number of channels which are activated by chemical irritants. TRPV1 channels, for example, are activated by capsaicin. In the present study two different behavioral assays were tested for their ability to determine whether Trpv1 knockout mice detect irritating compounds with the same efficiency as wildtype (C57Bl/6J) mice. In the first assay mice were presented with a chemical via a cotton swab and their response scored on a 5 level scale, with trigeminal reflex coded as -2 and feeding behavior coded as +2. In the second assay, mice were housed with two drinking bottles for 48 hours during which water consumption from each bottle was recorded. A felt washer was placed on each of the drinking bottle spouts and one of the washers was saturated with an irritant. The bottles were then switched and irritant reapplied after 24 hours to control for side bias. If a particular chemical is aversive to the animal then less water should be consumed from the bottle with the irritant washer. In the cotton swab test Trpv1 knockout mice were scored significantly less aversive to capsaicin and cyclohexanone but equally aversive to eugenol and benzaldehyde as wildtype mice. In the two bottle preference test Trpv1 knockout mice consumed significantly more water from water bottles treated with benzaldehyde, cyclohexanone and eugenol than wildtype mice. Based on these results it appears TRPV1 mediates at least some portion of benzaldehyde, cyclohexanone and eugenol induced trigeminal irritation. Furthermore, it appears that the two bottle preference has a higher resolution for detecting differences in irritant discrimination then the cotton swab test.

#P70 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Sweet Stimuli Elicit Differential Responses in the Chorda Tympani Nerve of Obesity-resistant Rats Compared to Obesity-prone Rats

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Previous research has shown that obesity-resistant, S5B/Pl rats differentially prefer carbohydrate diets compared to high-fat diet preference of the obesity-prone, OM rats. Furthermore, following a conditioned taste aversion, OM rats appear to be more sensitive in detecting fatty acids. *In vitro* recordings from taste receptor cells suggest further strain differences such that fatty acids appear to produce more depolarization in the S5B/Pl rats than OM rats; however, *in vivo* gustatory signaling has not been examined in either strain. This study characterized afferent gustatory responses of the whole chorda tympani nerve in these two strains of rat using an array of salt (NaCl, KCl), bitter (quinine-HCl), sour (citric acid), umami (MSG), and sweet (glucose, saccharin, glucose+saccharin mixture) taste stimuli at 3 concentrations each with and without the presence of a fatty acid in 20-s trials. The addition of 200 µM linoleate tended to produce subtle enhancements of the neural responses to select concentrations of the tastants with the most consistent effects on the sweet taste stimuli. The strains showed similar neural responsiveness within each taste category with the exception of the sweet taste stimuli. The S5B/Pl strain demonstrated greater differences in neural responses to glucose, saccharin, and a glucose+saccharin mixture compared to the OM strain which showed similar responsiveness across the three sweet stimuli. This difference in the neural responsiveness to sweet tastants may underlie the dietary preference for carbohydrates observed in the S5B/Pl strain.

#P71 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

The Perceptual Consequences Of Salt Appetite In Rats

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In rats and other mammals, a physiological sodium deficit leads to a robust appetite for sodium that is guided by the gustatory system. In 1993, Breslin et al. (Am. J. Physiol., 264, R312-8) demonstrated that the inverted-U intake-concentration function for NaCl was preserved (but elevated) in salt-deprived rats. They concluded, in contrast to some theories of salt appetite, that the perceived taste intensity of NaCl is unchanged during sodium deprivation, since a reduction in intensity should produce a rightward shift and increases in intensity a leftward shift of the response-concentration function. We tested the logic of this conclusion by examining the responses of salt deprived rats to Na_2CO_3, a salt that G.R. Morrison reported was ten times as intense as NaCl to rats (Physiol. Behav., 8, 25-28). Furosemide-injected rats were tested the following day for their licking responses to water and 7 concentrations of Na_2CO_3 in either low (0.0028 – 0.089 M) or high (0.028 – 0.89 M) concentration ranges. Consistent with Breslin et al. and Morrison, behavior towards Na_2CO_3 was an inverted-U shaped licking-concentration function leftward shifted from NaCl precisely one order of magnitude. Amiloride dose-dependently reduced the responses to NaCl (suggesting a change in perceived quality, not intensity), but had a more complex effect on Na_2CO_3 (suggestive of a change in perceived quality and a reduction in perceived intensity). Taken together with our previous work investigating behavioral responses to KCl and sodium gluconate, and combined salt appetite and taste aversion work, we conclude that sodium appetite is driven neither by shifts in quality nor intensity, but rather in a modification of the hedonic valence of a stable percept of salty taste.

#P72 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Rat as a model for the study of multimodal integration of flavor

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Neuroimaging studies in humans have revealed that formation of a flavor percept involves taste-odor integration. It has also been established that retronasal olfaction and flavor experience play key roles in this process. Little is known about the detailed neural circuitry and mechanisms underlying retronasal stimulus coding, experience and sensory integration of flavor. Since the use of humans precludes necessary invasive experimental procedures and control of flavor experiences, we are establishing the use of rats to explore the neuro-behavioral mechanisms of flavor perception. By optical calcium imaging of olfactory bulb responses to orally presented odorants in awake behaving rats we ask whether and how rats smell retronasally, and how this compares to orthonasal olfaction. We found evidence for bulbar responses to oral odorants. Both fast sniffing and fast licking attenuate ortho- and retronasal OB responses, but presumably by different means. Olfactory receptor neurons may be over-stimulated during rapid sniffing, and under-stimulated during licking and swallowing. Behaviorally, by employing conditioned odor and taste aversions, we asked whether the perceived smell of "sweet" odorants is similar to "sweet" tastants to flavor-naive rats. Retronasal odorants (benzaldehyde and tasteless amyl acetate, CS) did not generalize to the prototypical tastants in naive rats. Sucrose (CS) also did not generalize to the odorants. However, the prior experience of either odor-taste pair did result in specific generalization of the odorant to sucrose, suggesting a primary role of paired odor-taste experience in the formation of flavor objects. Both lines of enquiry provide direct evidence for retronasal olfaction in rats. Thus, the rat emerges as a useful model for extensive flavor research in mammals.

#P73 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Mouse Strain Differences in Conditioned Taste Aversion Formation, Generalization and Extinction using a Self-administration Paradigm

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Traditional approaches to conditioned taste aversion (CTA) learning have employed pairing of a novel taste with i.p. injections of LiCl. However, a self-administration model involving continuous licking of a LiCl solution is more comparable to naturally developed food aversions, and allows for temporal

analysis of CTA processing (Baird et al., 2005). In this study, we analyzed acquisition, expression and extinction of CTA in C57Bl/6J (B6) and DBA2/J (D2) mice by examining licking behavior to LiCl and NaCl solutions. In preliminary studies with water-restricted mice, we obtained baseline values for water licking and established that B6 and D2 mice respond equivalently to concentration series of NaCl and LiCl in brief access tests. ***Methods:*** Water-restricted mice were trained to lick water in the MS160 lickometer. On conditioning day, mice received a 20-min trial with either 0.12M NaCl or LiCl. For 6 days following acquisition, mice received a 20-min trial of 0.12M NaCl. ***Results:*** On conditioning day, mice from both strains responded similarly to NaCl and LiCl in the first minute, suggesting these stimuli are comparable in taste. All mice receiving LiCl rapidly formed an aversion beginning in the second minute; D2 mice displayed complete aversion by minute 3, while B6 showed this by minute 6. On the following day, both strains avoided NaCl relative to controls; B6 displayed comparable licks to controls by the 4th minute, while D2s did not show this until the 8th minute. However, D2 mice showed lick counts close to zero throughout the entire trial. ***Conclusion:*** B6 and D2 mice can rapidly develop a CTA to LiCl via self-administration in a lickometer. The CTA generalizes to NaCl the next day, but extinguishes, with B6 mice showing a faster rate of extinction than D2 mice.

#P74 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Beyond *Tas1r3*: Identification of other loci affecting consumption of sweet-tasting compounds

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Inbred mouse strains differ in their responses to sweet taste stimuli. Although allelic variation of the *Tas1r3* locus is partially responsible for these differences, our genetic analyses using C57BL/6ByJ (B6) and 129P3/J (129) strains suggests that other loci are also involved and that some of them are sweetener-specific. We have further studied such loci using an F2 intercross between B6 inbred and 129.B6-*Tas1r3* congenic mice and subsequent selective breeding. F2 mice varied in consumption of the 30 mM glycine and 20 mM saccharin and there was no correlation between these two traits. From the F2 generation, we started selective breeding of two pairs of lines: with high and low saccharin intakes, and with high and low glycine preferences. The phenotype-based selection resulted in divergence between these pairs of lines, which confirms presence of loci polymorphic between the B6 and 129 strains. To refine positions of these loci, we have genotyped mice from the 10th and 8th generations of selective breeding with markers on chromosomes, which were previously linked to glycine preference and saccharin intake, respectively. For all chromosomes, there was a significant divergence of frequencies of alleles in these regions. For glycine selection, mice from the High line accumulated B6 alleles in Chr2, 7 and 12, and 129 alleles in Chr14. For saccharin selection, mice from the High line accumulated B6 alleles in Chr1, 3 and 13, and 129 alleles in Chr2 and 7. Mice from the Low lines accumulated alleles from the opposite strain at these locations. This observation suggests a complex genetic architecture of behavioral taste responses to sweeteners. There are two distinct sets of genes regulating glycine and saccharin consumption and each parental strain contributes loci increasing or decreasing a trait value.

#P75 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

The Benzamil (Bz)-insensitive NaCl Chorda Tympani (CT) Taste Nerve Responses Demonstrate Increased Sensitivity to TRPV1t Modulators in Alcohol-preferring (P) Rats

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Ethanol (ETH), nicotine (NIC) and resiniferatoxin (RTX), modulators of TRPV1t, induce biphasic effects on rat Bz-insensitive NaCl CT responses. At low concentrations they enhance and at high concentrations inhibit the NaCl CT response. To investigate if chronic ethanol ingestion and genetic preference for ethanol are related to alterations in the Bz-insensitive NaCl CT responses, we investigated the effect of ETH, NIC and RTX on the Bz-insensitive NaCl CT responses in alcohol-preferring (P) and alcohol nonpreferring (NP) rats. In naïve P rats ETH, NIC and RTX concentration versus the magnitude of the Bz-insensitive NaCl CT response curves were significantly higher and were shifted to the left on the agonist concentration axis relative to NP rats. This suggests that alcohol preference increases the sensitivity of TRPV1t to stimulation by various agonists. P and NP rats were adapted to chronic oral ethanol ingestion using the sucrose fading paradigm. When adapted to 5% ethanol, P rats consumed significantly more ethanol than NP rats given free access to ethanol alone or when given a choice between ethanol and water. NP rats given oral 5% ethanol for 2 weeks the RTX concentration versus the Bz-insensitive NaCl CT dose response curve was higher and shifted to the left on the concentration axis and was not different from the response profiles observed in naïve P rats or P rats given 5% ethanol. These results suggest that upon chronic ethanol consumption NP rats develop the phenotype of P rats related to TRPV1t activity. We conclude that TRPV1t activity is modulated by both ethanol consumption and genetic preference for alcohol.

#P76 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Central neural sensitivity to ethanol and other taste stimuli in selectively bred ethanol-preferring and ethanol-non-preferring rats

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A strong positive relationship has been found in mammals for preference and intake of ethanol and sweet-tasting substances. Ethanol is an ingested drug and its first direct interaction with the body is orosensory. Data from randomly bred rats indicate that orally-applied ethanol stimulates neural substrates for sweet taste, which are known to activate forebrain systems associated with reward. Yet it is unknown how ethanol's appetitive sweet taste properties play into the array of variables that influence alcohol ingestive reinforcement. Here, we made in vivo electrophysiological recordings from central gustatory neurons in selectively bred ethanol-preferring (P) and ethanol-non-preferring (NP) rats (Lumeng et al. 1977) to determine if a relationship exists between genetically-mediated alcohol preference and the neural processing of ethanol taste. Rats were anesthetized and taste

responses (net spikes) evoked by applying ethanol (3, 5, 10, 15, 25 and 40% v/v) and standard stimuli (including [in M] 0.5 sucrose, 0.1 NaCl, 0.01 HCl and 0.01 quinine) to the tongue and palate were recorded from single neurons in nucleus tractus solitarius. Neurons were also tested with a concentration series of sucrose (0.01, 0.03, 0.1, 0.3 and 1 M) and various other stimuli (fructose, glucose, a sucrose/quinine mixture and individual components of this mixture, KCl, citric acid, $NaNO_3$, nicotine and $MgCl_2$). Thus far, 14 neurons have been recorded from P rats and 6 cells from NP rats. Preliminary analyses reveal that ethanol produces salient, concentration-dependent activation of NTS cells in both P and NP ($F_{5,90}$ = 24.48, P <.001) rat lines, although additional data need to be collected to determine differences in ethanol-induced activation of specific gustatory cell types.

#P77 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Integrative studies of the relationship between feeding condition and the rat olfactory bulb responses

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Olfaction is a major factor in evaluating food from distance and a major component for flavor perception of the food, effecting both the type and amount of food-intake. Psychophysical studies demonstrate that when people are hungry, their olfactory sensitivity will increase and their evaluations of the same food tend to be more positive. However, the mechanisms behind the phenomenon are not clear. Olfactory bulb (OB) is the first center dedicated to olfaction and is essential for all olfactory functions. Here, we have used multi-modal methods, including animal behavior, functional MRI (fMRI), optical imaging, electrophysiological recording, and NMR to reveal the relationship between blood glucose and the response in the rat OB. Data from these technologies so far agree with each other, although these methods provide information from different angles. The intensity, not the topography of the activity patterns elicited by odors and revealed by fMRI, changes significantly with blood sugar level and the effects are more significant in the deeper OB layers. The electrical recording data showed that effects of glucose level on local field potential (LFP) are different for some of the six frequency bands examined. The NMR studies on the metabolic rates of ^{13}C labeled glucose revealed that the TCA cycle rate is slower in fasted animal, but the glutamate recycling rate is increased. With odor stimulation, the increases of TCA and glutamate cycles in fasted animals are more significant, providing neurochemical basis for the increased olfactory sensitivity, BOLD signal, and LFP in fasted animals. The results from these methods provided insight into the mechanisms in the OB for the daily observed fact that olfactory responses change with feeding conditions.

#P78 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Resistance to Obesity Following Kv1.3-gene Targeted Deletion is Inhibited by Olfactory Bulbectomy

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Gene-targeted deletion of the voltage-gated potassium channel, Kv1.3, produces "super-smeller" mice that have altered mitral cell firing patterns, supernumerary and heterogenous glomeruli, and an increased ability to detect and discriminate odors. Kv1.3-null mice are thinner and weigh less than their wildtype (WT) counterparts. To determine if there was a correlation between Kv1.3, metabolism, and olfaction, 11 week old mice were maintained on a moderately high fat diet (MHF, 32% fat) for 26 weeks. Diet-induced obese (DIO) mice exhibited a 47% increase in body weight, a 32% increase in serum insulin, and a loss of 52% of M72-expressing olfactory sensory neurons (OSNs). DIO mice treated with intranasal insulin failed to demonstrate tyrosine phosphorylation of either the insulin receptor or the Kv1.3 channel. Kv1.3-null mice were resistant to DIO with a weight gain of only 10% and no change in adiposity. Gene-targeted deletion of Kv1.3 in melanocortin 4 receptor-null mice (a genetic model of obesity) reduced body weight by decreasing fat deposition and subsequent fasting leptin levels, without changing fasting blood glucose and serum insulin. Dark-phase locomotor activity and mass-specific metabolism were significantly increased, which resulted in increased total energy expenditure. In order to isolate an olfactory-specific contribution of the ability for Kv1.3 to regulate energy homeostasis, bilateral olfactory bulbectomy (OBX) was performed at 9 weeks followed by 26 weeks of a MHF-diet. OBX Kv1.3-null mice were no longer resistant to DIO and exhibited a 30% increase in body weight. These data suggest the energy homeostasis that is perturbed by loss of Kv1.3, interacts or is regulated via the OB and that obesity modifies the expression of OSNs as well as induces insulin resistance in the OB.

#P79 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Experimental anosmia abolishes avoidance of ethanol solutions in 129P3/J mice

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Several lines of evidence suggest that alcohol consumption in both humans and laboratory animals depends on genetic differences in chemosensation of alcohol (which involves taste, olfaction and chemosensory irritation). The aim of the present study was to evaluate the role of olfaction in avoidance of ethanol solutions in mice from the 129P3/J inbred strain known as a model of low alcohol consumption. Taste responses to ethanol (1.25-20%), sucrose (1-32%), and quinine (0.01-1 mM) were assessed in the brief-access licking tests using the Davis MS-160 gustometer in water-restricted control mice and mice with experimental anosmia

induced by intranasal application of 5% $ZnSO_4$ with 5% lidocaine. Then, conditioned taste aversion (CTA) to 10% ethanol was induced using LiCl, and testing in the gustometer was repeated. In separate groups of control and anosmic mice long-term consumption of ethanol solutions was assessed in 48-h two-bottle test. We have shown that 10% and stronger ethanol solutions were avoided in both long-term and brief access tests by intact naïve 129P3/J mice, but not by anosmic mice. This suggests that an odor of ethanol is a principle signal for ethanol avoidance in both brief-access and long-term tests. Anosmia did not change unconditioned licking responses to aversive quinine and palatable sucrose, and did not interfere with development of CTA to ethanol. Our data suggest that mice use both gustation and olfaction for chemosensory detection of ethanol, and that olfactory input plays the primary role in ethanol avoidance.

#P80 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Maillard Reacted Peptides (MRPs) Modulate Benzamil (Bz)-insenstive NaCl Chorda Tympani (CT) Taste Nerve Responses and Blood Pressure (BP) in Dahl Salt-sensitive Rats

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MRPs conjugated with galacturonic acid (GalA-MRP) produce biphasic effects on the rat Bz-insensitive NaCl CT responses. At low concentrations (0.1-0.25%) GalA-MRP enhances and at high concentrations (0.3-1.5%) inhibits the Bz-insensitive NaCl CT response. The maximum enhancement and inhibition of the NaCl CT response was obtained at 0.25% and 1.5% GalA-MRP. We hypothesize that at these concentrations GalA-MRP can be used as a salt taste enhancer and a salt taste suppressor, respectively. Accordingly, the effect of 0.25% and 1.5% GalA-MRP was tested on BP in Dahl salt-sensitive rats fed a diet containing 0.3% NaCl. Radio-telemetry blood pressure transmitters were surgically implanted in rats to monitor their mean arterial pressure (MAP) in conscious, unrestrained rats for 15s every 15 min over a 24 hr period. The average daytime (7 AM to 7 PM) and nighttime (7 PM to 7 AM) MAPs were recorded for each 24 hr period. Rats maintained on rat chow containing 0.3% NaCl demonstrated a slow rise in their nighttime MAP over a 2 week period (0.5-1.6 mm Hg/day). The spontaneous increase in MAP was inhibited when rats were fed 0.3% NaCl diet containing 0.25% GalA-MRP over a period of 4 weeks (0.05-0.17 mm Hg/day). Following removal of the MRP from the diet, MAP increased at the rate of 0.3-2.0 mm Hg/day for 2 weeks. In rats fed 0.3% NaCl diet containing 1.5% GalA-MRP the MRP increased at a rate of 0.89±0.17 mm Hg/day relative to 0.22±0.05 mm Hg/day in rats maintained on 0.3% NaCl diet alone (mean±SEM; $p<0.0001$). These results suggest that under chronic conditions, GalA-MRP modifies salt taste and regulates MAP in a rat model of salt-sensitive hypertension. Supported by DC-00122, DC-005981 and Kyowa Hakko.

#P81 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Regulation of release of endogenous opioids from duodenal brush cells requires Trpm5

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Objectives: We studied a special population of cells in the mucosa of mouse duodenum that appear as brush or caveolated cells and presumably function as chemosensory cells. These cells express the Trpm5 cation channel, do not contain secretory granules, yet produce several bioactive peptides. **Methods**: We used immunohistochemistry to study expression patterns of Trpm5 and other molecules in duodenum of transgenic mice that express green fluorescent protein from the Trpm5 promoter. We used ELISA to quantitate peptides produced by intestinal tissues from wild-type and Trpm5-null mice. **Results**: We identified a type of mucosal cell in mouse duodenum that is defined by the presence of the Trpm5, does not contain secretory granules typical of enteroendocrine cells, yet expresses endogenous opioids and uroguanylin in apical compartments close to the lumen of the gut. We determined that beta-endorphin is secreted into the gut lumen in response to various stimuli mimicking digestive products. **Conclusion**: Solitary chemosensory cells exist in mouse duodenum that co-express beta-endorphin, Met-enkephalin, uroguanylin and Trpm5. These cells are likely to secrete the bioactive peptides into the intestinal lumen in response to dietary factors. Release of the opioid peptides requires the Trpm5 ion channel.

#P82 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Differential Effects of GLP-1 Agonist on Brief- and Long-Access Sucrose Preferences in Lean and High Fat Diet-Induced Obese Rats

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Glucagon-like peptide-1 (GLP-1) is released by enteroendocrine L cells of the ileum in response to ingested nutrients, especially carbohydrates. In addition to its action as an incretin, recent studies have suggested a broader role for GLP-1 in control of food intake and body weight. To investigate plausible effects of GLP-1 on taste and preference functions for sucrose, we performed brief-access lickometer and 24-hr 2-bottle tests in lean and high fat diet-induced obese (DIO, 14 wks on 40kcal% fat, high-energy diet) male Sprague Dawley rats following a sub-chronic (7-day, BID, s.c.) administration of the GLP-1 agonist Exendin-4 (EX-4; 0, 1, 2, 3 µg/kg). EX-4 (2-3 ug/kg) resulted in decreased food intake (up to 40% on days 2 and 3) followed by sustained hypophagia (10-15%) irrespective of dietary fat content (i.e. low or high fat). Furthermore, EX-4 dose-dependently reduced 10-s lick responses to palatable sucrose solutions above 0.3M in lean and DIO rats equally compared to vehicle-treated controls. In contrast, 2-bottle preference for 1.0M over 0.3M sucrose was diminished only in lean rats at higher doses of EX-4. However, even the highest dose caused no aversion to sucrose.

These findings demonstrate the capacity of EX-4, a GLP-1 agonist to reduce preference for palatable sucrose solutions, and suggest diminished sensitivity to the postoral component of this effect in DIO rats maintained on high fat diet. These observations together with data showing an attenuated GLP-1 release after a meal and an increased preference for sweet in obese individuals suggest a causal link between diminished intestinal GLP-1 feedback and orosensory sensitivity to sweet, and may also help with promoting a novel application for GLP-1 agonists to combat sweet cravings and overeating.

#P83 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

State-dependent Yeast Intake in *Drosophila melanogaster*
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Specific hungers may arise as a result of metabolic stress. For instance, it has been shown that protein-malnourished human babies prefer soups that are high in protein over plain or sucrose-fortified soups. We aim to utilize the fruit fly as a genetic model of the human nutritional need and appetite for protein. Most infamously, female mosquitoes require a blood meal prior to egg laying. In the current study, we sought to determine if nutrient intake of *Drosophila melanogaster* is affected in a similar way. Mated, virgin, and male wild type *Drosophila melanogaster* were given a choice between equivalent concentrations of yeast and sucrose solutions. Mated flies showed greater consumption of yeast than virgin females or male flies, while sucrose ingestion remained similar among all three groups. The increase in yeast intake indicates an initial preference for yeast over sucrose. However, yeast preference was not stable throughout the experiment but rose and fell, behavior consistent with egg laying. These results suggest a state-dependent protein appetite in mated *Drosophila*, which may result from alterations in nutritional needs as the organism prepares for oviposition.

#P84 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

A Behavioral Assay using *Drosophila* to Test for Chemesthetic Irritants Activating TRPA1 Channels
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Chemesthesis is the sense of irritation produced by chemicals. In mammals, the trigeminal nerve mediates chemesthesis in the head and face. Chemical irritants stimulate the trigeminal nerve through a variety of receptor proteins, including TRPA1 which is activated by over 90 compounds. Fruit flies, *Drosophila melanogaster*, possess a *painless* gene which is the homolog of mammalian TRPA1. We compared behavioral responses to irritants in wild-type and *painless* mutant flies in a feeding choice assay (Al-Anzi et al, 2006) to determine whether irritants which activate trigeminal nerve responses in mammals also stimulate fly TRPA1. A 96-well plate served as the test arena with half the wells containing sucrose and blue dye and the other half containing sucrose plus irritant and red dye. In half of the tests, the color of the food choice was reversed to eliminate color preference. Fifty flies were starved for 24 hours, and then released into the test arena. After one hour of feeding, the flies were examined under a microscope to determine what they ate. Flies were scored for blue, red or purple abdomens. The number of flies consuming each food choice was quantified and a preference index was used to determine which chemicals the flies avoided. Eleven compounds were tested at a concentration of 5mM. Eight of these compounds elicited significantly different responses in the wild-type and *painless* mutant. These included allyl-isothiocyante, eugenol, nicotine, α-terpineol, amyl acetate, benzaldehyde, and d-limonene. Acetic acid, toluene, and cyclohexanone did not produce significantly different responses. These results are similar to descriptions of the chemical sensitivity of mammalian TRPA1. Thus, this fruit fly assay might serve as a useful screen for trigeminal nerve irritants which activate TRPA1.

#P85 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Intake of Fructose and Sucrose Solutions as a Function of Concentration
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There have been few direct comparisons of fructose (F) and sucrose (S) intake to determine differences in drinking patterns across multiple isocaloric concentrations. Adult male SD rats (275-300g) were divided into S and F groups (n=8/group), and individually housed in "hotel" cages which continuously monitored rats' access to powdered chow (3.6 kcal/g) and number of licks at each of 2 drinking bottles in 6-s bins. Drinking bottles and chow were weighed and replenished daily. Each week, rats were given access to water, a single concentration of sugar (S or F) and chow for 5 days, followed by a 2 day break with chow and water only. Rats were tested with S (0-1.0M) or F (0-2M) solutions in ascending order. Water intake was negligible when sugar was available. Sugar intake was significantly greater than baseline water intake at or above 0.03M S or 0.06M F. Intake as a function of concentration peaked at 0.25M S (136±15g/d) and 0.5M F (138±12g/d). When expressed as caloric density, the concentration-intake curves for S and F were not significantly different, with a peak at 0.34kcal/g. While S and F intake was similar, there was a significant effect of sugar type on chow intake and lick rate. Sucrose rats decreased chow intake as S increased above 0.03M (0.04kcal/g). Fructose rats did not significantly decrease chow intake compared to baseline and ate significantly more chow than S rats when drinking 0.08 kcal/g solutions and above. Thus, cumulative caloric intake was much greater in F rats (3607±63 kcal) than S rats (2025±107 kcal, $p<0.05$). Also, S rats showed an orderly increase in mean lick rate with concentration from 2.5 licks/s to 5 licks/s. The lick rate of F rats remained constant at ~3.5 licks/s. Thus fructose and sucrose elicit markedly different patterns of ingestion and caloric intake.

#P86 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Antennular Waving in Spiny Lobsters is Enhanced by Odorants: A 3D Kinematic Analysis
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A major goal of chemosensory guidance research is to elucidate how animals extract information from the complex spatial and temporal structure of odorant plumes. Studies using lobsters as model organisms have examined orientation and locomotion of the whole animal as it encounters odorant plumes, as well as the flicking behavior of the antennules, the olfactory organ. Antennules can also move independently of general body movements by adjusting the joints of segments of the antennules more proximal to the body than the lateral and medial flagella that contain chemosensilla. These movements, referred to as antennular waving, have been observed anecdotally to increase in the presence of chemical stimuli. This hypothesis was tested by stereoscopic filming of antennular waving of 10 spiny lobsters, *Panulirus argus*, towards control seawater and squid extract. 3D kinematic analysis software was used to measure the second by second coordinates of antennules and the body over 60 sec following stimulus introduction. Antennule coordinates were corrected for body position. Antennular waving increased about 50% upon introduction of squid extract relative to control responses. However antennular waving and body movement are positively correlated independent of stimulus type. Left and right antennular waving averaged over 60 sec were strongly correlated. However second by second velocities of the two antennules were not correlated. Thus left and right antennule movements, while not synchronized, are both tightly coupled to body movement. We also report preliminary results of ablation studies designed to determine contributions of nonolfactory and olfactory inputs to antennular waving. These results suggest that antennular waving contributes to increased spatial sampling in the presence of odorants.

#P87 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

The effect of sniffing frequency on odor behavior
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Many animals, such as horses, sheep, dogs, cats, rats and mice commonly exhibit the sniffing behavior when they are searching the foods or the odors. It is believed that the sniffing is one of the important behaviors in their life. It is possible that change in sniffing frequency and (or) strengthen, then the odor sampling behavior and the olfactory receptor cell responses may be changed and alter higher order coding. Standard respiration rate in rats is 1-2 Hz but sniffing varies between 4-12 Hz. Olfactory receptor cells are elicited the responses by the absorption of the chemical substances. We used continuously or intermittent (mimicked the sniffing respiration pattern) application of odor stimulation methods, and have investigated that changes in sniff frequency could be changed the level of odor behavior or not, and how sniffing alters odor responses during the different sniffing behavior.

#P88 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Detailed Analysis of the Effects of Periodic Input on Behavioral Measures of Odor Detection in the Moth *Manduca sexta*
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In mammals, sniffing is an active odor sampling process. In moths, the wing beat oscillates airflow over the antennae affecting olfactory input, which may affect sensitivity. To test this hypothesis, we generated concentration response functions using trained *Manduca sexta* moths. Moths were conditioned to associate a conditioning stimulus (odor; CS) with sucrose using classical conditioning. At 24 then 48 hours after conditioning, moths were tested with either a pulsed or continuous CS. Pulse trains were 4 s long, 20 Hz (the approximate wing beat frequency), with either a 10:40 ms (ON:OFF) or 40:10 ms duty cycle. Duration of continuous stimuli was normalized to the integrated pulse duration. Stimuli were presented across a 5 log-step dilution series from low to high. Flow velocity was ~30 or ~80 cm/s (speeds consistent with odor-guided flight). To control for extinction effects, 5 groups were tested with one dilution. To correct for the total stimulus duration, one group received the continuous stimuli at 1/5 the concentration (replicating the 10:40 duty cycle). We also statistically normalized responses to percent response/ms on-time. To control for stimulus-specific mechanical effects, responses to blanks were recorded for both stimuli and used for normalization of odor responses. Results of ANOVA indicated that the percentage of moths responding to the CS increased significantly as a function of concentration. Importantly, pulsed stimuli significantly enhanced sensitivity to odor; this was true across all control groups. Results of these behavioral experiments support the hypothesis that periodic stimuli increase odor salience in this moth species.

#P89 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Periodic Odor Stimulation Affects Antennal Input, Antennal Lobe Processing, and Behavioral Measures of Perception in the Moth *Manduca sexta*
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Biomechanical studies of the insect wing beat show that each down stroke increases air flow over the antennae. This could affect penetration of odor on the sensilla, thus, providing a temporally patterned antennal lobe (AL) input in a manner similar to mammalian sniffing. Does the antenna and AL respond to odor stimulation on a wing beat timescale (18-28Hz) and can this affect perceptual acuity? To address these questions, we varied stimulus frequency and measured antennal responses in *Manduca sexta* moths, via electroantennograms (EAGs). Spectral analysis of EAG responses indicated significant pulse tracking up to 30Hz. Responses to blanks were significant up to 25Hz, but were significantly smaller than pulsed odor. Using in vivo multi electrode methods, we show that both AL local field potential and unitary responses tightly track periodic stimuli at wing beat frequencies. Local field potentials tracked stimuli beyond the wing beat frequency (up to 72Hz). Furthermore ~25% of

recorded units tracked stimuli up to 30 Hz. Bicuculine (200μM bath applied) abolished AL pulse tracking, suggesting $GABA_A$ involvement. Intracellular replication indicated that both local and projection interneurons track pulses but the reduced "head only" intracellular method yielded tracking to only 14Hz. Finally, using a conditioned feeding response as a behavioral indicator of odor detection, we showed that moths are more sensitive to odor pulsed at 20Hz, relative to a continuous odor. These results suggest that the olfactory system of this moth has evolved to respond to olfactory input defined by the beating wing.

#P90 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Attraction of Female Round Gobies to Steroids Released by Males

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The objective of this study is to evaluate the female chemoattractant property of isolates derived from male urine or from water previously occupied by male round gobies, *Neogobius melanostomus,* which is an invasive fish species to the North American Great Lakes. Reproductive (RF) or non-reproductive phase female (NRF) round gobies were placed in isolated 5-L flow-through tanks and presented with odours via the water inflow after an acclimation period. The behaviour of the fish was recorded then analyzed. Swimming velocity and the amount of time spent near the odour inflow was determined. Neither parameter was affected by the delivery of vehicle blanks. The RF increased the time spent near the odor source when presented with urine collected from males previously injected with GnRH, and by fish water excluding urine. Both RF and NRF were attracted by methanol extracts of male-scented water. NRFs increased swimming velocity toward and the time spent near an isolate of male-scented water containing the steroid 11-oxo-etiocholanolone (11-oxo-ETIO), however RF showed an avoidance swimming reaction to this fraction. In contrast, both RFs and NRFs spent greater time near the inflow of an isolate containing an unknown conjugate of 11-oxo-ETIO. These studies demonstrate that females can exhibit reproductive-state specific attraction responses to steroids released by male round gobies, including 11-oxo-ETIO and an unidentified conjugate. We are working towards identifying the compounds responsible for this activity.

#P91 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Androstenone May Show Pheromonal Activity in Mice

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In mice reproductive and aggressive behavior is guided by odors and investigatory behavior provides the behavioral mechanism for evaluating sex, physiological status and social rank of another individual. Failure to detect certain biological odors may seriously disrupt behavioral reactions. Mice engage in anogenital and/or naso-oral investigation prior to either initiating sexual advances in the presence of a female or aggression with an unfamiliar male. We studied a possible relationship between sensitivity to androstenone (AND) and aggression in NZB/B1NJ (NZB) and CBA/J (CBA) mice. CBA mice are more than 2000-fold sensitive to AND than NZB mice. Atypical for mice in general, NZB males often attacked females. This may imply that chemosensory cues and social behavior are de-linked in NZB males. Alternatively, NZB mice may have deficits in olfaction that lead to failure to discriminate sex and social rank of conspecifics. Failure to process biologically important odors may lead to elevated aggression. In standard odor preference test CBA males showed strong preference for receptive female odor relative to male odor (n=8). However NZB males did not show preference for the odor from receptive female versus odor from male. CBA males showed significantly ($p<0.01$, n=8) higher investigatory activity towards biologically relevant odors then NZB males. Exposure of CBA males for 30 minutes to AND (0.1%) suppressed plasma testosterone (T) ($p<0.05$, n=7); in water treated animals we did not observe changes in plasma T ($p<0.05$, n=8); in mice exposed to receptive female urine we observed clear elevation of T ($p<0.05$, n=8). At the same time we monitored fecal corticosterone metabolites under long lasting AND exposures in mice. The data obtained indicate that AND known as sex boar pheromone may show pheronomal activity in mice.

#P92 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

The induction of pregnancy block in mice by saliva via the vomeronasal organ

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Animals have evolved specific communication systems to identify and attract mates, and to discern the social status of conspecifics. In mice these exchanges of information involves the emission and detection of pheromones. These pheromones are detected by the vomeronasal system. While urine has long been identified as the primary source of pheromones, including those responsible for pregnancy block, recent evidence indicates that there are other sources. The sources include pheromones such as ESP1 from an exocrine gland and MHC class I peptides from the immune system. These MHC class I peptides have been identified as compounds that elicit the pregnancy block effect *via* the vomeronasal system similar to the effect elicited by urine from castrated or juvenile males. Here we show that saliva, produced by a different exocrine gland, is capable of inducing the pregnancy block (Bruce Effect) paradigm in a manner equivalent to female mice exposed to whole urine or MHC peptides. Furthermore, we correlate this effect with the activation of the phospholipase C pathway by measuring the increase in inositol 1,4,5-trisphosphate. Therefore we conclude that saliva is capable of inducing pregnancy block and that the signal is transduced in a similar manner as urine or MHC peptides.

#P93 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Sea hares chemically defend themselves from predatory blue crabs and bluehead wrasse using light-harvesting molecules in their algal diet

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Sea hares, *Aplysia californica*, release a purple ink secretion when attacked by predators. This secretion is composed of two secretions, ink and opaline, and either alone or both together can defend them by affecting chemosensory system of predators. The molecular identities of the deterrent compounds in the ink remain largely unknown. We asked two questions: Is *A. californica* ink a chemical deterrent against two predators: blue crabs, *Callinectes sapidus*, and bluehead wrasse, *Thalassoma bifasciatum*? and if yes, What are the active molecules? Both species were tested in two behavioral assays: a feeding assay in which animals were offered food soaked (or not) in ink; and a cloud assay in which ink was squirted at the animals. Both assays revealed that fish and crabs are deterred by ink. The identity of the active components in ink was examined in bioassay guided fractionation using the feeding assay. Aplysioviolin (AV) was identified as the major active component and phycoerythrobilin (PE) was a minor one. AV and PE have similar activities at equimolar concentrations, but because aplysioviolin is approximately ten times more abundant in ink, it is the major active component in ink. The structures of these molecules were identified using NMR, MS, and UV spectra. PE is a chromophore covalently linked to a protein to form the photosynthetic pigment of red algae in the diet of *Aplysia*. AV is the monomethyl ester of PE. AV and PE are also present in ink of *Aplysia dactylomela*, a sympatric sea hare with blue crabs and bluehead wrasse, at approximately the same ratio. Thus, sea hares acquire light-harvesting proteins from red algae, cleave phycoerythrobilin from the protein, convert it into aplysioviolin, and store these molecules in the ink gland, and release it as chemical deterrents against predators.

#P94 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Role of Octopamine in Moth Olfaction

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Octopamine (OA) is a significant neuromodulator within the invertebrate nervous system. In many respects, the functional role of OA is similar to that of other aminergic compounds such as noradrenaline within vertebrates. This includes a direct influence on energetic activities, arousal, learning and memory. The current study investigates the effects of OA on the physiology and odour-mediated behaviours of the tobacco budworm moth, *Heliothis virescens*. First, a neuroanatomical study was conducted to determine the distribution of OA within this species brain, relative to other moth species. Second, OA injections were made into the brain and single-sensillum recordings were subsequently made from the antennae. In particular, the responses of male and female moths to female sex pheromones were compared to identify any intersexual differences in threshold or spiking evident from OA levels. Finally, male moth upwind flight ability was studied following OA injection, to identify differences in successful flight to artificial pheromone lures. Pending results of the current study, subsequent efforts will integrate learning paradigms such as the proboscis-extension reflex (PER) to decipher any influence of OA on odour learning.

#P95 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Flavor Identification and Memory in Children

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We have previously shown that identification and memory for jelly bean flavors is improved when flavor labels are provided during testing. The current study assessed whether similar effects would be found in children. Children between four and eleven years old were asked to identify and remember jelly bean flavors in a joint flavor identification/recognition memory task. In Phase 1 the children were given ten jelly beans and asked to identify the flavor of each. They were provided with four alternative flavor labels and accompanying pictures that matched the labels. Phase 2 commenced after a ten minute retention interval. Participants were presented randomly with the ten jelly beans from Phase 1 and ten new jelly beans, and asked to accurately identify each flavor and indicate whether the jelly bean flavor had been presented previously in Phase 1 (old) or was being presented for the first time (new). We found, as with adults, that identification and memory performance improved when labels were provided, but the effect was larger for adults. We had previously found that consistently labeled old flavors were remembered almost perfectly by adults. Interestingly, this same relationship was observed for children. This suggests that flavor knowledge and experience (rather than differences in cognitive processes) account for the observed differences between the performance of the children and adults.

#P96 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Millisecond Photoactivation of Bombykol Receptor Neurons Expressing Channelrhodopsin-2 Triggers Pheromone Searching Behavior in Male Silkmoths

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Odor-evoked spikes in olfactory receptor neurons (ORNs) induce behavioral responses within a short-timed latency. Therefore, controlling neural activity of targeted ORNs with high temporal resolution is important to clarify neural codes underlying computational circuit and odor-driven behavior. The silkmoth, *Bombyx mori*, is a useful model for studying the olfactory system because of the straightforward input-output

relationship in the characteristic response (pheromone searching behavior) of male moths to pheromone called bombykol released by conspecific females. However, little is known about the precise temporal characteristics of peripheral input in relation to the initiation of pheromone searching behavior because the difficulty in controlling and administering olfactory stimuli still remain to be solved. To address the problem, we generated a transgenic silkmoth expressing channelrhodopsin-2 (ChR2), a blue light-gated ion channel identified in green algae, under the control of a putative promoter sequence of *BmOR1*, the bombykol receptor gene in silkmoth. ChR2 expressing male displayed typical pheromone searching behavior in response to a single pulsed blue light stimulation. Probability of behavioral response was dependent on duration of stimulation, and response threshold time was 3 ms that evoked a single spike in bombykol receptor neurons in single sensillum recordings. When input information was decreased by covering a half region of antennae, probability of behavioral response was significantly reduced, which recovered to a normal level by a second stimulation given within a time window of approximately 120-240 ms. These results suggest that the triggering of behavior is simply determined by the collective amount of spiking information summated within few hundred milliseconds.

#P97 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Olfactory Thresholds of Elasmobranchs

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Olfactory capabilities of elasmobranchs are legendary. Although reputed to demonstrate remarkable odor sensitivities, this perception is based on surprisingly little empirical evidence. Olfaction plays an important role in the localization of prey, and amino acids in particular are effective odorants for elasmobranchs. However, olfactory sensitivity has been assessed for only four elasmobranch species using a handful of amino acids. Literature values for these species indicate thresholds to be approximately 10^{-7} to 10^{-8}M. The aim of this study was to survey the olfactory thresholds of five phylogenetically diverse elasmobranch species (*Dasyatis sabina*, *Urobatis jamaicensis, Raja eglanteria, Negaprion brevirostris,* and *Sphyrna tiburo*) in order to develop a representative picture of their olfactory capabilities. The electro-olfactogram (EOG) technique was used to assay the sensitivities of these species to a suite of twenty proteinogenic amino acids. Both the relative stimulatory effectiveness of the tested amino acids and their estimated thresholds (~10^{-7} to 10^{-9} M) across all five tested elasmobranch species were similar. These results indicate that elasmobranch species do not demonstrate greater olfactory sensitivity than teleost fishes.

#P98 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Cycloheximide: an effective taste aversion UCS

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A single oral exposure to 0.5 mM cycloheximide (Cyx) results in an increased aversive behavioral potency towards Cyx (Hettinger et al., 2007). To test if this increased potency is the result of de novo taste receptor induction or the ability of Cyx to act as an unconditioned stimulus in aversion learning, we behaviorally tested 4 groups (n=6) of golden hamsters (*Mesocricetus auratus*) and electrophysiologically recorded from 2 groups. All behavioral animals were maintained on a restricted water drinking schedule throughout the experiment. One group drank 100 mM sucrose before injection with 0.5 mM Cyx (mixed in 154 mM NaCl; 12.4 ml/kg bw; ip). This dose approximates the amount of 0.5 mM Cyx naïve hamsters ingest in 1 hr. A control group drank 100 mM sucrose before injection with 154 mM NaCl. A second control drank distilled water before injection with Cyx and the last group drank 0.5 mM Cyx before injection with NaCl. Hamsters exposed to sucrose and injected with Cyx showed robust behavioral avoidance to sucrose (~80% suppression) compared to all other groups, which did not differ from each other. The sucrose aversion remained robust through 4 weeks of testing. Chorda tympani (CT) responses were recorded in 2 groups (n=3) of hamsters culled from the 4 behavioral groups. One group was orally exposed to 0.5 mM Cyx and the other group was not exposed to Cyx at all. Cyx failed to activate the CT at concentrations up to 1 mM, regardless of Cyx exposure. These results suggest that the increased aversive behavior towards Cyx, following initial exposure, is due to the effectiveness of Cyx as an unconditioned stimulus in aversion learning.

#P99 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Enantioselective Odorant Receptor in the Yellow Fever Mosquito, Aedes aegypti

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Enantiomers, non-superimposable mirror image molecules, have been shown to drive important behaviors of a wide range of organisms from insects to humans. Enantiomer recognition is predicated on the organism's capability to discriminate these chiral olfactory signals. The existence of enantiomer-specific receptors in other molecular recognition processes and the extensive body of physiological evidence for such mechanisms in olfaction have long argued for the existence of enantiomer-specific odor receptors. Using the two-microelectrode voltage clamp technique with Xenopus oocytes expressing the mosquito *Aedes aegypti* OR8 (AaOR8), we were able to show for the first time direct evidence that an insect receptor discriminates the two enantiomers of 1-octen-3-ol. Not only is AaOR8 enantioselective, but proper chain length and degree of unsaturation are also important chemical features necessary for its full activation. This enantioselective odorant receptor provides a molecular basis for the chiral specificity exhibited by insect olfactory sensory neurons. This work was supported in part by a grant to J.C.D.

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#P100 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Relationships between Early Dietary Experiences and Acceptance of the Basic Tastes during Infancy

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To evaluate individual differences in acceptance and facial reactivity to the basic tastes, we studied an ethnically diverse sample of newly weaned infants (N=36), who were between the age of 5 and 10 months. The infants, all of whom had experience eating table foods, were tested for their acceptance of exemplars of the five basic taste qualities in a familiar food matrix (i.e., infant cereal). The methodologies used were developed and validated at the Monell Center and controlled for a number of factors to allow for the evaluation of infants' hedonic responses independently of the caregiver and experimenter. Our results indicate that there was a great deal of variability in the types of table foods that infants consumed at home as well as in their behavioral responses to the tastants. In general, the number of distaste expressions displayed during the first two minutes of feeding was predictive of infants' overall consumption (p's<0.05). Further, previous food experience was related to the infants' hedonic response to the particular taste. For example, those who ate cookies smiled more while eating the sweetened cereal (p<0.05), while those who consumed salty snacks, such as pretzels, wrinkled their nose (p<0.05) and squinted (p<0.07) less while eating the salty cereal. These results add to the growing body of scientific evidence that suggests that individual differences in food likes and reactivity are evident early in life.

#P101 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

How do taste and nutritional feedback from the gut interact to determine daily sugar intake?

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Most mammals consume sugar solutions avidly. This intake is stimulated by at least two types of sensory input: taste (oral) and nutritional feedback from the gut (post-oral). We asked how oral and post-oral stimulation interact to drive daily sugar intake in C57BL/6 mice. To this end, we exploited the fact that during a 24 h test, glucose (G) provides both oral and post-oral stimulation of intake, whereas saccharin (S) provides only oral stimulation of intake. Accordingly, adding S to a G solution should increase oral stimulation, while holding post-oral stimulation constant. We used water (W), one concentration of S (38 mM), three concentrations of G (167, 250 & 333 mM) and binary mixtures of G + S. Experiment 1 assessed oral stimulation from the solutions by (a) measuring chorda tympani nerve responses, and (b) determining their relative palatability in short-term choice tests, which minimized post-oral stimulation. Both measures indicated the following relative amounts of oral stimulation: 167G+S > S > 167G > W; 250G+S > S > 250G > W; 333G+S > S > 333G > W. Note that for each concentration of G, the relative oral stimulation was G+S > S > G > W. Experiment 2 determined whether the relative amount of oral stimulation reliably predicted daily intake of each solution. We observed the following relative intakes: 167G+S > 167G = S > W; 250G+S > 250G > S > W; 333G+S = 333G > S > W. Note that the measures of oral stimulation failed to predict the pattern of daily intakes, and that the discrepancy between oral stimulation and daily intake increased with concentration of G. In fact, 333G and 333G+S both stimulated daily intakes > 1.4 times the body weight of the mice. These data show that the contribution of post-oral stimulation to daily glucose intake increases dramatically with concentration.

#P102 **Poster session II: Chemosensory response to, and control of, feeding/Neuroethology**

Intensity of 6-n-propylthiouracil (PROP) Taste, Food Preferences, and Obesity: The Beaver Dam Offspring Study

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Background:The intensity of the response to PROP may influence food preferences and dietary intake, but few epidemiologic studies have evaluated taste. As part of the Beaver Dam Offspring Study (BOSS), a study of sensory aging, the associations of PROP taste intensity to food preferences and weight were evaluated. Methods:Adult children of participants in the population-based Epidemiology of Hearing Loss Study, a longitudinal study of aging, were eligible for the BOSS (n=3285; mean age =49 yrs). PROP filter paper disks (1.2-1.6 mg) were used to measure intensity on a generalized labeled magnitude scale (gLMS). Food dislikes/likes were assessed with a hedonic gLMS. Questionnaire data about lifestyle factors were obtained and height, weight, and waist circumference measured. Standardized PROP scores were analyzed as groups (low, mid and high responders). Results: The taste quality of the disk was called bitter by 83% of the high PROP group; 99.6% of those in the low group selected the "no taste" response. High PROP responders rated salted pretzels (3.16, p=0.04), sweets (3.87, p=0.02) and sausage (5.05, p=0.004) higher (liked more) on the dislikes/likes scale and rated dark chocolate (-6.09 p=0.02), and grapefruit juice (-4.93, p=0.03) lower (disliked more) than mid and low responders. High compared to mid/low PROP was associated with adding salt to food less often (Odds Ratio (OR)=0.74, 95%Confidence Interval (CI)=0.59, 0.92) and smoking (OR=1.50, 95%CI=1.17, 1.94). PROP was not associated with current Body Mass Index or waist circumference. Conclusions: PROP response in the general population was associated with differences in liking and disliking foods and may be associated with salt use and smoking. Longitudinal data are needed to understand the impact of PROP intensity on health.

#P103 Poster session II: Chemosensory response to, and control of, feeding/Neuroethology

Perception of Threshold and Suprathreshold Taste Stimuli in Obese and Normal-Weight Women

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The goal of the present study was to determine whether obese women exhibit altered umami and sweet taste perception when compared to normal weight women. To this end, each of 56 subjects participated in a two-day study separated by one week. Half of the women were evaluated using mono sodium glutamate (MSG; prototypical umami stimulus) on the first test day and sucrose on the second test day; the order was reversed for the remaining women. We used two-alternative forced-choice staircase procedures to measure taste detection thresholds, a forced-choice tracking technique to measure preferences, the general Label Magnitude Scale to measure perceived intensity of suprathreshold concentrations and a triangle test to measure discrimination between 29mM MSG from 29mM NaCL. We find that although obese women required a higher concentration of MSG to detect a taste, their perception of MSG at suprathreshold concentrations, their ability to discriminate MSG from salt, and their preference for MSG and sweets were similar to that observed for normal-weight women. Regardless of their body weight category, 28% of the women did not discriminate 29 mM MSG from 29 mM of NaCl (Non Discriminators). Surprisingly, we found that Non Discriminators at suprathreshold MSG concentrations had similar MSG detection thresholds than Discriminators. Taken together, these data suggest that different mechanism may be involved in the perception of threshold and suprathreshold MSG concentrations.

#P111 Poster session II: Cortical chemosensory processing/Receptor genomics and molecular biology

Group III metabotropic glutamate receptors modulate transmission of taste information in primary taste afferents

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Primary afferent taste fibers use glutamate as the major transmitter at their central terminals in the nucleus of the solitary tract (nTS). We investigated the role of metabotropic glutamate receptors (mGluR) in regulating transmission in the primary gustatory nucleus of goldfish, the vagal lobe (homologous to the vagal gustatory portion of the nTS in mammals). We used an *in vitro* slice preparation of the vagal lobe to determine the effects of mGluR agonists/antagonists in transmission of gustatory information. In this preparation, primary gustatory afferents were electrically stimulated, while evoked dendritic field potentials (fEPSP) were recorded in the sensory layers of the vagal lobe where the afferents terminate. We have previously shown that an mGluR agonist (L-AP4) attenuates synaptic components of the fEPSP to electrical stimulation of the primary taste afferent fibers. Here, we extended these findings by examining responses to an mGluR antagonist, MAP4. In Experiment I, we confirmed that L-AP4 significantly depressed the fEPSP. Then, the addition of MAP4 reversed the inhibitory effects of L-AP4 ($p < 0.01$). In Experiment II, we stimulated the afferent fibers with trains of electrical pulses (0.4, 3, 9, 24, and 48 Hz) and found that the amplitude of synaptic potentials decreased with an increase of frequency. This frequency-dependent depression was significantly attenuated in the presence of MAP4, the group III antagonist ($p < 0.05$). This indicates that glutamate activated group III mGluRs when the afferent taste fibers were electrically stimulated, and that this functioned to attenuate synaptic transmission. In sum, these results indicate that group III mGluRs at the primary afferent terminals reduce synaptic transmission during conditions of high afferent activity.

#P104 Poster session III: Cortical chemosensory processing/Receptor genomics and molecular biology

Odor Quality Coding and Categorization in Human Posterior Piriform Cortex

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Efficient recognition of odorous objects universally shapes animal behavior and is crucial for survival. To distinguish kin from non-kin, mate from non-mate, food from non-food, organisms must be able to create meaningful perceptual representations of odor qualities and categories. It is currently unknown where, and in what form, the brain encodes information about odor quality. We presented nine odorants (three each from three quality categories: minty, woody, citrus) to four subjects who underwent olfactory functional magnetic resonance imaging (fMRI). We used multivariate data analysis techniques to show that spatially distributed ensemble activity in human posterior piriform cortex (PPC) coincides with subjects' perceptual ratings of odor quality, such that odorants with more (or less) similar fMRI patterns were perceived as more (or less) alike. These effects were not observed in other brain areas linked to olfactory perception such as anterior piriform cortex, amygdala, or orbitofrontal cortex, providing evidence that ensemble coding of odor quality and categorical perception is regionally specific for PPC. Critically, there were no differences between conditions in the mean fMRI signal across voxels in PPC, demonstrating the utility of multivariate fMRI methods to assess sensory information content in the brain where more traditional univariate methods would fail. These findings substantiate theoretical models emphasizing the importance of distributed piriform templates for the perceptual reconstruction of odor object quality.

#P105 Poster session III: Cortical chemosensory processing/Receptor genomics and molecular biology

Anterior Olfactory Nucleus: A Golgi Study of Dendritic Morphology

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The anterior olfactory nucleus (AON) is the first bilaterally innervated structure in the olfactory system. It is typically divided into "*pars principalis*", a thick ring of cells that surrounds the remnant of the olfactory ventricle [usually subdivided into *pars medialis ("pm"), dorsalis ("pd"), lateralis ("pl")* and *ventro-posterior ("pvp")*] and "*pars externa*", a thin ring of cells encircling the anterior aspect of the structure. Little is known about the internal structure of either region. We performed a quantitative Golgi study to provide the first detailed look at the resident cells. Brains from 8 juvenile rats were stained with the Golgi-Cox method and sections counterstained with methylene blue. Neurolucida® software was used to reconstruct the cells and subject them to standard "branch" and "Sholl" analyses. A total of 206 "pyramidal"-type cells were examined in *pars principalis* (68 from deep, 71 from middle and 67 from the superficial thirds of Layer II, and further keyed as to location (19; *pm*, 73; *pd*, 86 ; *pl*, and 28; *pvp*). Preliminary analyses indicate no deep-superficial differences in total dendritic length or number of branches (medians: apical 855µm length and 18 branches, basilar: 17 branches, 430 µm length). Two varieties of cells in p*ars externa* were also examined: the typical cell with two apical dendrites extending into Layer Ib (sample = 50 cells: total dendritic length: 894 µm) and a second, complex cell with more primary apical dendrites plus basilar processes (26 cells: total apical length: 944 µm, basilar length 605 µm). Other less common cells were also observed, but due to the lack of a large sample were not subjected to a quantitative analysis. The results provide important information for understanding and modeling the circuitry of the AON

#P106 Poster session III: Cortical chemosensory processing/Receptor genomics and molecular biology

Detecting the taste-specific temporal type by fMRI – salty and sweet-

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Taste perception has a temporal dimension. The sensory evaluation in humans showed that, for example, the reaction time is shorter in salty than in that of sweet. The purpose of this study was to investigate the differences between salty and sweet in temporal neural responses in the human cortex. For this purpose, we used a new temporal model analysis to demonstrate the temporal parcellation of brain activity by whole brain analysis via fMRI. Healthy volunteers (ten males and ten females, 19-29 yrs of age) participated in this study, and all images were acquired with a 3.0-T MRI. Salty (0.1M sodium chloride) and sweet (0.5M sucrose) were used as tastants and tasteless artificial saliva (25 mM KCl plus 2.5 mM $NaHCO_3$) as the control. Image data analysis was performed using the SPM5. First, we analyzed fMRI datasets by using the standard approach model, and confirmed that the activated areas of both tastants were located on the putative human primary taste cortex (uncorrected $P < 0.001$). Second, for a new temporal model, we divided the actual stimulation period (for 6 sec) by three and constructed a box-car function with a 2-sec 'taste ON' duration. Based on its onset time at 0, 2, or 4 sec, each box-car function was described as a rapid (0-2 sec), medium (2-4 sec), or slow model (4-6 sec). Each model could detect the timing of the activation peak by each tastant. As a result, the salty-induced activation was fitted to the rapid model, and the sweet-induced activation was fitted to the medium model (uncorrected $P < 0.001$, respectively). These results revealed the taste-specific temporal type in the analysis of the temporal parcellation of human brain activity; the salty taste was represented as a rapid, and the sweet as a medium temporal type.

#P107 Poster session III: Cortical chemosensory processing/Receptor genomics and molecular biology

Low bulbar NE concentration modulates odor detection whereas higher concentrations modulate discrimination

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Although many neonatal rat studies have shown the importance of norepinephrine in species-specific odor dependent animal behaviors, very little is known about its effect in adult rats. Previous studies in our lab show that while blockade of NE receptors, in particular 1 receptors, impairs spontaneous discrimination, blockade of receptors improves discrimination (Mandairon, et al, 2008). Preliminary data from *in vitro* studies (see Nai et al., this meeting) suggest that inhibition of mitral cells is modulated by local NE application in a nonlinear manner, leading to dose-dependent effects on mitral cell activity. The current study used eight cannulated male Sprague-Dawley rats to investigate how odor detection and discrimination thresholds are modulated by NE release in the olfactory bulb. Using a habituation/cross-habituation task, we tested the animals' response to two similar odorants with varying partial pressures (10^{-6}, 10^{-4}, 10^{-3}, 10^{-2} Pa) after a direct infusion of NE to the olfactory bulb. The effect of different levels of NE input to the MOB was evaluated by using four NE concentrations (1 M, 100 M, 10mM, and 100mM). We found that detection and discrimination increase monotonically with NE concentration. In addition, detection is modulated by lower NE concentrations than discrimination. At very low odor concentrations, our results support the idea that low NE concentrations help odor detection whereas higher NE concentrations help odor discrimination. Given that noradrenergic locus coeruleus neuronal firing rate varies systematically across behavioral state and vigilance level, our findings would suggest that low LC firing rates may be optimal for odor detection while higher firing rates may enhance odor discrimination. *Support: PHS Grants: DC008702 and DC003195.*

#P108 Poster session III: Cortical chemosensory processing/Receptor genomics and molecular biology

The effect of unilateral naris occlusion on gene expression in the mouse olfactory mucosa and bulb

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Unilateral naris occlusion (UNO) has been the most common method of effecting stimulus deprivation in studies of olfactory plasticity. However, in the >100 years that have elapsed since the first reported UNO experiment, many contradictory results have accumulated. Early experiments focused on deleterious effects assumed to be due to the stimulus restriction that undoubtedly accompanies UNO. More recently, a number of studies have pointed to 'compensatory' effects of UNO. Unfortunately, few data are available on indirect UNO effects, i.e. those unrelated to odor deprivation. Modern high-throughput methods such as microarray analysis may help rectify the deficits in our understanding of UNO phenomenology as well as revealing new avenues to study olfactory plasticity specifically and neural plasticity more generally. Here we report the results of the first known analysis of genome-wide effects on olfactory mucosa and bulb induced by UNO using the Affymetrix 430.2 chip set. RNA was extracted from pooled tissue samples of 25-day-old female CD-1 mice using the Qiagen RNA easy kit. Some subjects had received UNO on the first postnatal day. The three treatment conditions: UNO open side, UNO occluded side, and untreated mice, were run in triplicate for mucosa and in duplicate for bulb using different groups of animals. Chip data were normalized using the gcRMA method and only genes that showed both a two-fold change and a p-value of <0.05 (BH corrected) were considered significantly regulated. This presentation will focus on the 87 genes in the mucosa and 82 genes in the olfactory bulb that met the significance criteria, comparing open and occluded tissues in mice that had received UNO. Network analyses of effected genes as well as our preliminary interpretation of selected results will be presented.

#P109 Poster session III: Cortical chemosensory processing/Receptor genomics and molecular biology

Anterior olfactory nucleus projections target the olfactory bulb

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The anterior olfactory nucleus (AON) is a cortical structure found caudal to the olfactory bulb (OB) and rostral to the piriform cortex (PC). Heavy reciprocal connections between the AON and the OB, the PC and other cortical regions associated with olfaction suggest that the AON plays an important role in olfactory processing. However, previous experiments examining these connections used large tracer injections that rendered a fine-scale analysis of individual projections impossible. Here, we used two methods to examine the projections to the OB from individual cells in the AON: a) small extracellular injections of *Phaseolus vulgaris* leucoagglutnin, and b) *in vivo* intracellular injections of biotinylated dextran amine. Injections were made into the large, central portion of the AON (i.e., *pars principalis*) in anesthetized male hooded rats (250-350g). Brains were extracted and processed 7-10 days later to allow complete filling of axon collaterals. Labeled fibers were found in both hemispheres, but were more heavily concentrated in the OB ipsilateral to the injection. Labeled axons were found in all layers of the OB. Quantitative analyses revealed that, although total axonal length and synaptic bouton numbers were highest in the granule cell layer, the ratio of synaptic boutons to axonal length was highest in the external plexiform and mitral cell layers. Notably, AON axons appeared to target mitral cells directly, suggesting synaptic contact with these principal output neurons. These results suggest that the AON participates in higher-order olfactory processing, and together with other morphological and functional evidence suggests that the AON may play a role in modulating activity in the OB and the piriform cortex.

#P110 Poster session III: Cortical chemosensory processing/Receptor genomics and molecular biology

Activation likelihood estimation (ALE) meta-analysis of human functional brain imaging data following trigeminal stimulation of the nasal mucosa with carbon dioxide (CO_2)

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Few functional imaging studies exploring the human neuronal correlates of intranasal trigeminal pain exist, and results are to some degree inconsistent. We utilized activation likelihood estimation (ALE), a quantitative voxel-based meta-analysis tool, to analyze functional imaging data (fMRI/PET) following intranasal trigeminal stimulation with carbon dioxide (CO_2), a stimulus known to activate solely the trigeminal system. Meta-analyses are able to identify activations common across studies, thereby enabling one to map activations with higher certainty. The contrast "CO_2 stimulation vs. Baseline" (207 foci) of 6 published studies, as well as 3 unpublished studies, was included in the meta-analysis. The ALE statistic was calculated by modelling a 3-D Gaussian distribution for each reported activation. Non-parametric permutation tests and subsequent corrections for multiple comparisons were used to test the null hypothesis that activation foci are distributed uniformly over the brain. We found significant ALE scores in the brainstem, ventrolateral posterior thalamic nucleus, anterior cingulate cortex, insula, precentral gyrus, as well as in primary and secondary somatosensory cortices – a network known for the processing of intranasal nociceptive stimuli. Additionally we detected significant ALE values in the piriform cortex, the posterior orbital gyrus and the cerebellum, areas known to process chemosensory stimuli, and in association cortices. The results of this meta-analysis map the human neuronal correlates of intranasal trigeminal stimulation with high statistical certainty and demonstrate that the cortical areas recruited during the processing of intranasal CO_2 stimuli include those outside traditional trigeminal areas.

#P112 **Poster session III: Cortical chemosensory processing/Receptor genomics and molecular biology**

Subnuclear organization of parabrachial taste neurons projecting to reward-related forebrain structures in C57BL/6J mice

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Sensations elicited by taste involve not only qualitative, but also hedonic evaluation of stimuli. In fact, feeding and drinking behaviors of animals including humans are largely based on this affective evaluative outcome. Although the neural pathways underlying these processes have not yet been well clarified, it is thought that taste information is conveyed to the reward system of the brain, whose essential components are the ventral tegmental area (VTA) and nucleus accumbens. Neurons in the pontine parabrachial nucleus (PbN), the second taste relay in rodents, send axons to the ventral tegmental area as well as to the forebrain gustatory areas such as the gustatory thalamus (VPMpc), central nucleus of the amygdala, bed nucleus of the stria terminalis and insular cortex. However, it is not clear whether 1) VTA-projecting neurons in the PbN also send axon collaterals to forebrain gustatory areas, and 2) these neurons are taste-responsive. In the current study, we performed functional neuroanatomical experiments combining c-Fos immunochemistry and retrograde tracing techniques to understand how the taste system interacts with the reward system in the mouse, an animal model with increasing importance in various fields of neuroscience. The retrograde tracers Fluorogold (FG) and cholera toxin subunit b (CTB) were iontophoretically injected into the VTA and VPMpc respectively, and retrogradely-labeled neurons and sucrose-evoked c-Fos expression in the PbN were visualized with immunofluorescence. Some neurons were double labeled with FG and c-fos or triple labeled with FG, CTB, and c-fos. These results suggest that taste neurons in the PbN project both to the taste and reward systems. This work was supported by DC000353.

#P113 **Poster session II: Cortical chemosensory processing/Receptor genomics and molecular biology**

Cloning and Localization of Four Putative Serotonin Receptors in the Primary Olfactory Pathway of the Moth *Manduca sexta*

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Serotonin (5-hydroxytryptamine, or 5HT) functions as a neuromodulator in the antennal lobes (ALs; the primary olfactory center) of the moth *Manduca sexta*. To elucidate the molecular and cellular mechanisms behind the physiological effects of 5HT in the AL, and to further our understanding of its modulatory role, we are studying 5HT receptors in the primary olfactory pathway of *Manduca*. We previously reported the cloning of two putative 5HT receptors from *Manduca*, Ms5HT1A and Ms5HT1B. We cloned a third putative 5HT receptor, Ms5HT7, and partially cloned a fourth putative 5HT receptor, Ms5HT2. Using sequence analysis, we demonstrate that the full length and partial clones exhibit high similarity to characterized 5HT7 and 5HT2 receptors (respectively) from other insects. Using RT-PCR, we have determined that all four putative 5HT receptors are expressed in both the AL and antenna of adult moth. We have generated antisera against Ms5HT1A and Ms5HT1B; Western blots of AL tissue using the Ms5HT1A and Ms5HT1B antisera produced single bands at 51kDa and 48kDa, respectively. In addition, we have generated riboprobes for Ms5HT1A, Ms5HT1B, and Ms5HT7 for *in situ* hybridization. Using these reagents, we are investigating the cellular expression patterns of the receptors in the AL through immunohistochemistry and *in situ* hybridization to identify specific AL cells or cell types where the receptors may mediate the effects of serotonin.

#P114 **Poster session III: Cortical chemosensory processing/Receptor genomics and molecular biology**

OR37 - receptors: a unique subfamily of olfactory receptors

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Olfactory sensory neurons (OSNs) which express the same odorant receptor (OR) gene are generally widely dispersed throughout the olfactory epithelium. In contrast, OSNs expressing a receptor of the OR37 family are assembled in a small central patch. Besides the unique topographic expression pattern, OR37 receptors display several other special features. All members of the subfamily share a considerable sequence identity, moreover, they all have an insertion of six amino acids in the third extracellular loop. Based on this unique structural feature it is conceivable that the OR37 receptors may be tuned to a defined group of ligands. This notion is supported by comparative studies indicating that orthologous receptors also exist in diverse mammalian species, e.g. mouse, dog, primates. Even in humans, this group of receptors exists, with a surprisingly high fraction of potentially functional genes, a scenario that is against the general trend of pseudogenization for OR genes in humans. When comparing the coding sequence of OR37 genes an unexpected high degree of sequence conservation across species border emerged. The bioinformatic data strongly suggest that the OR37 genes apparently are under a negative selective pressure, which is untypical for OR genes but supports the idea that the OR37 receptors are tuned to unique odorous ligands. Also the axonal projection pattern of OR37 expressing neurons was found to be unique. All neuron populations of the various OR37 subtypes project their axons onto a small, distinct area of the bulb, where each population formed a single glomerulus; all of them located in close vicinity. During development, from E15 to P0, axons of different subpopulations terminate in a common area; in a short postnatal period (P3) the formation of distinct glomeruli is accomplished.

#P115 Poster session III: Cortical chemosensory processing/Receptor genomics and molecular biology

Perceptual Decision-Making in the Human Olfactory Brain
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Little is known about how percepts of odor quality develop in the context of olfactory decision-making. Using functional magnetic resonance imaging (fMRI) and psychophysical testing we investigated the evolution of odor percepts in the human brain and the role of perceptual decision-making in the disambiguation of odor mixtures. A binary odor-mixture set of citral (lemon) and eugenol (clove) was assembled, systematically varying between pure citral and pure eugenol, with a total of nine discrete mixtures. Participants were instructed to make as many sniffs as needed to confidently identify which of the two odors was more prevalent in a given odor mixture trial. After making this binary decision, subjects made an analog rating on a continuum between pure lemon and pure clove, indicating to what extent they perceived the two odors contributed to the mixture. Psychometric data from nine subjects show that analog perceptual decisions reflected the ratio of the pure odorants, and reaction times increased with the ambiguity of the odor. At this time, we have completed fMRI data collection and analysis from one subject (additional subjects to be presented). Preliminary findings revealed increased activation for less ambiguous odor trials in primary olfactory (piriform) cortex, whereas an inverse pattern was observed in orbitofrontal cortex (i.e., increased activity for more ambiguous trials). These preliminary findings may reveal the different roles that these regions play during perceptual disambiguation of complex odors. Further analyses will specifically explore whether fMRI ensemble patterns in piriform and orbitofrontal cortices can predict participants' perceptual decisions on a trial-by-trial basis.

#P116 Poster session III: Cortical chemosensory processing/Receptor genomics and molecular biology

A novel chemical-informatics method to decode odor receptor chemical space
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Little is know about how odor receptors can detect a wide variety of volatile chemicals with high degrees of specificity and sensitivity. The problem is particularly complex due to the extreme diversity in both odorant structures and in receptor types. We have designed a novel chemical-informatics method to identify shared molecular features amongst odorants that may participate in binding to specific receptors. This ability to identify important molecular features of odorants was utilized to create a novel-ligand-discovery-pipeline that addresses one of the major challenges in olfaction. Functional assays for identifying ligands of odor receptors limit testing to hundreds of compounds which represents an extremely small fraction (<0.1%) of the vast volatile-chemical space. The first step in the ligand-discovery-pipeline starts with QSAR analysis of odorant structures for which receptor-activity data is available, to select the optimal molecular features. In the next step we use these features to computationally screen a library of ~240,000 potential odorants, including ones emitted by plants, fruits and humans. Hits are ranked by virtue of their distance in chemical space from known actives. Using this approach we have performed a systematic analysis for the majority of odor receptors present in *Drosophila melanogaster*. There are two main advantages of using *Drosophila* for the initial analysis; first the responses of most receptors to subsets of odors are available, and more importantly, we are able to validate the accuracy of predictions by directly performing single-sensillum electrophysiology assays. We plan to apply our discovery-pipeline to uncover novel agonists and antagonists for important odor receptors from various invertebrate and vertebrate species whose receptor decoding is underway.

#P117 Poster session III: Cortical chemosensory processing/Receptor genomics and molecular biology

Correlation between olfactory function and volume of hippocampus / amygdala
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Introduction: The amygdala is located in the frontal temporal lobe, ventral to the anterior part of the hippocampus and is an important part of the olfactory pathway. In the present investigation we studied the volumes of hippocampus and amygdala in correlation with olfactory function. Further the influence of age and sex on the respective volumes was investigated. **Methods:** We included 117 healthy normosmic subjects aged between 19 an 77 years (62 women, 55 men, mean age 37 years). First we tested olfactory function with the Sniffin' Sticks (lateralized threshold and discrimination, bilateral identification) and did the Mini Mental State Examination. MR scans of the head were performed using a 1.5 T system (Sonata, Siemens, Germany). The T1 weighted 1mm thick sagittal slices were analyzed and the volumes were measured manually using AMIRA (Visage Imaging). **Results:** There was a good reproducibility of volumetric measurements. The mean right hippocampal volume was 3.29 cm^3, the mean left hippocampal volume was 3.15 cm^3 ($p<0.001$). Such side differences do not exist in case of Amygdala (right: 1.60 cm^3, left: 1.59 cm^3; $p=0.39$). There is a reduction of hippocampal, amygdalal and the whole brain volume with rising age. For the right Hippocampus there exists a significant correlation between olfactory function and the volume. ($r=_{117}0.21$, $p=0.023$). We were unable to find such a correlation for the Amygdala. **Discussion:** Our data indicate a correlation between olfactory function and the volume of the right hippocampus. The resent data obtained in a relatively large sample may be used as standard values for the volumes of the hippocampus and amygdala.

#P118 Poster session III: Cortical chemosensory processing/ Receptor genomics and molecular biology

Characterizing Olfactory Sub-genome through Custom Microarrays

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The large number of olfactory receptors in rodents necessitates high-throughput methods to reveal their expression patterns. We designed a second-generation high-density oligonucleotide array containing all mouse and rat OR genes with different probe coverage. These arrays were approved to be reliable tools to monitor OR expression. About 1000 mouse ORs and 900 rat ORs were found to be specifically enriched in the olfactory epithelium. It also enabled us to identify the expression profile for the whole OR family in different tissues and at successive developmental ages. The onset of OR genes at early age and loss of OR genes at old age were found. The temporal expression profiles of all ORs were classified into five interesting patterns, indicating their possible behavior-related functions. We also applied our custom array to certain knockout mice and discovered the OR expression change, which is proved to be the most promising application of the arrays to explore OR regulation.

#P119 Poster session III: Cortical chemosensory processing/ Receptor genomics and molecular biology

Next Generation Sequencing as a Tool for Comprehensive Variation Analyses of Human Olfactory Receptor Genes

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Genomic variation in olfactory receptor (OR) genes most likely underlies odorant-specific phenotypes, as attested by reports of the respective linkage of *OR7D4* and *OR11H7P* gene variants to the perception of androstenone and isovaleric acid (Nature 449:468 '07; PLoS Biol 5:e284, '07). Genetic variations that result in a complete functional knockout of an OR gene in some individuals are eminent candidates to underlie threshold phenotypes, as exemplified by OR segregating OR pseudogenes (Nat Genet 34:143 '03) and whole-OR deletion alleles (PLoS Genet 4:e1000249 '08). Past work has been based on limited genomic sequence data and therefore conveys a partial view of the OR variation landscape. We now use the recently introduced Next-Generation DNA sequencing technologies to augment the list of deleterious and function-modifying OR variations in the human genome. As part of this effort we performed Solexa-Illumina deep sequencing of the entire coding region of 96 intact ORs (with 250bp flank at each end), in a pool of 21 HapMap individuals of different ethnic origin. We achieved average coverage of 40X per chromosome, thus accurate identification of rare variations present in the sample. We find a surprising amount of new polymorphisms, some causing premature stop codons or mutating highly conserved amino acids, thus presumably deleterious. In parallel, we initiate an exploration of novel entire-OR deletion alleles by generating new sequences and scrutinizing public data from large-scale sequencing efforts, e.g. the 1000 genomes project. Genotyping of current and future instances of OR gene inactivation events in >400 individuals that we have scored for odorant thresholds may help identify new genotype-phenotype correlations in human olfaction.

#P120 Poster session III: Cortical chemosensory processing/ Receptor genomics and molecular biology

OR5D3P, a pseudogene with a functional activity potential

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In humans, it is assumed that 2/3 of the olfactory receptor subgenome has succumbed to pseudogenisation. Although some OR genes may still be represented by either unaltered and pseudogenic alleles, others seems to be "old" pseudogene since the mutation accounting for their pseudogenisation is already recorded in the ortholog receptor of other primate species. The human receptor OR5D3P corresponds to this latter situation. It owes its pseudogene status to the lack of a start codon though no other alteration is observed in the rest of its sequence. This OR has been cloned into an expression vector in fusion with a tag corresponding to the 20 first amino acids of bovine rhodopsin to allow its expression into a heterologous cell model Using an automated calcium imaging-based assay, we identified anisyl acetate as a ligand for OR5D3P. The deorphanisation was further confirmed by concentration-response analysis performed with both a reporter gene-based assay and HTRF-based immunoassay allowing direct measurement of cAMP. These results indicate that, despite its pseudogene status, OR5D3P has not accumulated other mutations that would hamper its functionality and suggests that a positive selection pressure has been acting. A possible explanation for these observations is that the lack of a start methionine could be rescued by an upstream exon. Such an approach has already been proposed for the corresponding chimp ortholog, OR11-140. Further analysis of OR5D3P in the olfactory epithelium remains to be performed in order to test this intriguing hypothesis.

#P121 Poster session III: Cortical chemosensory processing/ Receptor genomics and molecular biology

Identification and characterisation of a carboxylic acid-responding human OR

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Short chain carboxylic acids constitute an important source of sweat malodours. Identifying the human olfactory receptors that recognize this class of molecules is therefore of interest. Understanding how the associated odour information is processed could possibly lead to the identification of receptor antagonists and/or modulators that could efficiently block the perception of these offensive smells. Here we describe the deorphanisation of OR51E1, a receptor that was initially found to respond to isovaleric acid (IVA). The activation by IVA was confirmed by three different functional assays (i.e. single cell calcium imaging,

luciferase-based reporter gene assay and cyclic AMP measurement with HTRF-based immunoassay) that all revealed the same range of sensitivity of the receptor for IVA (EC50 ~ 30 µM). A structure-activity relationship study further revealed that OR51E1 can be activated by carboxylic acids having a linear chain ranging from 4 to 6 carbons. Addition of methyl groups or introduction of double bonds into the main chain may hamper the activity of the ligand if these are located close to the carboxylic function. Unsaturated cyclic carboxylic acids, such as cyclohexanecarboxylic acid, were also found to activate the receptor whereas the aromatic carboxylic acids were inactive. This work represents one of the most extensive structure activity relationship analysis performed on a human olfactory receptor. Further studies linking in vitro analysis and genotyping of people anosmic to IVA would be required in order to determine the involvement of OR51E1 in IVA perception.

#P122 Poster session III: Cortical chemosensory processing/ Receptor genomics and molecular biology

High incidence of charged amino acids in the third extracellular loop of olfactory receptors: Linking receptor structure to olfactory perception

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The first principal axis of olfactory perception correlates best with the first principal axis of the physicochemical space [Khan et al. JNS 2007]. We set out to label this physicochemical axis, and identify receptor attributes that can account for the variance it explains. We applied principal components analysis to 1338 physicochemical descriptors (obtained with Dragon software) of 1519 monomolecular odorants. Detailed characterization of the descriptors that dominated this axis suggested that it reflects odorant molecular packing. Compact molecules will rarely form an induced dipole in the presence of a local charge or dipole, whereas molecules that are poorly packed will more readily depolarize. To test for a receptor attribute that can detect variations in molecular packing, we calculated the probability of occurrence of positively charged, negatively charged, polar uncharged and non-polar amino acids at four domains along the sequences of 391 intact human olfactory receptors (HORDE: http://bip.weizmann.ac.il/HORDE). We looked at the first (between TM1 and TM2), second (between TM4 and TM5), and third (between TM6 and TM7) extracellular loops. We found that the variability between proteins was largest in the third extracellular loop (F=60, P<.004). Strikingly, this loop also contained the most positively charged (mean=16%, F=82, P<.0001) and negatively charged (mean=17%, F=200, P<.0001) amino acids. Given that the third extracellular loop is a short sequence located in the vicinity of the binding site; this finding may suggest that the initial process of odorant binding may be facilitated by induced charged-dipole interactions between this loop and the odorant. This may underlie the link between the principal axis of perception and the principal axis of physicochemical variability.

#P123 Poster session III: Cortical chemosensory processing/ Receptor genomics and molecular biology

Plasticity in expression of chemoreceptor genes in *Drosophila melanogaster*

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Individuals interact differently with their chemosensory environments under different developmental, physiological and social conditions. *Drosophila melanogaster* presents an excellent model for assessing plasticity in expression of the chemoreceptor repertoire, as both the genotype and environment can be controlled precisely. To assess to what extent transcription of chemoreceptors responds to changing conditions, we constructed cDNA expression arrays that represent 50 *Odorant binding protein (Obp)*, 56 *Odorant receptor (Or)*, and 59 *Gustatory receptor (Gr)* genes, 4 genes that encode other antenna-specific proteins, 17 genes encoding components of neurotransmitter pathways, and 4 control genes. We compared transcriptional profiles under different environmental and physiological conditions. All experiments were done with isogenic *Canton S (B)* flies with sexes separately (except for larvae). We observed that chemoreceptor genes that are organized as clusters in the genome are independently regulated; sexual dimorphism in chemoreceptor expression is pervasive; and, chemoreceptor expression patterns are plastic throughout development as evident from larva-specific gene expression and altered regulation of gene expression during senescence. Furthermore, expression of subsets of chemoreceptors is modulated by physiological state (virgin vs. mated) and social context (solitary or group-reared). The observed plasticity in chemoreceptor gene expression reflects functional diversity of the chemoreceptor ensemble attuned to ecologically relevant environmental and physiological conditions.

#P124 Poster session III: Cortical chemosensory processing/ Receptor genomics and molecular biology

Role of Plasma Membrane Calcium ATPases in Mouse Olfactory Neurons

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Calcium rises transiently in olfactory sensory neurons (OSNs) when odorants bind to G protein-coupled receptors on their cilia. The elevated calcium feeds back on cyclic nucleotide gated channels, causing adaptation. Calcium clearance relieves adaptation and protects the cells. Plasma membrane calcium ATPases (PMCAs) are among known mechanisms of calcium removal, as are Na^+/Ca^{2+} (NCX) exchanger and ER calcium pump (SERCA). Our interest is in PMCAs in Ca^{2+} clearance in OSNs. We previously demonstrated that PMCAs are expressed in OSNs and we know which splice forms are present. We have also shown through kinetic analysis that the rate of Ca^{2+} removal from the dendritic knob after stimulation of OSNs with IBMX/forskolin is slowed when PMCAs are inhibited by carboxyeosin (CE) or by genetic knock out of PMCA-2. When we compromised the PMCAs, SERCA and NCX separately, removal slowed by about the same amount (30-34%). When all three removal mechanisms were compromised, Ca^{2+} was no longer reduced after stimulation. To investigate whether PMCAs affect adaptation, we measured the amplitude of the Ca^{2+} peak

following a second stimulation by IBMX/forskolin. We found that if stimulations occurred less than 300 seconds apart, the second peak was significantly smaller than the first peak in WT OSNs. The second peaks were reduced significantly more in the KO OSNs, providing initial evidence that PMCA-2 contributes not only to the duration of the transient but also to the duration of adaptation. PMCAs are not just housekeeping enzymes, and we have shown that they can contribute to the shape of the Ca^{2+} transient following stimulation.

#P125 Poster session III: Cortical chemosensory processing/ Receptor genomics and molecular biology

Expression of Canine b-Defensin (CBD103) and Olfactory Marker Protein (OMP) in the Canine Nasal Cavity

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Canine b-defensin 103 (CBD103) is a multifunctional antimicrobial peptidethat also has a strong effect on pigment type-switching in domestic dogs. A mutation in the CBD103 gene can lead to a black coat, while dogs carrying the wildtype CBD103 have yellow coat. Olfactory marker protein (OMP) is a cytoplasmic protein expressed in mature olfactory receptor neurons. The long term goal of our research is to determine whether the genetic mutation of CBD103 has any effect on olfactory function and the mucosal immunity protecting the olfactory epithelium in dogs. The objective of this study was to determine the expression pattern of CBD103 and OMP in the canine nasal cavity. Using RT-PCR, we have examined the expression of CBD103 and OMP in the nares/alar fold, ventral nasal concha (rostral and caudal regions), ethmoid labyrinth, olfactory bulb, and vomeronasal organ. It was found that CBD103 was strongly expressed in the nares/alar fold and rostral ventral concha, and OMP was strongly expressed in the ethmoid labyrinth and olfactory bulb. The strong presence of CBD103 at the beginning of the nasal cavity indicate that CBD103 may play a critical role in mucosal innate immunity against microbial infections. Future studies will determine whether CBD103 is present in the mucus covering the olfactory epithelium and whether CBD103 has any effect on the expression of OMP and other functions of olfactory neurons.

#P126 Poster session III: Cortical chemosensory processing/ Receptor genomics and molecular biology

Experience-Dependent Modulation of Odor Mixture Coding and Perception

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The majority of odorous objects encountered in the environment are composed of dozens, or even hundreds, of volatile molecules. As such, the olfactory system has been designed to optimize detection of the integrated assembly of odorants (configural encoding), rather than detection of the parts themselves (elemental encoding). Our study is designed to test the mechanisms by which odor elements are integrated into perceptual wholes. Using functional magnetic resonance imaging (fMRI) and multivariate analytical techniques, we investigated how aversive learning modulates perception and encoding of complex odors. We presented human subjects with seven stimuli: three monomolecular odorants (A, B, C), three binary mixtures (AB, BC, AC) and one ternary mixture (ABC). Odor-evoked patterns of brain activation to each stimulus were measured before and after conditioning. During the conditioning phase, one binary mixture (CS+) was repetitively paired with an electric shock while the other two (CS-) were not. We tested fMRI correlations of posterior piriform cortex (PPC) ensemble activity patterns between all possible stimulus pairs before and after conditioning. Results from one subject showed an increase in the correlation of PPC activity patterns between the binary CS+ and its two elements, but no correlation change between the CS+ and the third (irrelevant) odorant after conditioning. Conventional analysis of the neuroimaging data revealed increased amygdala activity during CS+ presentation from pre to post conditioning, consistent with the idea that aversive learning to the CS+ was successfully induced. These preliminary findings suggest that olfactory learning may induce perceptual and neural fusion of odor elements into a synthetic whole. Data will be presented from additional subjects.

#P127 Poster session III: Cortical chemosensory processing/ Receptor genomics and molecular biology

Endothelin modulates both short-term kinetics of odorant detection and long-term cellular population dynamics in olfactory mucosa

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In search of regulatory factors of the olfactory mucosa (OM), we have previously shown that endothelin is locally matured in the OM and that olfactory sensory neurons (OSNs) express preferentially ET_B receptors, while ET_A receptors are rather present in non-neuronal OM cells (nNCs). Using EOG recordings, we first observed that a direct application of endothelin (ET-1) could modulate the kinetics of odorant detection while the amplitude of the response was unchanged. As this effect was blocked by ET_A antagonist, we examined the modulation of GAP junctions on nNCs by electrical stimulation of primary cultured cells of OM by calcium imaging. ET-1 could limit the propagation of electrical stimulation in nNCs similarly to a known GAP junction antagonist. Thus, the observed modulation of odorant detection kinetics in EOG may be related to a change of electrical coupling between nNCs, due to the action of ET-1 on ET_A receptors. In addition to its vasoactive effect, the endothelin system is mainly known to act on cell population dynamics. While a serum deprivation leads to a massive decrease of OSNs in primary cultures of OM, a treatment with ET-1 rescued part of the cellular death through both ET_{AB} receptors. Interestingly, we observed that an ET_B receptors-deficient strain displayed a decreased sensitivity to odorants detected by EOG. Endothelin has thus at least a dual role on the OM. It can modulate the kinetics of odorant detection, but also affects the survival of OSNs on a longer term.

#P128 Poster session III: Cortical chemosensory processing/ Receptor genomics and molecular biology

Neuroanatomical correlates of olfactory function

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In recent years, objective whole-brain techniques (voxel-based morphometry, VBM) have become available that allow segmentation of brain structures into grey matter, white matter and cerebrospinal fluid. In the present study we used them to investigate the correlation between individual grey matter thickness and olfactory function. Forty-four subjects (25 women, 19 men) underwent extensive olfactory testing including odor identification, detection thresholds, intensity discrimination and quality discrimination. The behavioral results and subjects' anatomical MRI scans were analyzed using two MNI in-house programs CIVET and Surfstat. A global analysis demonstrated that general olfactory function was correlated with grey matter thickness in and around the right central sulcus and the right paracentral lobule. Moreover, a predicted regions analysis demonstrated a correlation with an area around the right olfactory sulcus. Of the individual olfactory tasks, odor discrimination was correlated with cortical thickness of right insula, right precentral gyrus, right superior parietal lobule and right parietal lobule. Odor identification was correlated with cortical thickness in the right superior temporal lobule, with an interaction with subjects' sex in occipital regions and the entorhinal cortex. These results indicate that performance on individual olfactory tests is reflected in brain anatomy.

#P129 Poster session III: Cortical chemosensory processing/ Receptor genomics and molecular biology

Mechanisms of constitutive and ATP-evoked release of ATP from neonatal mouse OE stores

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ATP, an important extracellular signaling molecule, is released constitutively and actively in many cell types. We previously showed the release of ATP from basally-situated ATP stores via purinergic receptor stimulation and vesicular fusion. Here, we further characterized the mechanisms of constitutive and evoked ATP release. Using confocal imaging of endogenous ATP fluorescently-tagged by quinacrine, the % ATP release was monitored in the absence (control, constitutive) or presence (evoked) of exogenous ATP (50 µM). In control conditions, ATP release occurred in the basal, middle and apical OE (34±2, 38±3, 32±1 % basal fluorescence (%Fo), respectively). Exogenous ATP significantly induced release of endogenous ATP in basal OE (18±1 %Fo; $p<0.05$), a small but significant effect on middle release (31±2 %Fo; $p<0.05$) and had no effect on apical release (31±3 %Fo; $p>0.05$). We hypothesized that ATP is constitutively released from the apical and middle OE, while modest ATP-evoked ATP release occurs in the middle OE and robust ATP-evoked ATP release occurs in basal OE. Removal of probenecid, an ABC transporter inhibitor, from our bath solution increased middle (24±6 %Fo; $p<0.05$) and apical release (22±6 %; $p<0.05$). Carbenoxolone, a hemichannel inhibitor, decreased release in middle (57±6 %Fo; $p<0.05$) and apical OE (52±2 %Fo; $p<0.05$) suggesting constitutive release is via ABC transporters and hemichannels. *Clostridium difficile* toxin A, an inhibitor of vesicle fusion, and removal of extracellular Ca^{2+} significantly decreased basal release in absence (50±5 and 41±2 %Fo; $p<0.05$) and presence of exogenous ATP (35±6 and 44±8 %Fo; $p<0.05$). Purinergic receptor agonists ATP, MeATP and BzATP significantly ($p<0.05$) increased basal ATP-evoked ATP release compared to control (17.6±1.3, 27.6±1.9, 24.4±2.5 v. 34.0±2.0 $\%F_0$, respectively), suggesting P2X receptor involvement. Collectively, these results show that ATP-evoked ATP release from basal OE stores is through Ca^{2+}-dependent exocytosis and suggests constitutive release of ATP is via ABC transporters and hemichannels.

#P130 Poster session III: Cortical chemosensory processing/ Receptor genomics and molecular biology

CNGA2 heterozygous mice with a knockout TRPM5 show fewer glomeruli targeted by OSNs with nonfunctional CNGA2

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The cyclic nucleotide gated channel A2 subunit (CNGA2) is critical for canonical olfactory signal transduction to detect airborne odorants and semiochemicals in the mouse main olfactory epithelium (MOE). The cyclic nucleotide gated channel is also important for the survival of olfactory sensory neurons (OSNs), and we found previously that there is regional variation in the survival of OSNs with nonfunctional CNGA2 in CNGA2 heterozygous mice (Dunston et al ISOT abstract, 2008). Since transient receptor potential channel M5 (TRPM5) is expressed in OSNs located preferentially in the lateral and ventral regions of the MOE, we examined whether TRPM5 expression influences the regional variation of the survival of OSNs with nonfunctional CNGA2. We use CNGA2 heterozygous mice that express GFP in OSNs with nonfunctional CNGA2. We find that these adult mice have many small ectopic GFP positive glomeruli, while newborn and 7 day old heterozygous mice rarely show ectopic GFP positive glomeruli. Using immunolabeling with a neuronal activation marker fos, we find that in adult CNGA2 heterozygous mice some ectopic GFP positive glomeruli are activated by soiled bedding. In addition, we have crossbred mice to generate CNGA2 heterozygous mice with a TRPM5 KO background, in order to determine the role of TRPM5 in the survival of GFP positive OSNs and their target glomeruli. Our results show that CNGA2 heterozygous mice with nonfunctional TRPM5 have fewer GFP positive OSNs in the lateral region of the MOE than CNGA2 heterozygous mice with functional TRPM5. Consistently, there are fewer GFP positive glomeruli in CNGA2 heterozygous mice with nonfunctional TRPM5. Our results suggest that both CNGA2 and TRPM5 play a role in maintaining the survival of adult OSNs in the MOE.

#P131 Poster session III: Cortical chemosensory processing/ Receptor genomics and molecular biology

Differences in Matrix Metalloproteinase-2 Expression Following Two Olfactory Injury Models

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We previously reported that matrix metalloproteinase-9 (MMP-9), an enzyme involved in extracellular matrix regulation, is elevated following olfactory nerve transection (NTx) and methyl bromide gas (MBr) injuries. In this study, we examined MMP-2. We used Western blot methods to measure the levels of MMP-2, GAP-43 and olfactory marker protein (OMP) in the bulb of mice at different time points following NTx and MBr injury. GAP-43 and OMP were used as markers to detect immature and mature olfactory neurons. In control animals, MMP-2 levels were not observed. After NTx, MMP-2 expression levels increased within hours and a large peak was observed at day 7. After MBr exposure, MMP-2 expression remained near control levels. Both GAP-43 and OMP decreased soon after NTx, reflecting degeneration of axon fibers and deinnervation of the bulb. There was a large increase in GAP-43 observed between recovery day 3 and 7, indicating regeneration and the growth of new axon fibers reinnervating the bulb. OMP levels began to increase around day 10, reflecting the maturation of olfactory neurons. GAP-43 and OMP levels decreased at a slower rate after MBr injury and did not increase until recovery day 40, indicating reinnervation of the bulb occurred later with MBr than for NTx. This is the first study to compare changes in MMP-2 levels after both NTx and MBr injuries. These findings suggest that MMP-2 may be associated with deinnervation and reinnervation processes in the olfactory bulb following NTx but not MeBr (in contrast to MMP-9), perhaps serving as a transition event signifying a lesion-specific switch from degradation to the recovery of olfactory neurons.

#P132 Poster session III: Cortical chemosensory processing/ Receptor genomics and molecular biology

Identification of Taste Bud-Associated Genes

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A systematic and comprehensive genome-wide screen was conducted to identify taste bud-associated genes. Taste buds and non-gustatory lingual epithelium were collected using laser capture microdissection and isolated RNAs were hybridized to microarrays to generate an expression database of over 2,300 taste bud-associated genes, including ~200 genes predicted to encode multi-transmembrane proteins with no known function in taste (ISOT XV Poster #132; p88-89, 2008). An important first step in elucidating the function of these gene products in gustation is to identify the specific cell types in which they are expressed. Using double label in situ hybridization analyses, we identified genes expressed in sweet, bitter, and umami cells (TRPM5-positive) and sour cells (PKD2L1-positive). CALHM1 (calcium homeostasis modulator 1), a component of a novel calcium channel, MCTP1 (multiple C2 domains, transmembrane 1), a calcium-binding transmembrane protein, and ANO7 (anoctamin 7), a member of the recently identified calcium-gated chloride channel family were expressed in TRPM5 cells. These proteins may modulate calcium signaling stemming from sweet, bitter, and umami receptor activation. SV2B (synaptic vesicle glycoprotein 2B), a regulator of synaptic vesicle exocytosis, was expressed in PKD2L1 cells, supporting the role of exocytic neurotransmitter release in this cell population. In addition, IKBKAP (inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein), a gene mutated in the disease familial dysautonomia that results in loss of taste buds was specifically expressed in PKD2L1 cells. Elucidating the functions of these and other taste bud-associated genes in gustation will advance our understanding of taste biology in normal and diseased states.

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Functional characterization of two fatty acid activated GPCRs expressed in the mammalian gustatory system

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Given that the epidemic of obesity appears to be driven, at least in part, through an increase in dietary fat intake, it has become increasingly important to identify the mechanisms the body uses to recognize dietary fat. Recently, we have identified a number of fatty acid (FA) activated G protein coupled receptors (GPCRs) that we hypothesize play a role in the initial recognition of free fatty acids, the prototypical fat stimulus, in the oral cavity. Two of these FA-GPCRs, GPR120 and GPR84, are highly expressed in the taste and somatosensory systems and are activated by long chain and medium chain fatty acids, respectively. To characterize these receptors in greater detail and explore the interaction of these receptors with another putative FA receptor, CD36, we have designed cell lines that inducibly express either GPR84+G_{qi9} or GPR120+G_{16} with and without CD36. Using FLIPR-based assays, we show that these cell lines expressing GPR84+G_{qi9} or GPR120+G_{16} alone respond with appropriate specificity to their cognate ligands. Cells expressing GPR84 respond to the medium chain saturated fatty acid, capric acid (5-40 µM), but not to a long chain unsaturated fatty acid (LCFA), linoleic acid, over a similar concentration range. Conversely, cells expressing the LCFA-specific receptor, GPR120, respond to linoleic acid, but not capric acid. Both receptors respond in the expected concentration ranges. Currently, we are using ratiometric (Fura2) calcium imaging to explore these receptors in greater detail and determine the effect that coexpression of CD36 has on the specificity and sensitivity of FA signaling in these cell lines.

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Genetic and Molecular Basis of Individual Differences in Human Umami Taste Perception

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During periods of human expansion into new environments, recognition of amino acids through taste may have conferred an important selective advantage. Umami taste is elicited by monosodium glutamate (MSG), and is one of five basic taste qualities that plays a key role in intake of amino acids. A particular property of umami is the synergistic potentiation of glutamate by purine nucleotide monophosphates (IMP, GMP). A heterodimer of a G protein coupled receptor, TAS1R1 and TAS1R3, is proposed to function as its receptor. Polymorphic sites and single nucleotide polymorphism (SNP) frequencies in these genes vary widely in human populations. If the diversification of these genes reflects adaptation to changing nutritional environments, the genetic variations of *TAS1R1* and *TAS1R3* may show correlations with differences in umami sensitivity and affect the receptor function. Here we showed the association between recognition thresholds for umami substances and genetic variations in human *TAS1R1* and *TAS1R3*, using taste tests and a heterologous expression system. This result suggests that natural selection on *TAS1R1* and *TAS1R3* alleles may have been particularly important, at least in part, in human evolution.

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Genetic mutations and bitter taste sensitivity to four substances

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Bitter taste perception is initiated by TAS2R receptors, proteins expressed in taste buds. Mutations in the TAS2R genes can cause variation in bitter taste sensitivity. We investigated the effects of mutations in three genes and their effects on the bitter perception of two artificial sweeteners (saccharin and acesulfame K) and two marker substances, salicin and denatonium benzoate. Our findings support earlier evidence that mutations in the *TAS2R43* and *TAS2R44* genes cause variable bitter perception of saccharin. We found that the same mutations account for variation in the bitterness of acesulfame K. In addition, we found that variation in the *TAS2R45* gene may be important in the bitter perception of these substances. Finally we found that these mutations differ in frequency among worldwide populations.

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Identification of the Interaction site for Gymnemic acid at the sweet taste receptor T1R2+T1R3

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Gymnemic acid (GA) is a triterpen glycoside that is isolated from the plant *Gymnema sylvestre*. It is known that GA selectively suppresses taste responses to various sweet compounds without affecting responses to salty, sour and bitter substances. Sweet-suppressing effect of GA is specific to humans and chimpanzees, but not to rodents. It has also been shown that the sweet-suppressing effect of GA is diminished by rinsing the tongue with γ-cyclodextrin (CD). In order to examine whether GA directly interact with T1R2+T1R3, we used the sweet receptor T1R2+T1R3 assay in transiently transfected HEK293 cells. Similar to previous studies in humans and mice, GA (0.1 mg/ml) inhibited the $[Ca^{2+}]_i$ responses of cells heterologously expressing hT1R2+hT1R3 to SC45647, saccharin and D-tryptophan. The sweet-suppressing effect of GA rapidly disappeared after rinsing the cells with 1% γ-CD. The mouse pair (mT1R2+mT1R3) was not sensitive to GA. One mismatched pair (hT1R2+mT1R3) behaved like the fully mouse heterodimer, showing no sensitivity to GA. These results demonstrate that hT1R3 is required for GA sensitivity. To identify the interaction site for GA, we examined the responses of the mouse/human chimeras of T1R2 and T1R3. The results suggest that the sensitivity to GA depends mainly on the transmembrane region of human T1R3.

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The role of the visual cortex in olfactory processing: an rTMS study

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It has become clear that crossmodal connections modulate unimodal perception to a larger extent than previously thought. An example is the often-reported but seldom-discussed finding from olfactory imaging experiments that higher-order odor tasks activate cortical areas commonly associated with visual processing. We are currently exploring this phenomenon using repetitive transcranial magnetic stimulation (rTMS), a technique thought to be capable of upregulating neuronal activity in stimulated areas, as demonstrated by improved low contrast detection following primary visual cortex (V1) stimulation. Through stimulation of V1, we are investigating the visual system's impact on olfactory processing. In a within-group design, either V1 (experimental condition) or the vertex (control condition) is stimulated while measures of olfactory identification,

supra-threshold discrimination, and peri-threshold intensity discrimination performance are obtained. Visual acuity is additionally measured to verify successful stimulation of V1. Though a limited number of participants have been tested to date, initial analyses indicate that rTMS stimulation of V1 primarily modulates supra-threshold discrimination and peri-threshold intensity discrimination performance and has less impact on olfactory identification performance. These initial results indicate that the primary visual cortex is capable of modulating higher-order olfactory processing.

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TAS1R1-intronic SNP Associations with Liking for Dietary Sources of Glutamate and for Orosensory Intensity

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Background: Gene products of the *TAS1R* family form heterodimeric receptors that appear to mediate umami (hT1R1+hT1R3) and sweet (hT1R2+hT1R3) sensations (Li et al, 2002). Limited information exists on the contribution of *TAS1R1* variation to functional differences in taste perception, although exonic variation has been described *in vivo* (Kim et al, 2006). Methods: DNA samples from 90 healthy adults were collected from whole bloods, isolated and genotyped for *TAS1R1* intronic SNP (rs17492553) by TaqMan genotyping. Results: Similar genotype frequencies were seen in our sample (33% CC homozygous for major allele, 41% were heterozygous, 26% TT homozygous for minor allele) to reference frequencies for European-Americans (50%, 21%, and 29%, respectively). Using the sensory and hedonic forms of the general Labeled Magnitude Scale, subjects rated the intensity of: liking and taste quality of sampled foods/beverages, prototypical tastants painted on fungiform and circumvallate papilla and tasted with the whole mouth; and 25% ethanol painted on the tongue tip. In analysis of covariance controlling for age, sex and intensity of tones as a cross-modal standard, CC adults reported greater liking for sampled soy sauce and white grapefruit juice (sources of glutamate) than did TT adults, with concurrent increases in sourness ratings for the juice. As expected, this SNP failed to explain differences in liking and sweetness of sampled sweet foods. However, this SNP explained intensity differences in the spatially-applied ethanol probe and tastants. The TT adults and, in some cases heterozygous adults, reported lower intensities than did the CC adults. Conclusions: These results support a role of *TAS1R1* SNPs in response to glutamate and further suggest their contribution to general variability in orosensory intensity.

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Key amino acid residues involved in multi-point binding interactions of sweet protein, brazzein, with the T1R2-T1R3 human sweet receptor

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Brazzein protein tastes sweet to humans through activation of the sweet sensing receptor heterodimeric GPCR composed of monomers T1R2 and T1R3. Brazzein's sweetness depends on both its three-dimensional structure and on distributed sites in its surface chemistry as we have shown by structural, dynamic and functional assays (both human psychophysical taste assays and functional heterologous expression assays) of wildtype and mutant brazzein proteins. Here we show data from our investigation of three "sweetness" sites on brazzein: loop 43 (Site 1); the N and C termini and the proximal Glu36 residue (Site 2); and loop 9-19 (Site 3). We have found that the presence of basic residues in Site 1, and acidic residues in Site 2 play significant roles for brazzein's sweet taste. We also find that position 54 (Site 2) requires a bulky side chain rather than one with hydrogen bonding potential for sweetness. We determined that proper disulfide bond formation in loop 9-19 (Site 3) is essential for sweetness. We also investigated several areas of the sweet receptor that modify the brazzein response. We confirmed the involvement of the CRD of T1R3 in brazzein activity by identifying an acidic residue that is essential for brazzein activity. We also demonstrated that the T1R2 VFTM participates in the ability of brazzein to activate the sweet receptor, suggesting that it too forms a point of interaction for brazzein. We have assessed several models for the brazzein binding site within the extracellular domains of T1R2/T1R3 receptor by mutating charged and polar residues within the small-molecule binding clefts as well as proximal residues in the interface between and along the backs of the T1R2 and T1R3 monomers and characterizing the resulting mutant receptors by a functional calcium mobilization assay.

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Orbitofrontal lesions and hypersensitivity to olfactory stimuli

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Hypersensitivity to odors has previously been reported in patients with adrenal cortical insufficiency (Henkin and Bartter, 1966). However, little is known regarding other clinical causes of hypersensitivity. We studied the olfactory profile two neurological patients complaining of increased sensitivity to olfactory stimuli. Patient 1, MP, is a 40 year old right handed man with multiple cerebral contusions in the frontal, including the bilateral orbitofrontal (OFC) and left temporal (TL) cortices. Detection thresholds for phenyl ethyl alcohol (PEA) were assessed monorhinally using an ascending staircase method. MP was able to detect our weakest dilution of PEA (i.e. 16th dilution step) in each nostril. He has tested significantly above his neurologically normal peers whose mean sensitivity for PEA has been reported to range between the 6th to the 7th dilution (Deems and Doty,

1987). MP's olfactory discrimination (Zatorre and Jones-Gotman, 1990) score was 16 out of 16 on both nostrils. UPSIT score was considered to be within normal range. Patient 2, CG, is a 59 year old right handed woman with bilateral OFC and TL lesions. CG had an increased sensitivity to PEA (detected the 16th dilution step) compared to aged matched controls, performed in the 99% percentile of all age groups on the UPSIT and got 16 correct out of a possible 16 on each nostril on the discrimination task. The current findings for threshold and discrimination were considered to be significantly higher than expect from age matched neurologically normal patients. Further, patients with frontal and TL damage are known to have deficits in odor identification and discrimination, as opposed to the heightened abilities observed in MP and CG. Additional analyses will be undertaken to assess the overlap in lesions of these two patients

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Structural requirements for bitter taste receptor activation
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At present, for 12 of the 25 human bitter taste receptors (hTAS2Rs) activating bitter substances were identified. In general, hTAS2Rs are activated by multiple different agonists. However, as all hTAS2Rs exhibit a unique agonist response profile, this observation is not simply caused by a reduced agonist-specificity. The responsiveness of hTAS2Rs to multiple low-affinity agonists is in sharp contrast to other GPCR-families with few, or even single, high-affinity agonists indicating differently organized binding pockets. With the present study we investigated which amino acid residues of hTAS2Rs are involved in agonist-interaction and how binding pockets of hTAS2Rs might be organized. We chose the broadly tuned receptor hTAS2R46 for this study because a number of closely related receptors with distinct agonist interaction profiles are available, and thus, allow focusing on few amino acid differences responsible for agonist selectivity. Guided by differences in amino acid sequences and *in silico* modeling studies, we identified hTAS2R46 residues, which are likely responsible for agonist selectivity and subjected the corresponding positions to point mutagenesis. Calcium imaging analyses of receptor mutants were performed to assess the functional consequences of amino acid exchanges. This approach enabled us to identify a number of positions that contribute to variable extents to the hTAS2R46-specific agonist profile. Introduction of these residues into the receptors hTAS2R43, -44, and -50 resulted in the transfer of hTAS2R46 agonist profile and, in part, pharmacological properties onto those receptors. Our findings allow the conclusion that the investigated hTAS2Rs possess, analogous to other GPCRs, a single, positionally conserved binding pocket accommodating the various tested agonists.

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Immunohistochemical Analysis of Human Fungiform Papillae
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Little data are available analyzing the cellular organization and immunocytochemical characteristics of human fungiform papillae. The basic cellular organization is similar to that of other mammals with respect to the types of cells and the number of cells within a taste bud. Taste buds contain two types of cells that are known to directly participate in taste detection: chemosensory transduction-receptor (Type II) cells, and presynaptic (Type III) cells. Receptor cells express G protein- coupled taste receptors and respond to sweet, bitter and umami taste stimulation. With antibody labeling, we find that the immunoreactivity for several taste transduction molecules was seen consistently in cells within fungiform papillae, including: Gustducin, Gg13, PLC (phospholipase C) and IP3R3 (Inositol triphosphate receptor type 3). Cells with neuronal characteristics were also observed, based on immunostaining for synaptic vesicle marker SNAP-25, Syntaxin and PGP9.5 (Protein Gene Product 9.5), which were detected in fibers and/or cell membranes within the taste bud. Cells immunostained with neural cell adhesion molecule NCAM, also reported to be present in Type III cells, were also detected. Double-labeling immunocytochemistry for Gustducin and IP3R3, PGP9.5 and Gg13 indicated separate cell populations. These results strongly suggest separate populations of synaptically communicating cells and taste-receptor expressing cells in human fungiform papillae.

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Subtypes of T Lymphocytes in Healthy Human Fungiform Papillae
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We previously identified the key immune cells (i.e. lymphocytes, macrophages, dendritic cells and Langerhans cells) in human fungiform papillae (FP). Of these cells, CD4 T cells were one of the most predominant types. CD4 T cells are divided to Th1 and Th2 cells based on the cytokines they produce. They also express different surface markers: e.g., CCR5 for Th1 and CCR4 for Th2 cells. Th1 cells are more related to inflammation and cytotoxic activities, while Th2 cells are more related to allergy, IgE production, and anti-inflammatory reactions. T-cells generally consist of a mixture of naïve (CD45RA+, having no immune function) and memory (CD45RO+, with capability of immune function) subsets. After antigen stimulation, some naïve T cells transition to memory cells and activated cells. Most T cells express the receptor (TCR) alpha/beta, but the TCR gamma/delta subset is relatively enriched within epithelia and plays a special role in mucosal immune. In this study, we studied the different T-cell subtypes in human FP by examining the surface antigens associated with T cell functions and activation via immunohistochemistry. Biopsies of human FP were taken from the anterior tongue. Interestingly, we found that CD45RO+ memory cells were the principal T cells in FP, while CD45RA+

naïve T cells were uncommon. This may reflect the activation of naïve T cells at the site of exposure to repeated antigen stimulation Th1 cells were more predominant in FP than Th2 T cells. This suggests that the local immune response in the gustatory system is Th1-type biased. Few gamma/delta TCR T cells were in FP. This study provides background for understanding inflammatory responses in gustatory tissue and clinical interactions between the immune and gustatory systems.

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Neutrophil infiltration impairs peripheral taste function

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Unilateral chorda tympani (CT) nerve sectioning induces rapid functional changes in the neighboring, intact population of taste receptor cells. Within one day after contralateral sectioning, neural responses to sodium are specifically decreased. We propose that these injury-induced changes in taste function are mediated by leukocytes. Neutrophils invade the taste system within hours of injury, in parallel with decreased responses to sodium in the intact CT nerve. Dietary treatments that amplify the neutrophil response extend the functional impairments. Importantly, depletion of neutrophils restores normal taste responses. In the current study, we tested the hypothesis that neutrophil infiltration in the *absence* of nerve sectioning also impairs sodium taste function. Specified pathogen-free (SPF) Sprague-Dawley rats received injections of the endotoxin, lipopolysaccharide (LPS; 10 µg in 10 µl sterile PBS), to the ventral tongue. This treatment significantly increased the number of neutrophils in the anterior fungiform field. Neurophysiological recordings from the CT nerve were performed at 24 hr post-injection. CT responses to NaCl and sodium acetate were suppressed in LPS-injected vs. PBS-injected rats. In contrast, neural responses to non-sodium stimuli did not differ between groups. These findings indicate that neutrophils have a negative impact on sodium taste function, whether they are elicited by nerve injury or endotoxin. We suggest that neutrophils responding to tissue damage, nerve injury, or bacterial infection release molecules that selectively downregulate sodium channel expression and/or function in taste receptor cells.

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Mouse taste buds express vesicular glutamate transporter type 2

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Various potential neurotransmitters are reported in taste buds, but their precise roles are mostly unknown. Previously, we presented evidence that ATP is necessary for communication between taste buds and taste nerves. In this study, we investigated the potential role of glutamate as a neurotransmitter by analyzing vescicular glutamate transporter (VGLUT) expression in peripheral taste tissue. VGLUTs are a family of 3 proteins that load glutamate into synaptic vesicles; the presence of VGLUTs in a cell is presumptive evidence that it is glutamatergic. Previous studies support the idea that glutamate may be a transmitter in taste buds. Various metabotropic glutamate receptors (mGluRs) are expressed in type II taste cells and in nerve fibers associated with taste buds. Some taste cells exhibit robust immunoreactivity for glutamate. To further explore whether glutamate might be a transmitter in taste buds, we used immunocytochemistry and RT-PCR for VGLUTs on tissues obtained from mouse circumvallate papillae. Surprisingly, we found VGLUT expression in nerves innervating the taste buds, but not in taste cells themselves. Immunocytochemical analysis shows that the VGLUT2+ nerve fibers are closely associated with gustducin-expressing type II cells. To test whether taste cells might express low levels of VGLUTs, we performed RT-PCR of pooled mouse circumvallate taste buds. No VGLUT PCR products were detected in RNA collected from taste buds, although there was robust expression of all VGLUT isoforms in the brain. Together, our results suggest that nerve fibers associated with taste buds are capable of releasing glutamate. This may indicate that glutamate serves as an intragemmal modulator of taste bud function rather than as a means of transmission of information from taste cells to nerve fibers.

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Serotonin Inhibits ATP Secretion in Mouse Taste Buds

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During taste stimulation, Receptor (Type II) cells secrete ATP. ATP, in turn, activates adjacent Presynaptic (Type III) cells to release serotonin (5-HT) and norepinephrine (NE) (Huang *et al*, J Neurosci, 2008). These interactions indicate feed-forward, cell-to-cell communication within taste buds. Here, we tested whether 5-HT and NE exert feedback onto Receptor cells. We measured ATP secretion from isolated taste buds and from Receptor cells using biosensors (CHO cells stably transfected with P2X2/P2X3 receptors and loaded with the calcium-sensitive dye, Fura-2; Huang *et al*, PNAS, 2007). As previously shown, taste buds isolated from mouse vallate papillae secreted ATP in response to stimulation with tastants (mixture of cycloheximide, 10 µM; saccharin, 2 mM; denatonium, 1 mM; SC45647, 0.1 mM). Bath-applied 5-HT (10 nM) abolished taste-evoked ATP secretion from isolated taste buds. 5-HT1A receptor agonists, 8-OH-DPAT (10 nM) or BP554 (10 nM), similarly inhibited taste-evoked ATP secretion. Finally, paroxetine (100 nM), a 5-HT reuptake inhibitor, reduced ATP secretion. In contrast, taste-evoked ATP secretion was *elevated* ~2X by methysergide (10 nM), a 5-HT1,2 receptor antagonist, and by WAY100635 (10 nM), a 5-HT1A receptor antagonist. We did not detect any actions of NE on Receptor cells, either positive or negative (Huang *et al*, J Neurosci, 2008). In sum, these findings indicate that during taste stimulation, 5-HT released from Presynaptic (Type III) cells exerts negative feedback onto Receptor cells by activating 5HT1A receptors and reducing ATP secretion. This suggests that 5-HT plays an important role in modulating peripheral taste responses.

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Expression of an Inwardly-Rectifying Potassium Channel (ROMK) in Mouse Glial-like Taste Cells

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Cells in taste buds are closely packed with little extracellular space. Tight junctions and other barriers further limit permeability and may result in the buildup of K^+ during action potentials. In many tissues, inwardly-rectifying K channels such as the Renal Outer Medullary K (ROMK) channel help to redistribute K^+. ROMK is an inwardly rectifying ATP-sensitive K channel, derived from the *kir1.1 (kcnj1)* gene. Using RT-PCR, we defined several splice variants of ROMK in mouse kidney, and report here the expression of a single one of these splice variants, ROMK2, in a subset of mouse taste cells. Using qRT-PCR, we found ROMK2 mRNA is expressed in taste buds in the following order of abundance: vallate > foliate >> palate >> fungiform. Immunocytochemistry revealed that the ROMK protein follows the same pattern as mRNA, and is essentially undetectable in fungiform taste buds. ROMK is localized to the apical tips of a subset of taste cells (~8.5+/-2.5 cells/vallate tastebud). Using tissues from PLCβ2-GFP and GAD-GFP transgenic mice, we show that ROMK is not expressed in PLC 2-expressing type II/Receptor cells or in GAD-expressing type III/Presynaptic cells. Thus, immunocytochemical data suggest that ROMK expression is limited to a subset of glial-like type I cells. Single-cell RT-PCR confirms this interpretation: ROMK2 mRNA was detected in 23% of NTPDase2-expressing cells, but not in either PLCβ2- or SNAP25-expressing taste cells. We propose that in taste buds, ROMK in supporting type I cells may serve a homeostatic function, excreting excess K^+ through the apical pore, and allowing excitable taste cells (types II and III) to maintain a hyperpolarized resting membrane potential.

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Cortical Processing of Learned Aversive Odors in Awake Rats

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Most naturally occurring odors are complex mixtures. These mixtures are hypothesized to be synthesized into odor objects through activity of olfactory cortical circuits. Work in anesthetized rats has demonstrated that neurons in the anterior piriform cortex discriminate between mixtures and their individual components. Furthermore, it has been shown that cortical odor processing is experience-dependent, with familiarity leading to enhanced discriminability of odorants. However, there is still limited data on cortical processing of odors in awake animals. The present experiment had two primary goals. First, compare activity of neurons in the anterior piriform cortex of awake rats to those in anesthetized rats in response to complex mixtures and second, examine how aversive conditioning can affect that processing. Long-Evans rats were chronically implanted with movable wire bundles aimed at the anterior piriform cortex. Responses of single-units to individual odorants and mixtures were tested. Odors were randomly presented from the top of the recording chamber to mimic natural odor plumes. Following several days of recording, one odor was chosen as the conditioning odor in an odor aversion conditioning paradigm. Unit data was recorded during the conditioning trials and for several days post-training. The electrode was moved over time to sample additional cells. Preliminary results (n = 148 units) suggest that 35% of the units responded to any one odor or odor mixture, which is comparable to response rates in anesthetized rats. Individual cells showed excellent discrimination of odors, including mixtures overlapping by as much as 90%. Finally, odor aversion appeared to enhance selectivity of anterior piriform cortex neuron ensembles, potentially enhancing the discriminability of learned aversive odors.

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Strategy for recombinant expression of functional N-terminal domain of human T1R3 taste receptor produced in *Escherichia coli*

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The sweet taste receptor is a heterodimer composed of two subunits called T1R2 and T1R3. Each subunit belongs to the class C of G protein-coupled receptors (GPCRs) and is constituted by a large extracellular N-terminal domain (NTD) linked to the transmembrane domain by a cystein-rich region. T1R2 and T1R3 NTDs are both able to bind natural sugars and some sweeteners (sucralose) with distinct affinities and undergo ligand-dependent conformational change. However, the relative contribution of the two subunits to the heterodimeric receptor function remains largely unknown. To study the binding specificity of each subunit using biochemical and structural approaches, a large amount of purified NTDs is suitable. Here, we report the production of functional human T1R3 NTD from insoluble aggregated protein (inclusion bodies) expressed in high level in Escherichia coli. Transferring this protein into its native state by in vitro refolding requires screening to find buffer conditions and suitable additives. We established a factorial screen to detect folded functional T1R3 NTD based on intrinsic tryptophan fluorescence quenching by sucralose. From the screen, we successively identified positive synergistic interactions between additives on refolding of T1R3 NTD. The soluble protein was then purified and characterized. Fluorescence and circular dichroism spectroscopy demonstrated that T1R3 NTD is properly refolded and able to bind saccharide compounds with physiological relevant affinities. To further validate our expression strategy, we introduced single amino acid changes in the predicted binding site using site-directed mutagenesis. The described production procedure in high quantity should be useful to perform structural and functional studies of human T1R3 and other T1R ligand-binding domains.

#P150 Poster session III: Cortical chemosensory processing/ Receptor genomics and molecular biology

Identifying TRPA1 agonists by monitoring intracellular calcium levels in HEK cells

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Nasal trigeminal chemoreceptors appear to be stimulated by virtually all volatile compounds if presented in high concentrations. Trigeminal nerve endings contain several different types of receptors; however, the specific receptors stimulated by many trigeminal stimuli are unknown. Transient receptor proteins (TRPs) are non-specific cation channels, associated with trigeminal nerve fibers, which display an affinity for calcium. TRPA1, found in a subset of neurons in the trigeminal ganglion, has at least 90 different known agonists and was considered a likely target of known trigeminal stimuli that work through an unidentified mechanism. The goal of this experiment was to determine if certain known trigeminal stimuli activate TRPA1. Both naive HEK and hTRPA1-HEK cells were allowed to grow in a 96-well plate for a minimum of 24 hours. Intracellular calcium levels were measured by a plate reader using the Ca^{2+}-sensitive fluorescent dye FLUO-3AM. Baseline fluorescence of each well was measured. A potential TRPA1 agonist was then added and fluorescence was measured again. The relative change in fluorescence elicited by the test stimuli from wells containing hTRPA1-HEK cells was compared to the relative fluorescent change elicited from wells containing naive HEK cells. In all, 11 stimuli were tested in various concentrations ranging from 0.1 mM to 100 mM. Based on preliminary data analysis, allyl isothiocyanate, alpha-terpineol, acetic acid, benzaldehyde, cinnamaldehyde, eugenol, and d-limonene stimulated TRPA1. It is presently unclear whether amyl acetate, cyclohexanone, nicotine or toluene did or did not stimulate TRPA1. This method identifies TRPA1 agonists and future studies examining behavioral responses will assess whether trigeminal irritation due to these stimuli is solely mediated by TRPA1.

#P151 Poster session III: Cortical chemosensory processing/ Receptor genomics and molecular biology

In Vitro* Nematocidal Activity of TRPA1 Active Compounds from *Perilla Frutescens

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Several food plants used in traditional cooking contain interesting bioactive compounds. We are particularly interested in chemestetic compounds, both for their use in gastronomy and for their medical and agronomical applications. *Perilla frutescens* Britton (Labiatae) is a native plant of eastern Asia, where it is popular as culinary and medicinal herb named *keaennip* in Korea and *shiso* in Japan. One of the major components of Perilla essential oil is perillaketone PK. We discovered that this molecule is a potent activator of TRPA1 in *in vitro* assays on rat cloned receptors [2]. TRP ion channels are important cellular sensors activated by several stimuli and involved in many aspects of chemical sensing [3,4]. In particular TRPA1 is involved in nociception and has an established role in sensing mechanisms in insects and invertebrates. This finding suggests that PK could be responsible of specific nematocidal activity of Perilla extracts. We isolated pure PK from the leaves and evaluated its nematocidal activity against second-stage larvae of cystic nematode *Heterodera daverti,* showing that it is characterized by a remarkable nematocidal activity. [1] Handbook of herbs and spices vol. 3, Peter K.V. Ed., CRC Press, Boca Raton Boston New York Washington, DC **2006**; [2] Bassoli A.; Borgonovo G.; Caimi S.; Scaglioni L.; Morini G.; Schiano Moriello A.; Di Marzo V.; De Petrocellis L., *J. Bioorganic & Med. Chem*., **2009**, (DOI 10.1016/j.bmc.2008.12.057); [3] Clapham D.E., *Nature*, **2003**, *426*, 517-524; [4] TRP ion channels in sensory trasduction and cellular signaling cascades, Liedtke, W.B.; Heller, S. Eds., Taylor and Francis, **2007**.

#P152 Poster session III: Cortical chemosensory processing/ Receptor genomics and molecular biology

Olfactory rivalry: Competing olfactory processing between the two nostrils and in the cortex

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When two different images are presented to the two eyes, we perceive alternations between seeing one image and seeing the other. Termed binocular rivalry, this visual phenomenon was recognized over a century ago, and its neural mechanism has been considerably studied. Here we report the discovery of alternating olfactory percepts when two different odorants are presented to the two nostrils. We show that both cortical and peripheral (olfactory receptor) adaptations are involved in this process. Our discovery extends the perceptual rivalry to olfaction, and opens up entirely new avenues to explore the workings of the olfactory system and olfactory awareness.

#P153 Poster session III: Cortical chemosensory processing/ Receptor genomics and molecular biology

Is there a difference in odor processing in response to left vs. right-sided odor stimulation?

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Objectives: The results of a previous localization study demonstrated, that humans need trigeminal perception to localize odors. Based on these findings a functional magnetic resonance imaging (fMRI) experiment was carried out to assess whether there are differences in odor processing in response to left versus right-sided odorant stimulation. **Methods:** We used two odors: 8ppm H_2S (hydrogen sulphide), which is known to be a pure odorant in this concentration, and 17.5% isoamyl acetate (IAA) as an olfactory-trigeminal stimulus. We tested 22 healthy subjects with H_2S and 24 subjects with IAA. Functional images were acquired using a 3T MR scanner. The odorant stimulation was performed using an olfactometer. The experiment was carried out based on an event-related design paradigm and the stimulus length was 500 ms. After every stimulus the participants were asked to

discriminate between the H_2S/IAA stimuli perceived either from the left or from the right nostril. **Results and Conclusion:** We found activations of brain areas specific for olfactory stimulation (piriform cortex, orbitofrontal cortex, insula) for both odors. Using ROI analysis we found differences in the primary olfactory cortex comparing left vs. right odorant stimulation in case of IAA odor, but not for H_2S odor. These results supported our previous findings and confirmed the hypothesis that nostril-specific differences in brain activation are functionally linked to successful odor localization.

#P154 Poster session III: Cortical chemosensory processing/ Receptor genomics and molecular biology

Brain representation of subjective pleasantness

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Neuorimaging data has implicated several brain regions in the representation of odor pleasantness. In previous studies, one set of odorants served as "pleasant odors" and another set as "unpleasant odors". Although differences in response to these odors may indeed reflect pleasantness, they may also reflect other unidentified differences between the odorants used. Here we built on individual differences in pleasantness perception and set out to test subjects that perceive the same odors differently. To date, two subjects perceived Anis as pleasant and Allyl-coparate as unpleasant, whereas the other two perceived Anis as unpleasant and Allyl-coparate as pleasant. Subjects sniffed Anis and Allyl-coparate inside the 3-Tesla Siemens scanner. The odorants were generated by a MRI-compatible olfactometer and were delivered in a block-design paradigm ("Clean", " Anis" and "Allyl-coparate" blocks). In the analysis we contrasted within and between subject analyses for pleasant vs unpleasant odorants. Within subjects analysis revealed increased activity for unpleasant odorants in the medial orbitofrontal gyri, anterior cingulate, and bilateral insula. Between subjects analysis revealed increased activity for unpleasant odorants in anterior cingulate and bilateral insula, though not in the medial orbitofrontal. Altogether our preliminary results suggest that some brain regions implied in pleasantness processing may in fact represent some other dimension of smell. Conclusions, however, depend on a larger number of subjects.

#P155 Poster session III: Cortical chemosensory processing/ Receptor genomics and molecular biology

Olfactory intensity coding: an fMRI study

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Odor intensity may be coded by either increased spatial extent of neural activity, change in amplitude of neural activity, or both. Whereas results obtained from anesthetised rodents pointed to the former, results obtained from wake animals provided a complex and inconclusive view. To probe cortical intensity coding we used fMRI (3T EPI, slice thickness=4mm, TR=1.5s, TE=23) in 12 human subjects. A block-design olfactory localizer was followed by a 3-consentration [(L) low, (M) medium, (H) high] event-related (22 events/condition) intensity estimation task using Allyl Caproate. Tones at three amplitudes served as an auditory control. The odorants varied in intensity as intended ($F= 228$, $P < e\text{-}10$). An exploratory analysis using a group conjunction (random-effects, $p < .05$) in search of brain areas that mirrored stimulus intensity (H>M>L) revealed left Globus Pallidus, Piriform and Cingulate and right Insula. A hypothesis-driven analysis used the localizer to functionally restrict frontal (PirF) and temporal (PirT) piriform cortex, and then analyzed the event-related response within these regions. There was an effect of intensity in left PirF, left PirT and right PirT (AUC for left PirF L-0.05%, M0.35%, H0.69% ($F(2,20) = 6.84$ $p < .01$), left PirT L-0.12%, M0.21% H0.41% ($F(2,20) = 4.63$ $p < .03$) right PirT L0.17%, M0.54% H0.76% ($F(2,20) = 4.13$ $p < .05$)), but not right PirF ($F(2,20) = 0.88$ $p < .43$). In order to estimate spatial extent we counted activated piriform voxels ($p < .005$) for L, M and H, and found a bilateral increase in voxel number with increase in concentration (all $F(2,20)>4.21$, $p<.03$). In conclusion, odor intensity influenced fMRI amplitude and fMRI spatial extent, and we do not rule out either encoding scheme at this stage.

#P156 Poster session III: Cortical chemosensory processing/ Receptor genomics and molecular biology

Development and Testing of a Neural Recording System for Chemosensory Behavioral Neuroscience

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While current technologies are available to record from behaving subjects, the reality is that there are technical issues that limit the usefulness of these techniques. First, typical multi-channel neural recording systems use single-ended recordings. Single-ended recording systems are susceptible to noise due to the half-cell potential, impedance mismatch, and electromyograms during movement, chewing and sniffing. The noise sources tend to be several orders of magnitude larger than the action potentials we are trying to record. Even with filtering, the noise makes it difficult to accurately isolate individual cells. Second, chronic recording quality begins to degrade immediately following implantation and is almost prohibitive by 4 to 6 weeks post-implantation. The recording quality degrades primarily due to encapsulation by microglia and astrocytes creating a high impedance sheath around the electrode. Third, chronic recording electrode arrays are bundled together preventing independent movement, typically resulting in low yield. For these reasons and others, multi-channel recording techniques make it difficult to perform high-quality neural recording during behavior. We have begun the development of a neural recording system for chemosensory behavioral neuroscience that addresses these concerns. The system is compatible with most commercial recording systems. The individual recording sites can be adjusted maximizing the duration and yield of the recording session. Our preliminary results demonstrate that this system significantly reduces the noise in behaving subjects compared to single-ended recording. Recordings can be obtained over a period of several weeks and between adjustments of the electrode positions.

#P157 Poster session III: Cortical chemosensory processing/ Receptor genomics and molecular biology

The effect of learning and attention on odor responses in piriform cortex

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The piriform cortex (PC) is the primary target of afferent signals from the olfactory bulb and is believed to be the site of odor perception. The anterior PC (aPC) receives more direct sensory input relative to the posterior PC, which has more intrinsic connections. Recently it has become clear that the aPC is more associational in nature than was previously believed, with cellular odor responses that can change based on learning (Schoenbaum et al, 2007). We have found that cells in aPC display robust odor responses, but only after an odor-reward association has been learned. We performed extracellular recordings from the aPC of awake-behaving mice receiving a water reward paired with an odor stimulus. Animals initially received odor-water pairs on every trial (all S+) and subsequently learned to discriminate one unrewarded odor (S-) from a series of rewarded ones (multiple S+). Preliminary results indicate that aPC cells respond to odor only when the animal has learned to identify an unrewarded odor in a series of rewarded ones. Passive sampling of odors or odor delivery paired with water reward did not elicit odor responses. Therefore, firing of cells in aPC may be modulated by attention, with cells only responding when the animal is attending to the odor stimulus. Comparing cellular responses when the animal made a correct decision to responses during mistakes indicates that some cells respond primarily to odor, but the response of most cells depends on both odor and reward. Individual cells were variable in the degree to which their response was modulated by reward or expectation of reward. In conclusion, cells in PC show context-dependent activity that is modulated by learning and attention.

#P158 Poster session III: Cortical chemosensory processing/ Receptor genomics and molecular biology

Mapping Local Inhibitory Circuits in the Rat Piriform Cortex using Photostimulation of Caged Glutamate

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The piriform cortex (PC) is considered the major cortical center for integrating olfactory inputs from the olfactory bulb (OB). Key to understanding PC computation is elucidating how principal excitatory neurons are functionally connected to local inhibitory circuits. To do this, we performed whole-cell recordings on semilunar (SL) and pyramidal (Pyr) cells. We then focally stimulated interneurons (IN) in each of the anatomic layers of the PC (Ia, Ib, IIa, IIb, and III) using flash photolysis of caged glutamate. Strength of inhibitory connections was assessed by measuring the peak amplitude, duration, and number of inhibitory postsynaptic currents (IPSC) recorded from each uncaging site. Our preliminary data indicate that PC-IN connections were stronger than SL-IN (mean IPSC number across all layers for Pyr vs SL, 3±0.5 vs 1±0.1; amplitude, 34.2±6.6 vs 19.5±1.5 pA; duration, 79.8±12.3 vs 62.9±10.3 ms; n=6). Also, for either SL or Pyr, strength of connectivity depended on the layer in which the uncaging beam was directed. SL and Pyr showed the most robust IPSCs in response to uncaging at layer IIa, the weakest when uncaging at layer Ia. SL and Pyr showed moderate responses to layer IIb uncaging. On the other hand, we observed contrasting results between SL and Pyr when we uncaged at layer Ib and III. SL were strongly connected to layer Ib cells, but weakly connected to layer 3 cells; the reverse was true for Pyr. Thus, our results suggest that SL and Pyr may be differentially connected to distinct IN subsets. As such, SL and Pyr would have unique input-output characteristics in response to the same OB input. Understanding these marked computational capabilities may be important in deciphering the processes involved in encoding and representing olfactory information.

#P159 Poster session III: Cortical chemosensory processing/ Receptor genomics and molecular biology

The olfactory bulb as cortical model system

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Model systems that reduce the scope and complexity of full-scale biological systems while retaining the principal variables of interest have a distinguished history in neuroscience. Some of the most productive reduced model systems are those that enable meaningful links between the cellular, circuit, and behavioral levels of analysis, enabling study of their interplay. Olfactory learning is a distributed phenomenon involving changes in synaptic weights and gene expression profiles throughout the central nervous system, thereby underlying a number of potential changes in animal behavior. My colleagues and I have sought to identify an aspect of this broader odor learning that can be localized within the olfactory bulb (OB). I here present the case for olfactory generalization gradients, in both their nonassociative (cross-habituation) and associative (rewarded) forms, as plastic behavioral measures dependent on learning and attention and mediated substantially by OB neural circuitry. Olfactory generalization gradients are regulated by multiple determinants of learning including the amount of training, CS salience, US reward value, and training-testing latency, as well as by neuromodulator activity within the OB. Aging and dementia model mice also exhibit shallower gradients after conditioning, indicative of reduced learning rates. Computational circuit models are used to illustrate how known neuromodulatory effects can mediate coordinated changes in OB odor responses and predict behavioral outcomes. I suggest that these effects are microcosms of larger-scale phenomena affecting cortical networks generally, and that insights gained from the reduced system of the OB therefore can be applied to broader questions of cortical function and dysfunction.

#P160 **Poster session IV: Chemosensory transduction and perireceptor events**

PI3K-gamma in olfactory signal transduction in mice

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Recent findings in rat olfactory receptor neurons (ORNs) suggest phosphoinositide 3-kinase (PI3K) -dependent signalling may play a role in mammalian olfactory signal transduction. In order to bring the power of genetically modified rodents to this question we tested whether PI3K has a similar effect on the output of mouse ORNs. We show that the pan specific PI3K inhibitor Wortmannin increases the calcium response of acutely dissociated mouse ORNs to a complex odorant mixture (Henkel 100) in approximately the same percentage of cells as with rat ORNs. The catalytic subunits of the alpha, beta and gamma isoforms of PI3K are expressed in the mouse olfactory epithelium (OE) and can be localized to the ORNs. As with rat ORNs, isoform-specific blockade of the beta (TGX-221) and gamma (AS252424) isoforms of PI3K implicate both isoforms in modulating odorant-dependent signalling. These results suggest that the PI3K dependent modulation of olfactory signal transduction originally characterized in rats generalizes to mice. Mice deficient in PI3K-beta are embryonically lethal, but mice deficient in PI3K-gamma are viable and do not show obvious differences in OE morphology and retain cyclic nucleotide-dependent responsiveness to odorants. These mice are being used to examine the specific role and mechanism of PI3K-gamma in olfactory signal transduction.

#P161 **Poster session IV: Chemosensory transduction and perireceptor events**

Differential sensitivity to monosodium glutamate in Type II and Type III taste cells

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Glutamate receptors are expressed in taste cells and allow the detection of umami taste stimuli via the G protein coupled receptor T1R1-T1R3. Glutamate receptors may also be involved in neurotransmission between nerve fibers and taste cells, or between different taste cell types. The aim of the present study was to compare the physiological response to monosodium glutamate (MSG) in identified types of taste cells. We isolated taste cells from circumvallate papillae and used calcium imaging to characterize MSG responses. We used T1R3-GFP mice to identify a subset of Type II taste cells, presumably those that express the umami taste receptor T1R1-T1R3. We used stimulation with high K^+ to identify Type III cells, as these are the only cells in the taste bud that possess voltage-gated Ca^{2+} channels. T1R3-GFP taste cells failed to respond to MSG at concentrations below 10 mM. On the contrary, in Type III cells, responses to MSG were obtained at 100µM. To determine whether these responses to low concentration of glutamate are mediated by ionotropic receptors, responses to glutamate were measured in the presence and absence of CNQX, an ionotropic AMPA/kainate glutamate receptor antagonist. CNQX partially blocked the responses to MSG in Type III cells, suggesting that AMPA/kainate receptors are functionally expressed in these taste cells. Hence, both Type II and Type III cells respond to MSG, but with different sensitivity. Further, responses to low concentrations appear to be at least partially mediated by AMPA/kainate receptors. This work was supported by NIH grant DC00766 and a 3ARP grant from Ajinomoto.

#P162 **Poster session IV: Chemosensory transduction and perireceptor events**

The Second Messenger Pathways in TRPC2 Knockout Mouse Vomeronasal Sensory Neurons

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In vomeronasal sensory neurons (VSNs), transient receptor potential cation channels (TRPC2) play a large role in responses to pheromones and odorants. TRPC2-/- mice fail to display the male-male aggressive behavior and mate indiscriminately (Stowers et al., 2002). However, pregnancy block, which requires a functional vomeronasal organ (VNO), does occur in TRPC2-/- mice (Kelliher et al., 2006). This suggests the presence of a TRPC2-independent pathway in VSNs. To reveal the mechanism for pheromone/odorant detection of VSNs, we investigated urine response in VSNs from wild-type and TRPC2-/- mice. Using perforated patch clamp technique, we recorded responses to dilute urine in wild-type & TRPC2-/- VSNs. These urine responses in both types appeared to be through the phospholipase C (PLC) pathway since they were completely blocked by the PLC inhibitor, U73122, although the response amplitude of TRPC2-/- VSNs was smaller than that for wild type. We asked which second messenger pathway mediates urine responses in knockout mice. Activation of the PLC pathway produces diacylglycerol (DAG), which directly gated TRPC2 chanels. However, DAG also produces arachidonic acid (AA) via DAG lipase. Thus, we tested if a DAG lipase inhibitor, RHC80267, altered urine responses. We found that RHC80267 blocked urine-induced inward current by ~60% in wild type VSNs while it abolished the urine responses in TRPC2-/-. Moreover, AA itself induced an inward current in both wild type and knockout VSNs that was dependent on extracellular calcium. Using inside-out patches, we recorded Ca2+-activated and AA-activated channel activity with a conductance of ~26 pS. Thus, our data suggested that in mouse VSNs, the pheromone/odor detection is carried out by both DAG-TRPC2 and Ca2+/AA-cation channels pathways.

#P163 **Poster session IV: Chemosensory transduction and perireceptor events**

PI3K mediated signaling in lobster olfactory signal transduction

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Odors are identified by the brain through a distributed process that begins with the primary olfactory receptor neurons (ORNs). In organisms in which odorant molecules bind to G protein-coupled receptors (GPCRs) on the ORNs to initiate signal transduction, involvement of phosphoinositide 3-kinase (PI3K) activity in olfactory signal transduction would suggest a potential role for the GPCR-activated class I PI3K β and γ isoforms. Using isoform-specific antibodies, we identified a protein in the olfactory signal transduction compartment of lobster ORNs that is immunoreactive with an antibody directed against mammalian PI3Kγ. The lobster PI3K co-immunoprecipitates with the α and βγ G protein subunits, and an odorant-evoked increase in phosphatidylinositol (3,4,5)-trisphosphate, the product of PI3K activity, can be detected in membranes prepared from the signal transduction compartment of the ORNs. The isoform-specific PI3Kγ inhibitor AS-252424 reduces the odor-evoked output of lobster ORNs *in vivo*. Additionally, the catalytic subunits of PI3Kβ and γ, as well as odorant-dependent PI3K activity, can be detected in a ciliary membrane preparation from rat ORNs. Collectively, these findings support a potential role for PI3K-dependent phospholipid signaling in olfactory transduction and suggest that PI3K-mediated signaling in olfactory signal transduction may be conserved across species.

#P164 **Poster session IV: Chemosensory transduction and perireceptor events**

Different response properties between Type II and Type III taste bud cells in mouse fungiform papillae

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Taste bud cells are classified into 4 groups (Type I ~ IV) according to their morphological and functional characteristics. Among them, Type II cells express sweet, bitter and umami taste receptors and transduction components, suggesting that Type II cells may be responsible for these taste qualities. Type III cells possess putative sour taste receptors, suggesting that these cells may be sour taste receptor cells. To clarify taste response properties of Type II and III cells, we investigated taste responses of individual mouse fungiform taste bud cells expressing gustducin (Type II) or GAD67 (Type III). We recorded taste responses from gustducin-GFP and GAD67-GFP taste bud cells, or examined the expression of gustducin and GAD67 by the single cell RT-PCR after recording of taste responses. Both Type II and III cells generated action potentials in response to taste stimuli, suggesting that these cells may transmit taste information to gustatory nerve fibers. Type II cells responded best to sweet, bitter, or umami stimuli whereas Type III cells responded to sour stimuli and electrolytes. These results are well consistent with those of previous molecular studies and suggest that Type II and Type III cells may contribute to detection and perception of different taste qualities. Supported by KAKENHI 18077004, 18109013 (YN) and 19791367 (RY).

#P165 **Poster session IV: Chemosensory transduction and perireceptor events**

Receptor-dependent PIP_2 resynthesis restores sweet and bitter inhibitions of potassium currents

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Phosphatidylinositol 4,5-bisphosphate (PIP_2), a lipid signaling agent, is a recognized component of sweet, bitter, and umami transduction cascades. It is also a well known activator of ion channels in other cellular systems. We previously established PIP_2 resynthesis as important in restoring activity to outward (K_V) and inward (K_{IR}) potassium currents in rat posterior taste receptor cells (TRCs) after caffeine-inhibition. Here we provide corroborative evidence that suggests PIP_2 resynthesis generalizes in adaption to bitter and sweet stimuli. Kv currents, recorded from rat posterior TRCs with standard patch clamp techniques, were inhibited approximately 35% by m-3M3FBS, a broad spectrum PLC activator that acts to globally deplete PIP_2 whether applied intracellularly (100 μM, n=32) or extracellularly (5 μM, n=57). o-3M3FMS (100 M, intracellular), an inactive enantiomer, was without effect. Unexpectedly, depletion of PIP_2 strongly influenced response profiles to cycloheximide (100 M; n=61), caffeine (20 mM; n=44), and SC45647 (100 M; n=56). After m-3M3FBS treatment, these tastants, known to inhibit K_V, now actually enhanced K_V. We hypothesize that this effect is due to PIP_2 resynthesis. To test this notion, polylysine, a PIP_2 scavenger, was added to the pipette solution and effectively reduced the enhancement caused by bitter (n=22) or sweet (n=19) stimulation. In addition, exogenous PIP_2 itself (10 M, n=33, 50 M, n=27) did not enhance K_V although 50 M PIP_2 was able to reduce K_V inhibition when combined with 5 M m-3M3FBS (n=20). Collectively, these data support the notion that potassium channels are subject to dynamic regulation by PIP_2 and that its resynthesis requires a signal, likely receptor-mediated, for its initiation.

#P166 **Poster session IV: Chemosensory transduction and perireceptor events**

Native TRPM5 currents recorded from posterior rat taste receptor cells

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The ion channel TRPM5 is expressed in a subset of taste receptor cells (TRCs) and is thought essential for bitter, sweet, and umami transduction cascades. It is permeable to monovalent cations and gated by intracellular calcium. This channel has mostly been characterized in heterologously expressed cells, where it is not

subject to native regulatory mechanisms. Here we present recordings of TRPM5 currents from native rat posterior TRCs using standard patch clamp techniques with cesium electrodes to block potassium current. Using flash photolysis (with caged-IP_3 or caged Ca^{2+}) or ionomycin (a calcium ionophore), a TRPM5-like current was consistently evoked in half of tested TRCs. The current displayed strong desensitization, rectification, dependence on extracellular sodium, and was unaffected by a TRPM4 channel blocker. Additionally, the tastants cycloheximide or SC45647 (each at 100 M) were demonstrated to evoke highly similar current in a subpopulation of TRCs. As expected, the IP_3-receptor blockers, 2-APB and heparin, and thapsigargin, an inhibitor of intracellular calcium stores, diminished the activation and amplitude of the TRPM5 current. Quinine, reported to inhibit TRPM5 currents, similarly had an inhibitory action on this current. Further the current was modulated by application of the lipid signaling agent PIP_2 which also promoted bitter and sweet tastant-elicited TRPM5 responses. The current could also be evoked by arachidonic acid (AA) further suggesting its modulation by lipid regulators. Preliminary data from HEK293 cells expressing mouse *trpm5* supports our results from native TRCs with evoked currents displaying similar activation and desensitization kinetics. Collectively, these data support the notion that we are recording native TRPM5 currents from a subset of TRCs.

#P167 **Poster session IV: Chemosensory transduction and perireceptor events**

Novel Insights into Odorant Recognition: A Computational and Functional Analysis of Ligand Binding to the Human Olfactory Receptor OR2AG1

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The process of odor perception begins with the binding of odorant molecules by specific odorant receptors (ORs). So far, only few ligand-OR interaction pairs have been characterized and most characterized receptors show a rather broad tuning and detect multiple, chemically similar odorants. Due to the limited knowledge on receptor – odorant interaction, the molecular details of ligand specificities of ORs are not well understood. Highly variable residues in the transmembrane domains III, IV, and V were postulated to form the basis for ligand specificity. To gain more insight into the molecular basis of odor recognition, we investigated ligand-receptor interactions for the human olfactory receptor OR2AG1 by combining dynamic homology modeling and ligand docking with functional analysis and site-directed mutagenesis. Basing on the rhodopsin crystal structure, we predicted the protein structure of OR2AG1 by homology modeling. The model was further subjected to free molecular dynamics simulations in an explicit membrane/solvent environment with various odorants introduced into the putative binding site. To verify the theoretical model experimentally we mutated amino acids predicted to be involved in ligand binding and compared activation of receptor variants heterologously expressed in Hana3a cells by Ca^{2+}-imaging. By combining theoretical and experimental techniques it was possible to characterize ligand binding of OR2AG1 on the atomic scale.

#P168 **Poster session IV: Chemosensory transduction and perireceptor events**

Olfactory Neuron Response Statistics: a Cross Species Analysis

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Although the olfactory systems of different species share remarkable organizational similarities, there are also marked differences between them, most notably the number of receptor types and their response dynamics. The reasons for, and implications of these differences, are largely unknown. Here we analyzed a large set of previously published olfactory neural response data from different olfactory neurons in six different species. We found that whereas species with a small number of olfactory receptor (OR) types have more broadly tuned response curves, species with larger number of OR types tend to have more narrowly tuned response curves. Consistent with this, species with larger number of OR types were better at discrimination of enantiomers. Comparing the response spectra of all olfactory neurons from the different species, we found that only a small set of olfactory neurons had similar response spectra. This lack of functional conservation is consistent with genetic analysis, that together suggest each species may have olfactory receptors that are optimized for its own ecological niche. Despite these differences, we found that in all species tested, and across 12 unrelated reported studies, the primary axis of the olfactory neuronal space was the "total neuronal response" elicited by the odors. In other words, summing the neural response elicited by an ensemble of olfactory neurons to a specific odor accounts for a large portion of the neural response variability. Critically, this primary axis of neural space was significantly correlated to the odor preferences of rats, mice and drosophila. We therefore suggest that the primary axis of neural response is related to odor hedonics.

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Determinants of Agonist Sensitivity in an Insect Olfactory Receptor

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To identify functionally important structural features of *Drosophila* olfactory receptors (DmOrs), we expressed DmOrs Or35a, Or85a, and Or85b in *Xenopus* oocytes (each with Or83b), and screened for susceptibility to methanethiosulfonate (MTS) reagents. These reagents may alter receptor function by covalently attaching to accessible cysteines, thus providing an approach for structural studies. One receptor, Or85b, was partially inhibited by MTSES (ES). We mutated each of the five cysteine residues (C124, C146, C208, C278, and C311) to serine and showed that only mutant C146S failed to be inhibited by ES, suggesting C146 as the site of ES covalent attachment and receptor inhibition. Comparison of current-voltage relationships before and after ES treatment indicated no effect on the ionic permeability properties of the receptor, suggesting that ES is not acting at the channel pore. Increasing agonist concentration reduced the inhibitory ability of ES, suggesting that ES was shifting the dose-response

relationship of the receptor and its agonist, 2-heptanone. Indeed, ES treatment increased the EC_{50} for 2-heptanone by 4-fold, indicating that residue 146 is at, or near, a region involved in agonist sensitivity. Next, we probed this region using the substituted cysteine accessibility method. Residues 134 to 154 were sequentially mutated to cysteine in an Or85b C146S background (the mutant insensitive to ES) and screened for susceptibility to ES, enabling identification of other residues important for agonist sensitivity. The M148C mutation restored susceptibility to ES in the C146S receptor. We conclude that the extracellular end of transmembrane domain 3 (the location of C146 and M148 in DmOr85b) is involved in determining agonist sensitivity of this insect olfactory receptor.

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Visualization of Assayed Olfactory Chemical Space
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The diversity of chemical ligands potentially detectable by olfactory receptors makes investigating how these proteins extract and encode information a particularly fertile area of study. Yet the same richness that makes this system so attractive often poses practical challenges for experimental design. In an effort to assist in selection of subregions of olfactory space, we present a visual map of the compounds that currently have been assayed on identified human and rodent olfactory receptors. This guide depicts the relationship between major "nodes of empirical focus" and provides a means of readily comparing the receptive fields between olfactory receptors. An accessory view organizes assayed compounds into a three-dimensional landscape based on physical-chemical descriptors to permit a more objective evaluation of the range of tested chemical space. We believe that this tool can help stimulate further evaluation of the coding logic employed by these receptors that have already proven amenable to expression in various systems.

#P171 **Poster session IV: Chemosensory transduction and perireceptor events**

Phosphoinositide-3-kinase Dependent Signaling in Mammalian Olfactory Receptor Neurons
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Phosphoinositide-3-kinase (PI3K)-dependent signaling can modulate the calcium response of rat olfactory receptor neurons (ORNs) to odorant stimulation (Spehr et al., Neuron, 2002). Here we report that Western blotting and immunohistochemistry confirms the presence of two isoforms of PI3K known to be activated by GPCRs in other systems (beta and gamma) in rat olfactory ciliary membranes and in most or all ORNs in fresh frozen sections of the rat olfactory epithelium (OE). The complex odorant Henkel100 transiently increases the level of PIP_3, generally assumed to be the primary product of PI3K *in vivo*, in rat olfactory ciliary membranes as fast as 2 sec following stimulation. Production of PIP_3 is significantly reduced by the pan-specific PI3K blocker, LY294002, as well as by the PI3K beta- and gamma-specific blockers, TGX221 and AS252424, respectively. The pan- and the gamma-specific PI3K blockers can modulate Henkel100-induced discharge in rat ORNs measured in the intact OE through loose-patch recording from dendritic knobs. PI3K-dependent modulation can account for almost a 10-fold shift in the overall sensitivity of the ORNs. Activation of P_2Y purinergic receptors, also thought to couple to phosphoinositide signaling, fails to modulate odorant responses in a PI3K-dependent manner. We conclude that odorants activate PI3K through a G-protein coupled pathway, and do so sufficiently fast to modulate the electrophysiological output of rat ORNs in a transduction-dependent context.

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The Effects of Membrane Permeant and Impermeant Carbonic Anhydrase Inhibitors on the EOG and NMP Responses to CO_2 in Mice
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Physiological concentrations of CO_2 (less than the 4-5% CO_2 in expired air) have been shown to stimulate a subset of olfactory receptor neurons, while noxious CO_2 concentrations (25% or above) are known to stimulate trigeminal nerve endings in the nasal epithelia. Although the mechanism by which CO_2 stimulates olfactory receptors or trigeminal nerve endings is not known it appears that the enzyme carbonic anhydrase (CA) plays a role in the transduction mechanisms. CA is located in the nasal mucosa as well as in a small percentage of olfactory receptor neurons. The objective of this study was to record electro-olfactograms (EOG) and negative mucosal potentials (NMP) in response to CO_2 before and after topical application of membrane permeant (acetazolamide - AZ) or membrane impermeant (quaternary ammonium sulfanilamide - QAS) CA inhibitors. A range of CO_2 concentrations, from 0 to 50%, was used in this study. Topical application of mammalian Ringers did not affect the EOG responses to CO_2 while 0.1mM QAS caused a small decrease in EOG amplitudes and 0.1mM AZ eliminated the EOG responses to all CO_2 concentrations. Topical application of mammalian Ringers, 0.1mM QAS, or 0.1mM AZ caused a small decrease in the NMP at each CO_2 concentration. These results indicate that intracellular CA plays a critical role in the detection of CO_2 by olfactory receptor neurons. Although inhibition of extracellular CA caused a small decrease in the EOG responses to CO_2 it is not clear whether this indicates a specific role for mucosal CA or is due to non-specific effects on mucosal pH. The results from the NMP experiments indicate that extracellular and intracellular CA play no role or only a minor role in the detection of CO_2 by trigeminal nerve endings.

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Identification of a novel Calcium Activated Chloride Channel in the Cilia of Olfactory Sensory Neurons: TMEM16b

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Chloride channels are a ubiquitous group of ion channels involved in many physiological processes, including sensory signal transduction. The chloride channel involved in olfactory signal transduction belongs to the family of chloride channels that are regulated by the cytosolic Ca^{2+} concentration. These Ca^{2+}-activated chloride channels (CaCCs) can conduct Cl- ions upon activation by calcium, but only little is known to date about their structure and function.

We performed a proteome analysis of the olfactory epithelium membrane proteome and identified so far uncharacterized membrane proteins as candidate channels. One of the most abundant membrane proteins was TMEM16b, a member of a recently identified family of CaCCs. We show here with in-situ hybridization and RT-PCR that TMEM16b is expressed in mature olfactory sensory neurons. Western blot analysis with an antibody specifically designed to detect TMEM16b revealed that its expression is different from the closely related TMEM16a protein, which shows a broad expression pattern in secretory epithelial cells. TMEM16b expression was found to be highly specific for the olfactory epithelium and the retina, the protein was not detected in other tissues such as lung, kidney, liver, testis, trachea, colon, and tounge. TMEM16b localization is restricted to the cilia of the olfactory receptor neurons. The localization in the olfactory neurons is in agreement with electrophysiological recordings showing expression of CaCCs in the ciliary membranes. Since the channels were shown to be involved in generating the receptor current during odor detection, we are currently investigating odor responses upon TMEM16b siRNA transfection of the olfactory epithelium with electro olfactogram recordings (EOGs).

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Biotransformation of odorants modifies the olfactory signal

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The olfactory epithelium contains several families of enzymes involved in biotransformation of chemicals, such as cytochrome P450-dependent monooxygenases (CYP) and transferases. Some of them are specifically expressed in olfactory tissues. While these enzymes are mostly responsible for the detoxification of inhaled toxic compounds, at this peculiar location, they could also play a role in the biotransformation of odorants and contribute to the olfactory signal termination. Olfactory tissue displays high CYP and UDP-glucuronosyltransferase activities. The biotransformations catalyzed by these enzymes could therefore lead to the inactivation of odorants. In the present study, we studied the impact of odorant glucuronidation and oxidation on olfactory signal in rat using a submerged electro-olfactogram (EOG) technique. EOG amplitudes produced by two compounds, 4-methyllumbelliferone and quinoline, were compared to those generated by the glucuronide and oxidized forms. The native forms elicited dose-dependent responses. No EOG responses were recorded when stimulating the same preparation with the glucuronides. The hydroxylated metabolite, 8-hydroxyquinoline, produced a response half-way between those obtained with the non-hydroxylated native form and the conjugated form. The signals of mixtures containing both the native and the glucuronide forms were equivalent to those observed with the native molecules, indicating that metabolites do not compete with unmetabolized molecules. These data are consistent with the hypothesis that biotransformation of odorant molecules, particularly glucuronidation, can lead to a decrease of the olfactory input.

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Homeostatic Control of Sensory Output in Basal Vomeronasal Neurons: Activity-Dependent Expression of *Ether-à-Go-Go* Related Gene Potassium Channels

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Conspecific chemosensory communication controls a broad range of social and sexual behaviors. In most mammals, social chemosignals are predominantly detected by sensory neurons of a specialized olfactory subsystem - the vomeronasal organ (VNO). The behavioral relevance of social chemosignaling puts high demands on the accuracy and dynamic range of the underlying transduction mechanisms. However, the physiological concepts implemented to ensure faithful transmission of social information remain widely unknown. Here, we show that sensory neurons in the basal layer of the mouse VNO dynamically control their input-output relationship by activity-dependent regulation of K+ channel gene expression. Using large-scale expression profiling, immunochemistry and electrophysiology, we provide molecular and functional evidence for a role of ether-à-go-go related gene (ERG) K+ channels as key determinants of cellular excitability. Our findings indicate that an increase in ERG channel expression extends the dynamic range of the stimulus-response function in basal vomeronasal sensory neurons. This novel mechanism of homeostatic plasticity in the periphery of the accessory olfactory system is ideally suited to adjust VNO neurons to a target output range in a layer-specific and use-dependent manner.

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Movement of Pheromone Inside Insect Olfactory Sensillae
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Pheromones of the order Lepidoptera (butterflies and moths) are commonly straight-chain fatty acids. Hence, they are quite insoluble in the aqueous layer of the sensillar lymph. The current theory of insect olfaction asserts that in order to become soluble a pheromone must bind with a pheromone binding protein, or PBP. This pheromone/PBP complex then diffuses across the sensillar lymph to eventually bind with a putative protein receptor. A review of the literature was conducted to ascertain if diffusion is a sufficient mechanism in order to support the current theory. The review finds that the diffusion coefficient is too slow (0.12 square micrometers/millisecond) when considering a 250 dalton pheromone and the bound PBP at 14,000 daltons. Already too slow to explain the electrophysiological evidence (1-10 ms) there are other reviewed factors to consider which will further slow the diffusion coefficient. These include a non-linear mathematical extrapolation, a concentration of 10 mM PBP in the sensillar lymph, no diffusion gradient within the lymph, bound water molecules to the PBP, and changes in concentration or viscosity over time. Based on known physical laws, diffusion cannot be the sole mechanism involved in insect olfaction. If the electrophysiological evidence supports pheromone detection in from 1-10 ms, then either a different mechanism must be occurring, or additional mechanisms must be proposed.
A scientific reassessment of the possible mechanisms is warranted, but new theories must be able to explain the electrophysiological evidence, not contradict it.

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Pathophysiological role of ENaC in a mammalian model of diabetes
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Untreated diabetes is a profound disease that is reflected in a severe impairment of systemic salt and water balance. Based on our earlier work demonstrating the insulin regulation of epithelial sodium channels (ENaC), we hypothesize that ENaC in mouse taste receptor cells (TRCs) plays a central role in the restoration of salt and water intake by virtue insulin's effect on the gustatory system. To investigate whether ENaC function is altered during the onset of diabetes, we performed functional ratiometric Na^+ imaging in isolated taste cells from a mouse model of Type 1 (insulin-dependent) diabetes. Taste cells from diabetic mice exhibit Na^+ responses and amiloride sensitivity similar to non–diabetic littermates. However, insulin enhancement of Na^+ influx via ENaC was abolished in diabetic taste cells. In contrast, taste cells from diabetic mice, especially those in the posterior mouse tongue, evoke greater responses to 140 mM NaCl (60±5.3 AUC) than those from non-diabetic mice (26.4±5.4). To evaluate the effects of the diabetic state in salt taste, we next characterized behavioral responses to NaCl using a brief access test. Diabetic mice showed avoidance of NaCl at significantly lower concentrations than the non-diabetic group. In contrast, diabetic animals showed no significant avoidance to these NaCl solutions ($p<0.01$) when amiloride (100 µM) was added to NaCl solutions indicating a role for ENaC in this increased sensitivity. Currently, we are using qRT-PCR to quantify relative differences in the expression of α, β, and Y ENaC subunits in TRCs from the diabetic mouse. Our results are consistent with the hypothesis that ENaC alterations during diabetes may be an example of the ability of the gustatory system to respond to nutritional changes.

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Response Latency to Lingual Chemical Stimulation Distinguishes Neuron Types within the Geniculate Ganglion
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In anesthetized male rats, we recorded single-cell 5-s responses from geniculate ganglion neurons (N = 47) simultaneously with stimulus-evoked summated potentials (Electrogustogram; EGG) from the tongue to signal when the stimulus contacts the taste receptors. Artificial saliva (15mM NaCl, 22mM KCl, 3mM $CaCl^2$, 0.6mM $MgCl^2$) at 35°C served as the rinse solution and solvent for the basic taste stimuli (0.5 M sucrose, 0.1 M NaCl, 10 mM citric acid, and 20 mM quinine hydrochloride (QHCl), as well as 0.1 KCl. Cluster analysis separated neurons into 4 groups (Sucrose-specialists, Na+-specialists, Na+/QHCl-generalists and Acid-generalists). Artificial saliva elevated the spontaneous firing rate of all neurons. Consequently, there were a few cases of stimulus-evoked inhibition. Specialist neurons as well as Na+/QHCl-generalists were largely unresponsive to citric acid or KCl, except in a few weakly responsive cases. As a rule, the best stimulus elicited the highest frequency response at the shortest latency, with Acid-generalists the only exception. Although Acid-generalists responded with the highest frequency to citric acid over 5-s, the average response latency was 1828ms to citric acid, but 1782, 622, 547, and 667ms to sucrose, NaCl, QHCl and KCl, respectively. For specialist neurons and for Na+/QHCl-generalists, the average response latency to the best taste stimulus was 2 to 4 times shorter than the latencies to non-preferred stimuli. The shortest latency responses for all neuron types were to NaCl in Na+-specialists (374 ms) and Na+/QHCl-generalists (200 ms) neurons. Collectively, these latency data indicate the direct and indirect influences from Type I (amiloride-sensitive), Type II (receptor) and Type III (pre-synaptic) cells within the taste bud onto chorda tympani fibers innervating the bud.

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Cell-Cell Communication in Intact Taste Buds Through ATP Signalling
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Isolated taste cells and strips of lingual tissue from taste papillae have been observed to secrete neurotransmitters, including ATP, upon taste stimulation (Finger *et al* 2005, Huang *et al* 2007; Romanov *et al* 2007). Taste bud Receptor (Type II) cells possess

G protein-coupled receptors for bitter, sweet and umami compounds, and are the source of ATP secretion. These cells are believed to secrete ATP through gap junction hemichannels. Based on studies from isolated taste cells, we have postulated that ATP secreted from Receptor cells acts within the taste bud to excite adjacent Presynaptic (Type III) cells (Huang *et al* 2007). This however, remains to be established in more intact preparations. Here we use lingual slices containing intact taste buds to test the hypothesis in detail. We used confocal Ca^{2+} imaging to track taste stimulation of Receptor and Presynaptic taste cells. Incubating the tissue with an ecto-ATPase (apyrase, 30 units/ml) reversibly blocked signal transfer from Receptor to Presynaptic cells, consistent with ATP being the transmitter. Inhibiting pannexin 1 (Px1) gap junction hemichannels with CO_2-saturated buffer reduced cell-cell signaling (*N.B.,* intracellular acidification with CO_2-buffer is a potent blocker of Px1 channels). Moreover, probenecid (1 mM), an antagonist of Px1 channels (Silverman *et al* 2008; Ma *et al* 2008) significantly reduced cell-cell signaling between Receptor and Presynaptic cells. These findings strongly support the hypothesis that *in situ*, gustatory Receptor cells secrete ATP via Px1 hemichannels when taste buds are stimulated and that the released ATP excites adjacent Presynaptic cells. These findings are being used to develop a model for signal processing in mammalian taste buds.

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Taste cells express and secrete glucagon-like peptide 1
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We previously observed that gustducin, T1r receptors and several other taste signaling elements are expressed in duodenal enteroendocrine L cells that express glucagon-like peptide 1 (GLP-1). We determined that gustducin and T1r3 are critical to enteroendocrine L cell release of GLP-1 in response to glucose. A number of years ago we speculated that if the gut's L cells expressed taste elements then taste cells might in turn express GLP-1 and other L cell hormones. We have found that this is indeed the case and have examined the function of hormones released from these "endocrine taste cells." METHODS: RT-PCR, in situ hybridization and immunohistochemistry were used to examine expression of GLP-1 and other gut hormones in taste cells. ELISA was used to monitor in vivo release of GLP-1 from taste cells into the bloodstream in response to glucose and other tastants. Esophagealectomy/vagotomy was done to eliminate direct or indirect stimulation of enteroendocrine L cells in gut. Circumvallate papillae explants in culture were examined for the ability to release GLP-1 after tastant stimulation. RESULTS: Taste cells were found to express GLP-1, glucagon, PYY and other gut hormones. Patterns of expression indicated that gustducin-expressing type II cells and other subtypes of taste cells express GLP-1. In wild-type mice, with or without vagotomy, application of glucose to the tongue induced a rapid elevation of GLP-1 in the bloodstream. Stimulation of taste cell explants with glucose led to release of GLP-1 into the medium. Glucose stimulation of gustducin-null mice did not lead to significant release of GLP-1 from taste cells in vivo or in explants. CONCLUSIONS: The cephalic phase rise in circulating GLP-1 depends on direct release of GLP-1 from gustducin-expressing taste cells into the bloodstream.

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Sodium/calcium exchangers selectively contribute to the regulation of cytosolic calcium levels in mouse taste cells
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The detection of gustatory stimuli depends on the activation of diverse signaling pathways that are selectively expressed in taste receptor cells in the oral cavity. Some taste stimuli activate G-protein coupled receptors (GPCRs) that cause calcium release from intracellular stores while other stimuli depolarize taste cells to cause calcium influx through voltage-gated calcium channels (VGCCs). We have recently shown that activation of these two types of signaling pathways generate significantly different calcium responses within taste cells (Hacker *et al.*, 2008) and we predicted that the mechanisms needed to regulate these different calcium loads may also differ. To date, however, the calcium buffering mechanisms in taste cells have not been well studied. We recently demonstrated that mitochondria make significant contributions to the regulation of cytosolic calcium in taste cells (Hacker & Medler, 2008) but no other calcium buffering mechanisms in taste cells have been identified. In this study, we used calcium imaging to characterize the role of sodium/calcium exchangers (NCXs) in regulating cytosolic calcium in taste cells. We found that NCXs make important contributions to the maintenance of resting calcium levels in taste cells and that these proteins selectively contribute to the regulation of evoked calcium responses. RT-PCR analysis revealed that multiple NCX and sodium/calcium/potassium exchangers (NCKX) are expressed in taste cells.

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The multiple PDZ domain protein 1 (MUPP1) – Role in the olfactory signal transduction cascade
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The complex network of the olfactory signal transduction pathway, found in olfactory sensory neurons (OSNs), enables mammals to detect and discriminate between thousands of different odorants. Until now, the importance of organizing the various interaction partners of olfactory receptors (ORs) is not well understood. For diverse cell signalling cascades interactions with PDZ domain containing proteins, which assemble defined protein networks and thereby regulate signalling events, are characterized. The Multiple PDZ Domain Protein 1, MUPP1, consists of 13 individual PDZ domains and interacts with different GPCRs such as the serotonin receptor 5-HT_{2c} and the $GABA_B$ receptor. We demonstrate that this scaffolding protein is highly expressed in the dendritic knobs and cilia of olfactory sensory neurons. We further found that ORs and MUPP1 interact *in vitro* and in the recombinant expression system. Therefore MUPP1 represents a possible nucleator or regulator of the olfactory response by acting as first building block of a putative "olfactosome". The physiological role of MUPP1 in chemosensory systems and the identification of unknown interaction partners are currently investigated.

#P183 **Poster session IV: Chemosensory transduction and perireceptor events**

Gurmarin inhibits the Sweet Receptor by Binding to the Venus Fly Trap Module of T1R3

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Gurmarin is a polypeptide of 35 amino-acids that suppresses behavioral and gustatory neural responses of rodents to sweet compounds without affecting responses to salty, sour, or bitter substances. Gurmarin has no detectable effect in human psychophysical studies. Here, we show that gurmarin acts on mouse T1R3 to antagonize the heterologously expressed mouse sweet receptor's response to a panel of sweeteners. Co-expressing the non-taster allele of mT1R3 with human T1R2 yields a functional sweet receptor that is sensitive to gurmarin, indicating that these allelic variations in mT1R3 does not affect gurmarin binding. Studies with human-mouse chimeras of T1R3 indicated that the first 150 amino acids (aa) of T1R3 must be from mouse to maintain sensitivity to gurmarin. Additional chimeras narrowed the region of importance to aa 40-80 of mT1R3. Based on the crystal structure of mGluR1, we created a homology model of the Venus Fly Trap Module of mT1R3. According to this model a 41-66 aa loop (loop1) is present within the upper lobe1 of the receptor's cleft. In our model, gurmarin docks to the receptor within the open cleft of the VFTM, directly in contact with the upper lobe. The difference in the 3D shape of the corresponding loop1 of human T1R3 suggests that steric hindrance prevents gurmarin from binding to human T1R3's VFTM cleft. We hypothesize that gurmarin may inhibit activation of the sweet receptor by preventing proper adoption of T1R3 VFTM close state. In addition, analysis of the interactions in the docked model between gurmarin and the receptor accord with previous work identifying key aromatic residues necessary for gurmarin function. Finally, point mutants altered at residues of mouse T1R3 predicted to interact with gurmarin displayed reduced sensitivity to gurmarin in in-vitro assays.

#P184 **Poster session IV: Chemosensory transduction and perireceptor events**

Effect of inosine monophosphate (IMP) on taste perception of methionine and valine by mice

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In vitro heterologous expression studies showed that most L-amino acids, including L-methionine (Met) and L-Valine (Val), activate the mouse T1R1+T1R3 receptor when they are mixed with IMP. However, Met and Val differ in their ability to activate the mouse T1R1+T1R3 receptor without IMP. While Met strongly activates this receptor, Val evokes only negligible activation (Nelson *et. al*., 2002). The goal of our study was to examine whether addition of IMP changes taste quality perception of Met and Val. We have addressed this question using a conditioned taste aversion (CTA) technique. Separate groups of C57BL/6J mice were exposed to one of four conditioned stimuli (50 mM Met, 50 mM Met + 2.5 mM IMP, 50 mM Val or 50 mM Val + 2.5 mM IMP) or to water (control) and injected with LiCl to form CTA. Conditioned mice were presented with five basic taste solutions, Met and Val, and their lick responses were recorded. An aversion to Met generalized to quinine, while an aversion to Met+IMP generalized to Met, 150 mM sucrose, a mixture of 50 mM MSG and 30 M amiloride (Ami; added to block sodium taste) with or without 2.5 mM IMP (i.e., MSG+IMP+Ami and MSG+Ami), but not quinine. An aversion to Val generalized to quinine, while an aversion to Val+IMP generalized to MSG+IMP+Ami and MSG+Ami, but not quinine. This suggests that addition of IMP changes the taste quality of Met and Val *in vivo*, which is consistent with results of *in vitro* experiments. Supported by Fisheries Research Agency (Yokohama, Japan) research grant (YM) and NIH grant DC 00882 (GKB and AAB).

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Mitigation of irradiation effects on taste epithelium in the Protein Kinase C delta null mouse

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Radiotherapy for head and neck cancer can result in taste loss; yet how radiation influences taste is unknown. One idea is that disruption of taste cell renewal may be causal. Taste cell turnover spans 10-14 days (Beidler and Smallman, 1965) driven by production of transit amplifying (TA) cells in or near taste buds from as-of-yet unidentified stem cells. We propose that taste loss after irradiation is due to apoptosis of replenishing TA cells, and consequent attrition of mature taste cells. In support of this, we find that proliferating TA cells are absent at 3 days post irradiation (dpi) including cells in S (BrdU-IR) and M (phospho-histone3 (pH3)-IR) phases, in fungiform (ffp) and circumvallate (cvp) papillae. Thus, one strategy for averting taste loss may be to reduce epithelial cell death, with the prediction that cell division would not be interrupted. Protein kinase C delta (PKCd) is a multifunctional kinase, which positively regulates apoptosis. To test if radiation effects on taste epithelium are mitigated in PKCd-/- mice, the heads of wild type (WT) and PKCd-/- adults were irradiated with a single 8Gy dose and lingual epithelia examined for BrdU- and pH3-IR at progressive dpi. As in our initial results, in ffp and cvp, BrdU-IR cells are absent at 3 dpi, increase at 5 dpi, then decrease after 9 dpi. Cells in M phase (pH3-IR) are missing at 3 and 5 dpi, reappear by 7 dpi, then decrease after 9 dpi. By contrast, the proliferative profile of irradiated PKCd-/- mice does not drop; BrdU- and pH3-IR cells are present at 3 and 5 dpi and dividing cell number is dramatically higher from 7-11 dpi compared with WT mice. Our data suggest that PKCd is required for apoptotic cell death and/or represses proliferation in irradiated taste epithelium.

#P186 **Poster session IV: Chemosensory transduction and perireceptor events**

Effect of Nicotinic Acetylcholine Receptor (nAChR) Blockers, Mecamylamine (Mec) and Dihydro-β-erthroidine (DH E) on the Chorda Tympani Responses to Nicotine in TRPM5 Knockout (KO) Mice

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The TRPM5 cation channel is an essential downstream signaling effector in taste cells for bitter, sweet and umami taste transduction. Accordingly, when the tongues of Trpm5 KO mice were stimulated with10 or 20 mM quinine (bitter stimuli) the CT responses were not different from those of the rinse. However, a robust CT response was elicited by 10 and 20 mM nicotine, also reported as bitter by humans. The magnitude of the tonic CT response to nicotine in TRPM5 KO mice was 40% smaller than the response in the wildtype (WT) control mice, suggesting that 60% of the CT response to nicotine is TRPM5-independent. To determine if the TRPM5-independent component of the CT response to nicotine is dependent upon the presence of nAChRs, we investigated the effect of the nAChR blockers Mec and DHβE (0.05 – 0.5 mM) on CT responses to nicotine in TRPM5 KO mice. Both Mec and DHβE inhibited the CT response to nicotine in a dose-dependent manner. 0.3 mM Mec and 0.4 mM DHβE inhibited the CT response to 10 mM nicotine by 40% and 58%, respectively. Using a combination of 0.3 mM Mec and 0.4 mM DHβE further inhibited the CT response by 96%. At 20 mM nicotine Mec+DHβE inhibited the CT response to 20 mM nicotine by 84%. We conclude that nAChRs composed of a3, a4, 2 and 4 may be responsible for the CT response in TRPM5 KO mice. The presence of a3, a4, 2 and 4 nAChR subunits was further confirmed by RT-PCR with cDNA made from fungiform taste buds derived from WT mice and using specific primers for the above subunits.

#P187 **Poster session IV: Chemosensory transduction and perireceptor events**

1,3-N-Acetylglucosaminyltransferase 1 (3GnT1) regulates signaling in olfactory neurons

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3GnT1 is a glycosyltransferase that is highly expressed by sensory neurons in the mouse olfactory epithelium (OE). We previously showed that 3GnT1 expression is required for proper formation of connections between subsets of neurons in the OE and their targets in the main olfactory bulb (OB). P2 neurons fail to form glomeruli in 3GnT1 null mice, while the positioning of M72 glomeruli is displaced towards the rostral/medial OB. This phenotype is remarkably similar to defects reported for mice deficient in AC3, a heavily glycosylated adenylyl cyclase isoform that determines intracellular cAMP levels and glomerular positioning in the OB. The phenotypic similarities between these mouse lines suggest that 3GnT1 influences on targeting could be mediated through direct glycosylation of AC3. We show here that AC3 of wildtype mice strongly reacts with both the anti-lactosamine monoclonal antibody 1B2 and the poly-N-acetyllactosamine binding lectin LEA. Western analysis of AC3 isolated from olfactory cilia of 3GnT1 null mice shows a dramatic reduction in size, and a loss of 1B2 and LEA reactivity. Furthermore, AC3 protein expression overall is greatly reduced in the OE and the OB of 3GnT1 null mice, suggesting that the proper glycosylation of AC3 is required for its expression and function in olfactory neurons.

#P188 **Poster session IV: Chemosensory transduction and perireceptor events**

Penetrating the Permeability Barrier that Surrounds Mouse Taste Buds

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All epithelia contain specialized junctions between cells. These junctions are formed by interacting membrane proteins and by cytoskeleton and extracellular matrix components. Proteins that make up junctions include claudins, occludins, tricellulin, and junction adhesion molecules. In taste buds, tight junctions join the apical tips of taste cells and constitute an apical barrier. Apical tight junctions provide a selective barrier that separates the mucosal surface of the tongue from the underlying basolateral spaces within the taste bud. Tight junctions are thought to limit tastant (and drug) permeability into taste buds (Michlig *et al* 2007). We have developed a simple assay for determining how well compounds permeate into mouse taste buds (Pereira *et al* ISOT 2008). Our assay uses lingual slices incubated in lipophilic or hydrophilic fluorescent dyes. Surprisingly, we discovered that the permeability barrier is not limited to apical tight junctions. Entire taste buds are surrounded by a permeability barrier. Here, our goal was to find methods to break down the barrier. We used a number of different treatments, including Ca^{2+}-free solutions, Na caprate, verapamil, and C-terminal fragments of *C. perfingens*. However, dye penetration into taste buds was most reliably achieved by briefly treating lingual slices with 75% DMSO. We tested whether exposure to such high concentrations of DMSO affected the physiology of taste bud cells. We measured Ca^{2+} responses of taste cells in lingual slice preparations (confocal Ca^{2+} imaging) before and after DMSO. DMSO did not compromise taste- and depolarization-evoked Ca^{2+} responses. Indeed, responses to bath-applied stimuli such as KCl were enhanced. These results show a quick and effective way to enhance drug penetration into taste buds.

#P189 **Poster session IV: Chemosensory transduction and perireceptor events**

Electrophysiological Responses to Tactile Stimulation of the Tongue in the Presence of Oils and Gustatory Stimuli

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Fats and oils are highly preferred by humans and rats and are necessary components of a balanced diet, yet little is known about the sensory detection of lipids. The responses from individual axons of the chorda tympani (CT) and lingual nerve (LN) were recorded during separate and concurrent stimulation of the tongue with mineral oil (MO), corn oil (CO) and 25% emulsions of each. Caseinate (1%) was the emulsifier. Gustatory responsiveness was tested with 0.3M sucrose, 0.1M NaCl, 0.01M citric acid, 0.003M QHCl, and 0.1M MSG. A motorized, rotary brush was used for somatosensory stimulation of gustatory and tactile receptive fields on the anterior tongue. We hypothesized that the lubricating properties of the oils would modify the neural responses evoked by stroking the tongue with the brush. Data collected from thermal fibers, unresponsive fibers, and those not fully tested were excluded. Based upon preliminary analysis, passive application of the oils and oil emulsions did not activate CT or LN axons. Tactile responses were reduced by 15% in the presence of either MO or CO, but not by the oil emulsions. In several instances, mechanical stimulation of the tongue caused a transient inhibition of taste-evoked responses in the CT. Similarly, in several fibers, application of sapid stimuli to the tongue altered the time course or effectiveness of tactile stimulation. More detailed analysis of the present data and, if need be, a different experimental design will be used to determine if mechanical modulation of gustatory responses is the basis for oral detection of lipid stimuli.

#P190 **Poster session IV: Chemosensory transduction and perireceptor events**

Optimization of the production of recombinant brazzein secreted by the yeast *pichia pastoris*

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Brazzein is a small (6.5 kDa), heat- and pH-stable sweet protein originating from the fruit of *Pentadiplandra brazzeana* Baillon, a plant found in West Africa. Brazzein isolated from its natural source exists in two forms differing in sweetness intensity. The major form (54 amino acids, ~80%), called pGlu-bra, contains a pyroglutamic acid (pGlu) at is N-terminus, while the minor form (53 amino acids, ~20%), called des-pGlu-bra, lacks the N-terminal pGlu. It has been reported that des-pGlu-bra is twice as sweet as pGlu-bra. Heterologous expression of brazzein in bacteria is complicated by the presence of a pyroglutamic acid (pGlu) in the major form of brazzein. Here we report the heterologous expression of brazzein using the methylotrophic yeast *Pichia pastoris* as major form under the control of the methanol-inducible alcohol oxidase (AOX1) promoter. Brazzein was secreted into the extracellular medium using the alpha-factor preprosequence peptide of Saccharomyces cerevisiae without the Glu-Ala-Glu-Ala spacer. We found that brazzein regularly accumulated in the culture medium reaching approximately 40 mg per liter of culture over an expression period of 6 days. After dialysis, the yeast culture filtrate containing recombinant brazzein was submitted to cation-exchange chromatography and two brazzein isoforms were isolated. MALDI-TOF mass spectrometry revealed that the first isoform was pGlu-bra, while the second isoform, called Gln-bra, was assigned to the same molecule with a N-terminal glutamine residue instead of the pyroglutamic residue. 1D NMR spectroscopy revealed that both brazzein isoforms were properly refolded. Moreover, both brazzein isoforms were able to stimulate hT1R2/hT1R3 taste receptor in agreement with their sweetness properties.

#P191 **Poster session IV: Chemosensory transduction and perireceptor events**

Autocrine Regulation of ATP Secretion in Mouse Taste Buds

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Receptor (Type II) cells secrete ATP and stimulate adjacent Presynaptic (Type III) cells to release serotonin (5-HT, Huang *et al*, PNAS, 2007). We have shown that 5-HT mediates negative feedback onto Receptor cells and inhibits ATP secretion (Huang & Roper, AChemS 2009). Here we examine the autocrine effects of ATP back onto Receptor cells. Single Presynaptic and Receptor cells isolated from mouse circumvallate taste buds were loaded with calcium indicator dye, Fura-2. When we bath-applied ATP onto isolated taste cells, Presynaptic and Receptor cells alike showed robust Ca^{2+} responses. ATP-evoked Ca^{2+} signals were not affected when extracellular Ca^{2+} was removed from the bath, indicating that responses were generated by intracellular Ca^{2+} release. Further, ATP-evoked Ca^{2+} responses of Receptor cells were abolished by MRS2179, a selective P2Y1 receptor antagonist. Thus, metabotropic P2Y, not ionotropic P2X purinoceptors are mainly responsible for the actions of ATP. Because Receptor cells were excited by ATP, we tested whether ATP exerts positive feedback (autocrine) during taste stimulation. We used ATP-sensitive and 5-HT-sensitive biosensor cells to detect taste-evoked transmitter release from isolated taste buds. Indeed, ATP and 5-HT secretion were significantly *decreased* in the presence of MRS2179 (10 µM). Moreover, Receptor cells isolated from P2Y1/P2Y2 double knockout mice failed to respond to ATP and ADP. These findings suggest that P2Y receptors, particularly P2Y1, exert positive autocrine feedback during taste bud signaling. Taken together with findings described in Huang & Roper (AChemS 2009), these data show that complex cell-cell interactions take place during taste stimulation. ATP exerts positive, and 5-HT exerts negative feedback onto Receptor cells.

#P192 **Poster session IV: Chemosensory transduction and perireceptor events**

Acidic substances added in the oral cavity reduce our bitter taste sensation by pH-dependent inhibition of hTAS2R response

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Some acidic peptides are known to reduce our bitter taste sensation, although the mechanism remains to be elucidated. The recent progress in taste molecular biology has revealed that G protein-coupled receptor members of the TAS2R family act to receive bitter tastants; 25 members have been identified as bitter taste receptors including hTAS2R16 that responds to β-glucopyranosides. We investigated the bitterness-masking effects of acidic dipeptides by calcium imaging analysis using HEK293T cells that transiently expressed hTAS2R16 along with G 16gust44. Salicin, a cognate bitter tasting ligand, was used in the presence or absence of acidic dipeptides, with the result that acidic dipeptides significantly reduced the receptor response to this ligand. Interestingly, a variety of acidic substances including amino, organic and inorganic acids as well inhibited the response of hTAS2R16 to salicin, while no such effect was observed with neutral peptides and amino acid, and as well as acidic amino acid salts. The inhibition took place depending on the pH values as a result of the addition of acids but not on their concentrations. We also confirmed the inhibition of hTAS2R38 response to *N*-phenylthiourea and 6-propyl-2-thiouracil at low pH. Our results suggest that the reduction of our bitter taste sensation by acidic dipeptides in particular and sour taste substances in general can be attributed to their inhibitory effects on hTASA2R molecules and also that the receptor-environmental pH in the oral cavity is a critical factor responsible for this sensory event.
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#P193 **Poster session IV: Chemosensory transduction and perireceptor events**

Unraveling the Signal Transduction Cascade Mediated by the Olfactory Receptor hOR51E2 in Prostate Cancer Cells

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Olfactory receptors (ORs) are expressed not only in the sensory neurons of the olfactory epithelium but also in various other tissues. The functions of ORs in these tissues are largely unknown. We previously reported that the human OR51E2, which is endogenously overexpressed in prostate cancer cells, can be activated by androstenone derivates as well as the odorant ionone. We could also show that exposure to ionone resulted in inhibition of cell proliferation as well as induction of apoptosis. Here, we characterized the signal transduction mechanism induced by activation of OR51E2 in LNCaP cells (prostate cancer cell line) using a combination of calcium imaging, patch clamp and biochemical techniques. Ionone stimulation leads to an increase of intracellular calcium, which at least in part depends on extracellular calcium. Electrophysiological measurements revealed an ionone dependent opening of a calcium conductance in the plasma membrane. Further biophysical and pharmacological analysis identified TRPV6 as transduction channels, a finding that was confirmed by RNAi experiments. The expression of TRPV6 in prostate cancer cells has been described before but the function as well as the activation mechanism was still elusive. Currently, we investigate how activation of the OR51E2 leads to an opening of TRPV6 channels. In summary we report the first endogenously expressed OR that couples to a signal transduction cascade different from the one used in olfactory sensory neurons. Namely we show that OR activation leads to an opening of TRPV6 channels.

#P194 **Poster session IV: Chemosensory transduction and perireceptor events**

Expression and Functionality of Oxytocin Receptor in Mouse Taste Cells

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Oxytocin (Oxt), in addition to its effects on reproduction and certain behaviors, may also influence taste and feeding. For example, $Oxt^{-/-}$ mice overconsume sweet solutions regardless of caloric content. And mice lacking the cognate receptor, i.e. $Oxtr^{-/-}$, are obese. We asked if Oxtr is expressed and functional in mouse taste buds. Using RT-PCR, we detected Oxtr mRNA in vallate, foliate, palatal and fungiform taste buds. In vallate taste buds, Oxtr mRNA is 1000-fold less abundant than β-actin mRNA. Immunocytochemistry revealed that Oxtr is expressed in cells that are neither Type II/Receptor cells nor GAD-expressing Type III/Presynaptic cells. In taste buds from Oxtr-YFP(Venus) knock-in mice also, we found that YFP-labeled (that is, Oxtr-expressing) taste cells did not overlap with PLC 2 immunofluorescence. Thus, Oxtr expression might be limited to Type I taste cells. RT-PCR on isolated single taste cells confirmed that Oxtr is expressed only in Type I/glial-like (NTPDase2-expressing) cells. Using Fura-2 Ca^{2+} imaging, we observed dose-dependent increases of intracellular $[Ca^{2+}]$ in vallate taste cells and this was attributed to release from stores. Responses were detectable at 10 nM Oxt, saturated at 1μM, and displayed an EC_{50} of ~30 nM, similar to the sensitivity of uterine smooth muscle, a known target of Oxt. The Oxtr antagonist, L-371,257, significantly decreased the Ca^{2+} response. None of the isolated cells that responded to Oxt expressed PLCβ2. In sum, our data indicate that Oxtr is expressed in a subset of taste cells that are likely Type I/glial-like, and that these cells can respond to physiological concentrations of Oxt via Oxtr. Therefore, it is possible that Oxt may exert at least some of its effects on taste and feeding at the level of the taste bud.

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Inhibition of bitter taste receptors

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In humans, bitter taste is mediated by ~25 G protein-coupled receptors of the hTAS2R family. For most TAS2Rs, several cognate agonists have been identified suggesting that the TAS2Rs possess broad ligand spectra. Specific TAS2R inhibitors would allow for pharmacological analysis of the bitter response *in vivo* and reduction of unwanted bitterness in food and medicine. By screening a chemical compound library in cell-based receptor assays we found that 4-(1,1,2-trimethyl cyclopentanoyl-) butanoic acid (GR-81-3727) concentration-dependently blocked activation of hTAS2R44 by the sulfonyl amides saccharin and acesulfame-K, as well as by several other agonists. GR-81-3727 also abolished, with distinct potencies, agonist-induced responses of cells expressing various other hTAS2Rs, including hTAS2R43, which is highly similar to hTAS2R44 and is also activated by the sulfonamides. In human sensory trials, GR-81-3727 effectively reduced the bitterness associated with ace-K and saccharin, indicating that it has physiological efficacy as a bitter taste inhibitor. I*n vitro* analyses employing chimeric receptors between hTASR44 and hTAS2R46 revealed that GR-81-3727 caused rightward shifts of concentration-response functions and likely acts as a competitive antagonist. Notably, we also discovered that GR-81-3727 acted as an agonist at one hTAS2R. In similar experiments, we also identified additional TAS2R antagonists with properties similar to those of GR-81-3727. Together, our data demonstrate that the property of GR-81-3727 to interact with multiple bitter receptors is a feature it shares with numerous TAS2R agonists. Our results also suggest unexpectedly complex interactions between chemicals released from food in the mouth during a meal with the family of TAS2Rs to elicit the bitter response.

#P196 **Poster session IV: Chemosensory transduction and perireceptor events**

Solitary chemosensory cells (SCCs) in the pancreas

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Solitary chemoreceptor cells (SCCs) are specialized cells of the gastrointestinal and respiratory tracts which detect and respond to a variety of compounds, including nutrients and irritants. For example, SCCs of the gut detect glucose by transduction mechanisms similar to those used by taste cells of the tongue to regulate secretion of insulin and hormones that affect appetite (Margolskee et al, 2007; Jang et al, 2007). In contrast, airway SCCs respond to irritants and certain bitter substances (Lin et al. 2008; Gulbransen et al 2008). These diverse SCCs express many markers typical of taste cells including the G-protein gustducin, PLC 2, TrpM5, IP3R3, and bitter (T2Rs) and sweet/umami receptors (T1Rs). The major pancreatic excretory ducts contain a large number of specialized epithelial cells, named brush cells, which express gustducin (Hofer D & Drenckhahn D, 1998). The function of pancreatic SCCs is unknown. Here we report that many of the SCCs in the pancreatic excretory ducts also express GFP driven by the promoter for choline acetyltransferase (ChAT) or by the promoter for TrpM5. Further, TrpM5GFP+ SCCs are detected in gall bladder, common bile duct and papilla of Vater which sits at the exit of the pancreatic duct into the intestine. Many of the GFP+ SCCs also express gustducin and are sparsely innervated by peptidergic (CGRP) sensory fibers. These findings suggest that SCCs may release acetylcholine upon stimulation, thereby affecting the sensory afferents and/or surrounding tissues. Thus, chemosensory control of pancreatic secretion might occur via two independent mechanisms: hormonal (from the gut) and neural/paracrine from local SCCs. The SCCs associated with the pancreatic ducts and papilla of Vater may provide chemoreceptive feedback to regulate pancreatic secretions.

#P197 **Poster session IV: Chemosensory transduction and perireceptor events**

The prenyl binding protein PrBP/δ impedes trafficking of G_{olf} in olfactory sensory neurons (OSNs)

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The ubiquitous prenyl binding protein, termed PrBP/δ, is important in processing and targeting of prenylated proteins. We recently deleted the murine *Pde6d* gene (encoding PrBP/δ), and observed that transport of GRK1 and PDE6 from the endoplasmic reticulum to the cilium of photoreceptors was disrupted and led to severe alterations in photoreceptor physiology [1]. We used *Pde6d*$^{-/-}$ mice to identify the role of PrBP/δ in olfactory sensory neurons (OSNs). We found a dramatic decrease in immunoreactivity for G_{olf} in the cilia of *Pde6d*$^{-/-}$ OSNs but normal localization of ACIII. Initial behavioral tests showed that most *Pde6d*$^{-/-}$ mice were capable of discriminating between R and S carvone however investigation time was reduced. Despite modest behavioral changes, electrical responses to odorants were significantly reduced in *Pde6d*$^{-/-}$ mice. Peak amplitudes of electro-olfactograms (EOGs) of the odorant responses to 2-heptanone, n-amyl acetate, and (-)-menthone in *Pde6d*$^{-/-}$ mice (ages 16, 20, 23, and 28 months) were only 23± 10% of EOG responses in approximately age-matched control mice (4 knock out and 4 wild type; Student's *t*-test $p<0.001$). Our studies suggest that PrBP/ plays a role in trafficking of heterotrimeric G_{olf} to the cilia of mouse OSNs and is required for normal transduction of odorant information in OSNs. Despite the significant decrease in EOG responses, *Pde6d*$^{-/-}$ mice appear to retain some odorant detection capabilities as demonstrated using a simple habituation behavioral assay. 1. Zhang et al., 2007. *PNAS* 104: 8857-8862

#P198 **Poster session IV: Chemosensory transduction and perireceptor events**

Odor-stimulated phosphoinositide signaling in mammalian olfactory receptor neurons

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Odors can modulate rat olfactory receptor neurons (ORNs) in a phosphoinositide (PI)-dependent manner (Spehr *et al.,* 2002). To support the assumption that odors indeed activate PI signaling, we created an adenoviral vector carrying two different PI activity markers, the pleckstrin homology (PH) domain of phospholipase C 1 (PLC 1) for monitoring the activity of PLC and the PH domain of the general receptor of phosphoinositides (GRP1) for monitoring the activity of phosphoinositide-3-kinase (PI3K) in the dendritic knobs of mouse ORNs *in vivo*. Two different complex odors (Henkel 100, Symrise 100) caused translocation of PI3K and PLC in 9.6% and 17.4%, respectively, of the ORNs tested. We subsequently used a phospholipid overlay assay and ELISA to measure odor stimulation of PLC and PI3K in mouse and rat olfactory ciliary membranes *in vitro*. The same odor mixtures could activate PLC and PI3K as fast as 2 sec of odor stimulation, with PLC being activated more robustly. Odor-dependent activation of PLC and PI3K could be blocked by PLC- and PI3K-specific inhibitors, respectively (U73122, Edelfosine, LY294002). These results collectively provide compelling evidence for the presence of PI signaling in the transduction compartment of mammalian ORNs and suggest that odors can activate both PLC- and PI3K-mediated signaling sufficiently fast to account for modulation of the electrophysiological output of the cells.

#P199 **Poster session IV: Chemosensory transduction and perireceptor events**

A Proteomic Screen of Mouse Cilial Membranes Reveals TMEM16B as an Olfactory Calcium-Activated Chloride Channel

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A major amplification step in olfactory signal transduction is the efflux of chloride ions in response to elevated calcium concentration in the olfactory sensory neuron (OSN) cilia. However, the molecular identity of the olfactory calcium-activated chloride channel remains elusive. To identify this channel, we isolated OSN cilial membranes and analyzed the proteins in the preparation by mass spectrometry. We identified 53 proteins by two or more peptides as being present, including all of the known signal transduction components. TMEM16B and CLIC6 were the only two proteins found to be putative chloride channels. While CLIC6 was found to originate from supporting cells, TMEM16B mRNA localized specifically to olfactory sensory neurons by in situ hybridization. Transfection of HEK-293 cells with C-terminal GFP-tagged TMEM16B showed plasma membrane localization. Expressing this construct using an adenoviral vector, TMEM16B::GFP fusion protein localized to the OSN cilia. Patch-clamp analysis of TMEM16B expressed in HEK-293 cells revealed channel properties similar to those of the native calcium-activated chloride channel recorded from OSN membrane patches. Together, we propose that TMEM16B is a major component of the olfactory calcium-activated chloride channel.

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WITHDRAWN

#P201 **Poster session IV: Chemosensory transduction and perireceptor events**

TRPM5 Expressed in Solitary Chemosensory Cells is Involved in Regulating Chemical Access to the Vomeronasal Organ

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The mouse vomeronasal organ (VNO), a peripheral sensory organ, detects semiochemicals. Complex stimuli containing semiochemicals are drawn into the lumen of the VNO, some of which may be contaminated by irritating and harmful environmental substances. Mechanisms that monitor and control chemical access to the VNO are not well understood. In the VNO, the majority of the solitary chemosensory cells (SCCs) expressing transient receptor potential channel M5 (TRPM5) is localized in the anterior vomeronasal duct. Previous research has shown that SCCs respond to stimuli known to activate the trigeminal system (Ogura et al ISOT abstract 2008). We hypothesize that TRPM5 in SCCs plays an important role in controlling chemical access to the VNO. To monitor chemical access to the VNO we exposed both wild type (WT) and TRPM5 deficient (TRPM5KO) mice to chemical stimuli mixed with a

rhodamine dye and measured the fluorescent intensity in the VNO. In WT mice the fluorescent intensity of the VNO was negatively correlated with the concentrations of the exposed irritants indicating the stronger the irritants the lesser amount drawn into the VNO. In KO mice the fluorescent intensity was significantly higher for both control and irritant stimuli indicating greater chemical access to the VNO. In addition the pH of the solution affected chemical access to the VNO, decreasing as the pH deviates from neutral. This pH dependent pattern is distorted in TRPM5KO mice. Our data strongly indicate that TRPM5 expressed in SCCs of the VNO is involved in monitoring and controlling chemical access to the VNO.

#P202 **Poster session IV: Chemosensory transduction and perireceptor events**

Transient receptor potential V1 is directly activated by nickel ions

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TRPV1 is a member of the transient receptor potential (TRP) family of cation channels. It is expressed in sensory neurons of trigeminal and dorsal root ganglions, as well as in a wide range of non neuronal tissues, including cells of the immune system. As a polymodal receptor, TRPV1 can be activated by various chemical and physical stimuli, including divalent cations in concentrations >10 mM. Searching for further activators and modulators of TRPV1, we were interested in the effect of Ni^{2+} ions ($NiSO_4$), known to induce allergic contact dermatitis. Using Ca^{2+}-imaging and whole-cell voltage-clamp recordings we observed that micromolar doses of $NiSO_4$ induced Ca^{2+} transients in cultured capsaicin-sensitive trigeminal neurons of mice. Moreover $NiSO_4$ led to an activation of recombinant rat and human TRPV1 heterologously expressed in CHO-cells, inducing significant outwardly rectifying currents. Outside out recordings revealed an increase in open probability paralleled by a decrease in single-channel conductance. Both events resulted in an increased net activity of TRPV1 which became manifest in macroscopic currents. The effect of Ni^{2+} on capsaicin-induced currents depended on the capsaicin concentration. Outward currents induced by low doses of capsaicin were sensitized by $NiSO_4$ in low concentrations, whereas currents induced by higher doses of capsaicin were inhibited. Using TRPV1-mutants with specific point mutations, we identified several positively charged amino acids localized at the channels pore region which are apparently involved in the TRPV1 activation by Ni^{2+}. Future experiments will focus on the detailed molecular mechanisms of TRPV1 activation and modulation by Ni^{2+} and the impact of TRPV1 in the development of pathophysiological changes in neuronal and non-neuronal tissues.

#P203 **Poster session IV: Chemosensory transduction and perireceptor events**

Cetylpyridinium Chloride Effects on Sodium and Potassium Taste Stimulus Sensing in Hamster

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We sought to obtain neuropharmacological evidence for the existence of a general salt-sensing pathway in taste receptor cells of the hamster anterior tongue. Previous work has suggested that salty taste in rat depends partly on detection of sodium and potassium by a variant form of the type I vanilloid receptor (VR1). We recorded integrated hamster chorda tympani nerve taste responses to sodium and potassium solutions (100 and 250 mM) in the presence and absence of VR1 agonist-antagonist ligands cetylpyridinium chloride (CPC) and SB-366791. Lingual application of CPC modestly, but significantly, inhibited chorda tympani nerve taste responses to salts of sodium and potassium in a concentration-dependent, reversible manner. While 2 and 5 mM CPC caused significant suppression of 100 and 250 mM sodium chloride (NaCl), sodium gluconate (NaGlu), and potassium responses (KCl) (ts ^{3} -.7.08, ps <0.001, N = 4-6), suppression by 200 µM CPC was significant only for CT responses to 250 mM KCl (t = -9.43, p = 0.0007, N = 5). In general, though significant, suppression of CT responses to the stimuli tested was modest and never exceeded about 30% of the response to the native stimulus. While these findings are consistent with the presence of a VR1-like receptor in hamster taste receptor cells, another more specific blocker of the VR1, SB-366791, failed to affect chorda tympani nerve taste responses to either salt at any concentration tested. We are currently exploring the effects of additional VR1 drugs at elevated stimulus temperatures to reconcile these divergent results.

#P204 **Poster session IV: Chemosensory transduction and perireceptor events**

Establishment and Optimization of Antigen Capture Polymerase Chain Reaction Utilizing the Epithelial Sodium Channel Subtype Delta Antibody

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Antigen capture – PCR (AC-PCR) is an alternative to single cell PCR (SC-PCR) for identifying genes expressed in single cells. Here we report the establishment of an AC- PCR procedure in single cells of a human fungiform taste cell culture using the epithelial sodium channel (ENAC) subtype delta. Initially, we demonstrated that some cells in human fungiform taste cell cultures exhibited increases in intracellular calcium in response to application of sodium chloride at concentrations above that in the physiological saline bath solution. We then demonstrated that ENAC delta antibody can be used to obtain an enriched population of ENAC delta positive cells from the human fungiform taste cell cultures using the sandwich immunocytochemistry technique (sICC). Double labeling immunocytochemistry with α-gustducin and phospholipase -Cb_2 (PLC-b_2) antibodies, showed surprising co- localization with ENAC delta positive cells. ENAC delta positive cells displayed both molecular and physiological features characteristic of mature taste cells, including other ENaC subunits (ENAC alpha, ENAC

beta, ENAC gamma), b-actin, PLC-b_2, and tryptophan hydroxylase II (TPH) II mRNA, which were detected by RT-PCR, indicating expression of ENAC delta in different taste cell types. Immunoprecipitation - Western blot analysis demonstrated protein-protein interaction among ENAC subtypes. Antibody capture PCR has the potential enriching cells expressing a particular protein for studies to explore the heterogeneity of cells expressing the same gene. It will also find application for studies of taste cell development, proliferation, differentiation and function in an *in vitro* preparation.

#P205 **Poster session IV: Chemosensory transduction and perireceptor events**

Direct evidence of the role of TRPM5 in bitter transduction in enteroendocrine cells

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Enteroendocrine cells (EECs) in the GI tract have been shown to express members of the bitter (T2R) taste receptor family and to release satiety hormones like CCK and GLP-1 in response to bitter stimuli. Moreover, EECs express all the signaling components identified in Type II taste receptor cells including alpha gustducin, PLC-ß2 and TRPM5. While the role of TRPM5 in bitter taste transduction is unequivocal, bitter-activated TRPM5 currents have never been recorded in native cells. In the current study, we have used the enteroendocrine cell line STC-1 to elucidate the bitter transduction pathway. To explore functional responses to bitter compounds in STC-1 cells, we have used patch clamping and ratiometric Ca^{2+} imaging. Denatonium benzoate (DB) depolarized STC-1 cells and this depolarization was significantly reduced in absence of extracellular sodium ions. DB also elicited rapid, Na^+ dependent inward currents at -100 mV. Ion substitution experiments revealed that these DB-induced currents were carried by monovalent cations. DB-induced inward currents were greatly reduced when upstream proteins like G proteins and PLC were blocked with GDP-ß-S and U73122, respectively, implying that these inward currents are activated downstream of G proteins and PLC. DB-induced inward currents were inhibited by the TRPM5 blocker, triphenylphosphine oxide (TPPO, 100 µM) and were significantly reduced when expression of TRPM5 was knocked down using RNA interference. These results are consistent with a role of TRPM5 in bitter transduction. Consistent with these electrophysiological experiments, similar results were found using fura-2 based calcium imaging to characterize the role of TRPM5 in the generation of bitter-induced changes in intracellular calcium in EECs.

#P206 **Poster session V: Chemosensory memory/ Central synaptic physiology/Neurogenesis**

ATP Promotes Proliferation of Olfactory Sensory Neuron (OSN) and Sustentacular Progenitor Cells in Adult Mouse Olfactory Epithelium (OE)

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Extracellular ATP exerts multiple neurotrophic actions in the olfactory system including the synthesis and release of growth factors and cell proliferation, but the role of ATP in cell differentiation is unknown. We hypothesized ATP could induce proliferation of OSN and sustentacular progenitor cells. Adult Swiss Webster mice were intranasally instilled with ATP (200 µM) or vehicle. After three BrdU injections (60 mg/kg ip) between 42-46 hours, tissue was collected at 2, 7 or 14 days after ATP instillation. ATP significantly increased BrdU+ cells compared to vehicle controls by 125, 49 and 46 % at 2, 7 and 14 days ($p<0.01$; n=3 each group). The ATP-induced increase in BrdU+ cells was comparable at 2, 7 and 14 days ($p>0.5$), indicating ATP produces a transient sustained increase in proliferation. We counted BrdU+ cells in the apical sustentacular cell layer, the middle OSN layer and the basal cell layer. At 2 days, 97% of total ATP-induced BrdU+ cells were in the basal layer and BrdU+ cells co-localized with the neuronal progenitor marker MASH1, but not the immature neuronal marker GAP43. At 7 days, ATP significantly increased BrdU+ cells v. control in the apical and basal layers (3.4 v. 1.4 and 47.2 v. 32.9 BrdU+ cells/mm OE, $p<0.001$), but not the OSN layer (8.6 v. 5.0 BrdU+ cells/mm OE), suggesting proliferation of a sustentacular cell progenitor. At 7 days, BrdU+ cells co-localized with GAP43 but not mature OSN marker OMP. At 14 days, ATP-induced a significant increase in BrdU+ cells v. control in the apical and OSN layers (4.5 v. 2.6 and 29.3 v. 17.8 BrdU+ cells/mm OE, $p<0.001$) but not in the basal layer (20.4 v. 16.7 BrdU+ cells/mm OE). At 14 days, BrdU+ cells co-localized with OMP. These data indicate that ATP induces proliferation of both OSN and sustentacular progenitor cells in adult mouse OE and lends support for a neurotrophic function of ATP.

#P207 **Poster session V: Chemosensory memory/ Central synaptic physiology/Neurogenesis**

Sniffing out fear: Anxiety enhances olfactory discrimination learning via aversive conditioning

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Recent data from our lab demonstrate remarkable olfactory learning in humans to the extent that initially undistinguishable odor cues become distinct after a smell is associated with classical conditioning. Such keen olfactory plasticity renders critical ecological benefits, alerting an organism to impending threat. However, extreme sensitivity to threat often accompanies overgeneralized conditioning and anxiety. Towards that end, we examined the role of anxiety in olfactory discrimination learning via conditioning. Similar to our previous paradigm, we presented two pairs of enantiomers, constituting a conditioned pair in which one enantiomer was presented with an electric shock, and a non-conditioned baseline pair. Twenty-seven college students completed a set of questionnaires at the outset of the experiment and performed a triangular test discriminating between

enantiomer counterparts before and after the conditioning phase. In support of the previous study, there was a trend of improvement in enantiomer discrimination at post- relative to pre-conditioning ($P = 0.15$). Importantly, increased enantiomer discrimination was significantly associated with anxiety ($r = 0.38$, $P = 0.05$) and perceived control of threat-relevant situations ($r = -0.42$, $P = 0.03$). That anxiety in this high-functioning nonclinical sample enhances odor discrimination learning suggests that non-clinical anxiety facilitates emotional learning via aversive conditioning, thereby enhancing perceptual discrimination. This mechanism may serve to compensate for hyper-arousal and excessive sensitivity to threat in anxiety, preserving the cognitive and social functions of these individuals. Further evidence is being collected with measures of brain event-related potentials and autonomic responses.

#P208 **Poster session V: Chemosensory memory/ Central synaptic physiology/Neurogenesis**

Expansion, Engraftment and Multi-Lineage Potency of Mouse Neonatal Olfactory Neurospheres

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The olfactory mucosa (OM) of humans and rodents exhibits ongoing neurogenesis and epithelial reconstitution following injury and may have potential for cell-based therapies. Unfortunately, conventional 2-D cultures of OM have limited potency following transplantation. The precedent set by CNS stem cell neurospheres have led us to investigate the use of a 3-D olfactory neurosphere (ONS) culture system in which OM harvested from neonates was used as a proxy for stem and progenitor activity. In our hands, ONS are comprised of multiple, marker-defined cell types as well as proliferating cells like those found in OE, confirming previous work (Barraud 2007). ONS were passaged and exposed to varying growth factors and media conditions to determine their effects on expansion and renewal. Culture of passaged ONS in NIH3T3–conditioned media increases the number of secondary spheres suggesting that expansion of ONS is driven by selected components of the culture media. To test the engraftment potential of ONS–derived cells, OM from constitutive GFP–expressing neonatal mice was dissociated, cultured for 8 DIV as ONS and infused into the nasal cavity of host animals 1 day after exposure to the olfactotoxin methyl bromide. At two weeks after transplantation, ONS-derived cells have engrafted into the host, are integrated in the regenerating olfactory epithelium and formed colonies. The donor–derived colonies are composed of multiple marker– and morphology–defined OE cell types, demonstrating that culture as ONS maintains the capacity for engraftment and the multipotency of ONS–derived cells. These data imply that the recapitulation of the rich milieu of inherent cell state, cell-to-cell communication, and growth factor influences are required to maintain transplantation potential.

#P209 **Poster session V: Chemosensory memory/ Central synaptic physiology/Neurogenesis**

Comparison of incidental and intentional learning of olfactory and visual stimuli

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Objective: To investigate if memories of incidentally and intentionally learned olfactory and visual stimuli differ with respect to modality, mode of learning, gender and age. **Methods**: One hundred and seventeen young and 114 elderdy Ss participated in the experiment which had 2 sessions on 2 separate days. Stimuli consisted of 12 odorants and 12 images of objects emanating the odorants used. Half of each set of 12 stimuli were pleasant and the other half less pleasant. Young and elderly Ss were divided into two groups who learned the visual and olfactory stimuli to be remembered either incidentally or intentionally. Each of the learning groups were split into 4 groups in which the olfactory and visual stimuli were either congruent or incongruent with respect to pleasantness. In the first task Ss evaluated pleasantness of 6 odours and 6 images. In the memory test which followed after 5 min, the stimuli evaluated for pleasantness in the first task served as targets and the other 6 odours and 6 images served as distractors. On day 2 Ss performed 3 tasks: discrimination of odours and images used in the memory test, a congruency test where each odour was presented with the two corresponding images and the task was to pair the odour with one of the images and vice versa for images and a hedonic test of all 24 stimuli. **Results and conclusions:** Overall, there is no difference in memory of incidentally and intentionally learned stimuli and there is no difference between men and women. Young Ss, however, remember the stimuli significantly better than elderly Ss ($p<0,001$). At the meeting we will present detailed results of the experiment and compare visual and olfactory memory and particularly discuss the extent to which congruency between visual and olfactory stimuli influences memory in the two modalities.

#P210 **Poster session V: Chemosensory memory/ Central synaptic physiology/Neurogenesis**

Characterizing the Relationship between Odor Memory and Identification Performance using Generalized Linear Modeling

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The decline in people's ability to recognize and recall common olfactory stimuli has been consistently linked to the development of neurodegenerative diseases. As a result, olfactory memory has been studied extensively over the last three decades with mixed results. Previous work in our laboratory has shown that facilitating odor identification by providing alternative odor labels during the inspection and subsequent testing phase of an odor memory task yielded nearly perfect memory performance among healthy adults. Conversely, providing odor labels during just one of the phases or not at all resulted in memory performance that rarely exceeded 74 %. The purpose of the current research was to develop a model of the memory performance, and the relationship between odor memory and identification, from data described above. Data from remembered ("old") and novel ("new") odors

presented in a recognition memory paradigm were analyzed independently. We used a Generalized Linear Model approach and logistic regression to predict recognition memory performance. The probability of correctly remembering an "old" odor was very strongly predicted by consistent odor labeling. Correctly categorizing "new" odors was best predicted by a combination of two predictors- accuracy of odor identification and general memory performance across the "old" odors. It is proposed that odor knowledge (represented statistically as the interaction of the individual and an odor stimulus) has a critical influence on recognition memory performance.

#P211 **Poster session V: Chemosensory memory/ Central synaptic physiology/Neurogenesis**

Embryonic Chicks Can Learn the Scent of a Putative Predator
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Research on the response of birds to the scent of predators is scarce, as are studies of avian chemosensory learning that occurs *in ovo*. We incubated domestic chicken eggs (*Gallus gallus domesticus*) in three different odor environments: (1) putative predator odor (fox urine), (2) novel odor (vanilla), or (3) no odor control. After hatching we measured the response of all chicks to the aforementioned odors via two methods. The first measured the degree to which a chick in the hand roused from sleep when exposed to odors (a simple discrimination test, Porter et al. 1999). The second involved recording the movement of pairs of chicks placed in an unfamiliar arena and exposed to different odor conditions (a test of odor familiarity; Jones et al. 2002). Three results were notable. First, birds in the hand showed strong evidence of discrimination between all scents (35 <5 7, 29.3 <78.4, P <0.0001) with predator odor causing the greatest response. Second, chicks incubated in predator odor (n = 46) showed evidence consistent with odor learning. During discrimination tests birds roused less to predator scent than those that had been incubated in vanilla (n = 35, Z = 5.30, P = 0.02). Third, chicks incubated in predator odor (n = 15 pairs) were more apt to move around an unfamiliar arena when it contained predator odor than were controls (n = 11 pairs, Z = -3.15, P =0.002), suggesting they were less fearful when the arena contained a familiar odor. Combined, our results support the notion that birds can habituate odors they experience *in ovo*, including the aversive scent of a putative predator. Our data also provide a useful side-by-side comparison of two important behavioral metrics used to measure avian responses to odor.

#P212 **Poster session V: Chemosensory memory/ Central synaptic physiology/Neurogenesis**

Role of Glucagon-Like Peptide-1 in Conditioned Taste Aversion
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Glucagon-like peptide-1 (GLP-1) is involved in satiety, glucose homeostasis, and visceral illness. Prior studies suggest that GLP-1 receptor agonists reproduce the unconditioned effects of toxins such as lithium chloride (LiCl), producing a conditioned taste aversion (CTA) when delivered to the forebrain ventricles, and that GLP-1 antagonists block LiCl induced CTA. Interestingly, GLP-1 receptor agonists delivered to the hindbrain do not produce a CTA, though lesions of hindbrain nuclei containing GLP-1 receptors such as the area postrema (AP) abolish CTA responses to LiCl. To further explore this issue, rats in separate experiments were injected with GLP-1 receptor agonist exendin-4 (EX4; 1 ug) in either the lateral (LV) or the fourth (4V) ventricle and were tested for the formation of a CTA. The GLP-1 receptor antagonist des-His(1),Glu(9) exendin-4 (dHGex-4; 10 ug) was also injected into the LV and 4V in rats injected either with i.p. vehicle, LiCl, or lipopolysaccharide (LPS). Consistent with prior studies, LV injections of EX4 produced a robust CTA, while 4V injections did not. However, EX4 produced stronger unconditioned malaise than seen after LiCl injection. Antagonist infusions to either LV or 4V failed to block the formation of a CTA to LiCl, however LV but not 4V infusions of dHGex-4 partially blocked a mild CTA induced by LPS. Additional doses of DHGex-4 are being tested. Preliminary data also suggest that AP lesions do not abolish LV EX4-induced CTA. Overall, the results suggest that the unconditioned effects of GLP-1 are mediated at least in part by neural mechanisms different than those stimulated by LiCl. The results also appear to discount a role for GLP-1 in hindbrain viscerosensory systems that process gastrointestinal distress.

#P213 **Poster session V: Chemosensory memory/ Central synaptic physiology/Neurogenesis**

Altered Gene Expression In Brainstem And Forebrain Nuclei Following Acquisition Of A Learned Taste Aversion
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A learned taste aversion (CTA) alters taste palatability and is manifest behaviorally by the avoidance of a previously accepted gustatory stimulus following association with visceral malaise. Although the central pathways involved in such learning and its dependence on immediate early gene transcription are reasonably well understood, the modulation of downstream gene expression is not. The present study used oligonucleotide microarrays to identify altered gene expression in the parabrachial nucleus (PBN), central and basolateral nuclei of the amygdala (CeA and BLA) and lateral hypothalamus (LH) following CTA. One set of animals had two pairings of sucrose intake and intraperitoneal saline injection (control group), while in another set sucrose intake was paired with LiCl injection (conditioned group). Immediately following a single-bottle intake test with sucrose two days after the second CS-US pairing, the total RNA from each

brain region was isolated. Then, Cy3 labeled cRNA was hybridized to whole rat genome chips. Out of 28,142 genes present in each brain region, 251 (PBN), 113 (CeA/BLA), and 103 (LH) showed up or down regulation of at least 2 fold and a p value ≤ 0.05. When considering only p values ≤ 0.001, the number of differentially expressed genes in PBN, amygdala, and LH decreased to 37, 27, and 22, respectively. Relative to control animals, CTA acquisition altered the expression of various peptides, transcription factors, phosphatases, kinases, receptors and ion channels. Directional change in expression of subsets of genes was confirmed using quantitative real-time RT-PCR. The present data provide a starting point for understanding gene expression specific to gustatory and aversive visceral stimulation, as well as learning dependent modulation of gustatory palatability.

#P214 **Poster session V: Chemosensory memory/ Central synaptic physiology/Neurogenesis**

Expression of Transient Receptor Potential (TRP) Channels in the Mouse Main Olfactory Bulb (MOB)

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TRP channels are a large family of cation channels with wide distribution in the CNS. TRP channels are involved in sensory processing in the visual, gustatory, olfactory, auditory and somatosensory pathways. Currently, 28 TRP gene subtypes have been identified and subdivided into 6 families (TRPC1-7, TRPV1-6, TRPM1-8, TRPP2-3, 5, TRPML1-3 and TRPA2). The MOB has been reported to express several TRP channel subtypes. However, the full expression profile of the TRP channel family in the MOB has not been investigated, and very little is known about TRP channel expression in different MOB cell types. In present study, we employed MOB tissue and single-cell RT-PCR methods to investigate the expression of 28 TRP channel mRNAs in the mouse MOB. Our results showed that TRPC1-7, TRPV2, 4, TRPM2-4, 6-8, TRPP2-3, 5 TRPML1-2 and TRPA2 mRNA were expressed in the MOB. To date, TRPC4-5 were detected in electrophysiologically identified external cells and periglomerular cells whereas TRPC3, TRPC5, TRPV4 and TRPM2-3, 7-8 were detected in electrophysiologically identified mitral cells.

#P215 **Poster session V: Chemosensory memory/ Central synaptic physiology/Neurogenesis**

Cholinergic modulation of glomerular circuits

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The olfactory bulb receives cholinergic (ACh) afferents from the basal forebrain. These inputs are activated in a behavioral state-dependent manner. There are open questions about the impact of ACh inputs, particularly at the level of glomerular circuits. ON synapses activate external tufted (ET) cells, which strongly excite GABAergic periglomerular (PG) cells. PG cells provide presynaptic inhibition of ON terminals, postsynaptic inhibition to MT cells as well as feedback inhibition to ET cells. Thus intraglomerular circuits generate inhibition at several points to shape the transfer of sensory signals to output neurons. With $GABA_A$ and $GABA_B$ Rs blocked, either nicotine (N) or a muscarinic (M) receptor agonist, milameline, significantly reduced spontaneous EPSCs in PG but not ET cells, indicating that ACh suppresses ET cell excitation of PG cells via both N and M ACh receptors. With fast glutamate Rs blocked, carbamylcholine, which activates both N and M receptors, significantly enhanced spontaneous IPSCs in both ET and PG cells. Scopolamine blocked and milameline replicated these effects indicating that activation of M receptors increases intraglomerular release of GABA. ACh appears to reduce ET cell excitatory drive on PG cells, yet increases PG cell release of GABA. We are investigating whether ACh increases PG cell GABA release by action potential-dependent/-independent mechanisms. Increased intraglomerular GABA release may presynaptically inhibit ON inputs via $GABA_B$ receptors and postsynaptically inhibit MT output firing via $GABA_A$ receptors. Behavioral state-dependent activation of cholinergic inputs might contribute to increased contrast among glomeruli differentially activated by sensory signals.

#P216 **Poster session V: Chemosensory memory/ Central synaptic physiology/Neurogenesis**

Synchronization of spike activity in tufted cells of mouse olfactory bulb

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Synchronization of principal cell spiking in the olfactory bulb is proposed to play a role in odor coding. Synchrony occurs in mitral cells linked to the same glomerulus, but has not been analyzed in tufted cells. We recorded spontaneous spiking from mitral and tufted cell pairs in mouse olfactory bulb slices and performed cross correlation analyses on spike trains. Synchronous bursting and spiking were manifested as broad and narrow peaks in spike cross-correlograms from mitral-mitral, mitral-tufted and tufted-tufted pairs linked to the same glomerulus, but not to different glomeruli. Slow EPSCs were synchronized in mitral-tufted and tufted-tufted pairs linked to the same glomerulus. Spike synchrony was uncorrelated with burst synchrony, suggesting independent synchronization mechanisms. On average, spike synchrony was stronger and spike lags shorter in tufted-tufted, than in mitral-tufted or mitral-mitral pairs. All pair combinations exhibited electrical coupling, with largest mean coupling coefficient in tufted-tufted pairs. Spike synchrony was not fully blocked by the AMPA receptor antagonist NBQX for any pair combination, and was abolished by NBQX plus NMDA receptor antagonist dichlorokynurenic acid only in mitral-mitral pairs. Our results suggest that glomerulus-specific spike synchrony in olfactory bulb principal cells depends primarily on gap junctions, and may be enhanced by mutual excitation via ionotropic glutamate receptors. Glomeruli may split sensory information into parallel data streams conveyed by mitral and tufted cells, encoding different aspects of odor stimuli. Spike synchronization provides a substrate for temporal binding of olfactory receptor identity for these parallel streams, even if they are received and decoded by distinct cortical circuits.

#P217 **Poster session V: Chemosensory memory/ Central synaptic physiology/Neurogenesis**

Adult neurogenesis in the mouse accessory olfactory bulb
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The olfactory bulb is one of the few regions in the brain that exhibits adult neurogenesis. In adult mice newly born cells originate in the subventricular zone and migrate through the rostral migratory stream to the olfactory bulb. These cells mature into inhibitory neurons, the granule and periglomerular cells, and are functionally integrated into the existing neuronal network. Adult neurogenesis has been extensively characterized in the main olfactory bulb (MOB) and it has been shown to vary under different physiological conditions (i.e. mating, exposure to the conspecifics odors and odor-enriched environments among others). However, little is known about the role and extent of adult neurogenesis in the accessory olfactory bulb (AOB), a region of the bulb involved in the processing of pheromonal information. Here, we determined the extent of adult neurogenesis in the mice AOB using immunohistochemical techniques and BrdU to label newly born cells. We found abundant labeled cells in the granule cell layer (GCL) of the AOB (4,191±564 and 2,759±573 $BrdU^+$cells/mm^3, in male and female mice respectively) with no significant differences between genders. The majority of the $BrdU^+$ cells corresponded to neurons (93%), as determined by double labeling with NeuN, a marker of mature neurons. Interestingly, the number of $BrdU^+$cells in the GCL of the AOB was significantly lower compared to the number of $BrdU^+$cells in the GCL of the MOB (10,390±105 and 9,274 ±1,084 $BrdU^+$cells/mm^3; males and females respectively; $p<0.01$). These results indicate that a considerable number of granule cells are born in the adult AOB, although this number might be lower than the number of newly generated granule cells in the MOB.

#P218 **Poster session V: Chemosensory memory/ Central synaptic physiology/Neurogenesis**

Gap junction coupling and granule cell connectivity both contribute to long-range synchrony in the olfactory bulb
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Recent studies have shown that a major driving force for synchronizing olfactory bulb mitral cells are GABAergic inputs from granule cells. The question of how granule cells synchronize mitral cells, however, has not been fully resolved, especially with respect to how synchrony might vary with spatial separation of the mitral cells. Physiological studies have shown that distal inhibitory inputs from granule cells onto mitral cells do not affect the mitral cells' firing, which should limit how far apart different mitral cells can be to synchronize. Furthermore, the probability of two mitral cells sharing some number of granule cells is quite low, especially as the mitral cells diverge spatially. We were therefore interested in quantifying the degree of synchrony in biologically realistic networks and describing the underlying mechanisms that could induce synchrony. Through computational modeling, we quantified synchrony between two glomeruli with respect to their distance while varying connectivity of granule cells along the mitral cell lateral dendrites and, also, while varying gap junction connectivity of mitral cells sharing a glomerulus. We show that only when incorporating intraglomerular gap junction coupling, interglomerular synchrony could be attained with biologically plausible synaptic weights and sparse connectivity. Furthermore, we show that synchrony falls off with respect to interglomerular distance, mainly because of a reduction in the number of granule cells they share. However, when the number of shared granule cells did not change with respect to distance, resolvable synchrony could be obtained between glomeruli separated by the full length of the mitral cell lateral dendrites.

#P219 **Poster session V: Chemosensory memory/ Central synaptic physiology/Neurogenesis**

Synaptic markers show heterogeneous mitral and tufted cell synapse distributions
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The mammalian olfactory bulb (OB) processes chemosensory stimuli through modular units, the glomeruli. Previous viral transsynaptic tracing results suggested that the modules extend to the granule cell layer and form columnar units. Further, the columnar patterns in the granule cell layer from focal injection in the glomerular layer were widely distributed in the ispilateral half of the OB. This finding led to the interpretation that Mitral and Tufted (M/T) cell lateral dendrites are only connected to specific columns of granule cells with gaps in between. Alternatively, virus transfer across synapses could be activity dependent, and therefore pass relatively inactive synapses without labeling the connected granule cells. In order to test these alternative anatomical configurations at the level of the individual cell, we use a post-synaptic density scaffold protein fused to GFP (PSD95-GFP) as a marker of synapse density. In these studies, the plasmid bearing PSD95-GFP was injected into the external plexiform layer of the OB in 12 week old C57BL6 mice and delivered to M/T cells through in vivo electroporation. Punctate PSD95-GFP fluorescence was clear following a 24 hour incubation period, indicating the synapses were labeled and the lateral dendrite synapse distribution could then be assayed. Preliminary results indicate a heterogeneous distribution of synapses along the lateral dendrites of M/T cells.

#P220 **Poster session V: Chemosensory memory/ Central synaptic physiology/Neurogenesis**

Neuronal Survival and Replacement in the Neuron-depleted Olfactory System
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Various models of injury to the olfactory system have been used to study neuronal replacement in the adult rodent. The purpose of the present study was to measure the life-span of olfactory sensory neurons (OSNs) born after removal of postsynaptic target neurons with N-methyl-D-aspartic acid (NMDA). We also examined the proliferation, migration and survival of subventricular zone (SVZ) cells in the same subjects after bulb

neuron depletion. Adult mice were given 5-bromodeoxyuridine (BrdU) 3 weeks after unilateral bulbar injection of NMDA. They were killed 4 days, 2 wks, and 5 wks later. Similar to the effects of bulbectomy, we found increased numbers of BrdU(+) cells at 4 days in the epithelium on the treated side (112 vs 52 cells/mm contralaterally). Surprisingly, there were also more BrdU (+) survivors on the treated side at 2 wks (32 vs. 24 cells/mm). By 5 wks, numbers were similar to control counts, and surviving mature neurons persisted in the deprived epithelium as seen with double-labeling for olfactory marker protein. Within the forebrain, bulb damage caused increased proliferation of SVZ progenitors, and these continued to migrate to the bulb in the rostral migratory stream (RMS). By 2 wks, most BrdU (+) cells on the normal side had reached the bulb, while on the lesioned side, most BrdU (+) cells were still contained within the RMS. Increased proliferation was accompanied by an increase in RMS volume on the treated side (39.9 vs 19.1 $mm^{3 \times 10^{-3}}$), and an increase in TUNEL labeling. Labeling with neuronal markers showed that new neurons were added to the damaged bulb. These results indicate that some OSNs are capable of long-term survival after depletion of targets. Moreover, bulb injury significantly alters the proliferation and migration of SVZ/RMS precursors.

#P221 **Poster session V: Chemosensory memory/ Central synaptic physiology/Neurogenesis**

Glomerular Regulation of Mitral Cell Responses to Sensory Input

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Olfactory signals are initially processed in glomeruli, where olfactory nerve (ON) axons form excitatory synapses onto principal output neurons, mitral/tufted (M/T) cells. M/T cells are thought to be regulated mainly by inhibition at their lateral dendrites from GABAergic granule cells (GC) but less is known about inhibition occurring at their glomerular tuft from GABAergic periglomerular (PG) cells. We examined the relative contributions of glomerular and infraglomerular inhibition of mitral cells in bulb slices. ON stimulation produces an initial monosynaptic EPSC in mitral cells that is interrupted by a short latency burst of IPSCs followed by a protracted train of intermittent IPSCs. Addition of APV, which has been shown to block the M/T-GC feedback circuit, significantly reduced late IPSCs suggesting that they derive from GCs, but had little effect on the early IPSC barrage. In contrast, microinjection of gabazine (GBZ) into glomeruli blocked the early burst of IPSCs but had little effect on the late IPSCs. How does this affect mitral cell output? Mitral cells respond to ON input with a long lasting depolarization (LLD) upon which rides an initial short latency spike burst followed by sparse later spikes. Removal of M/T-GC inhibition with APV had little effect on M/T spike responses to ON stimulation. However, microinjection of GBZ to block glomerular inhibition increased M/T spike output ~30-fold. Glomerular inhibition also dramatically regulated the magnitude and duration of mitral cell LLDs. These data suggest glomerular inhibition is a much more potent regulator of mitral cell responses to sensory input that previously considered.

#P222 **Poster session V: Chemosensory memory/ Central synaptic physiology/Neurogenesis**

Characteristics of spontaneous and evoked EPSCs of interneurons in the superficial external plexiform layer of olfactory bulb

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Interneurons in several olfactory bulb layers are excited by mitral/tufted cells and provide feedback/lateral inhibition that shapes M/T cell spontaneous spiking/bursting and responses to olfactory input. At some M/T cell-interneuron synapses, AMPA receptors mediate fast interneuron excitation that results in fast M/T cell inhibition. At other synapses, NMDA receptors mediate slower interneuron excitation that results in slower, prolonged M/T cell inhibition. Here, we characterized spontaneous EPSCs of interneurons in the superficial EPL of mouse olfactory bulb slices and responses of cells in this region to glomerular-layer stimulation. Most superficial interneurons (26/27) spontaneously generated EPSC clusters. EPSC frequency within clusters (112.2 ± 9.4.0 Hz) was significantly higher than before clusters (37.5 ± 3.8 Hz, $P < 0.01$). The NMDA receptor blocker, APV, reduced cluster EPSC frequency by ~half. The AMPA receptor blockers, CNQX/NBQX, had more dramatic effects, however, eliminating clusters (4/4 cells) and evoked responses (8/10 cells). The evoked responses had ~2 ms longer latency than pair-recorded tufted cell responses. Spontaneous EPSCs and evoked responses were blocked by TTX. Thus, spontaneous activity of superficial EPL interneurons is distinguished by EPSC clusters, and these interneurons could presumably provide both fast and prolonged feedback/lateral inhibition in response to M/T cell spiking/bursting. Supported by NIH (DC007876).

#P223 **Poster session V: Chemosensory memory/ Central synaptic physiology/Neurogenesis**

1 and 2 Noradrenergic Receptor Exert Opposing Effects on the Excitability of Rat Main Olfactory Bulb (MOB) Granule Cells

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The mammalian MOB receives a dense noradrenergic innervation from the pontine nucleus locus coeruleus. This noradrenergic input plays important roles in neonatal odor preference learning and odor discrimination in mature animals (Doucette et al., 2007; Mandairon et al., 2008). These effects are due, at least in part, to noradrenergic modulation of GABAergic inhibition of mitral cells. In the present study we investigated noradrenergic modulation of GABAergic granule cell excitability using electrophysiological approaches in rat MOB slices. NE and the a1 receptor agonist phenylephrine depolarized granule cells; the depolarization was associated with increased membrane resistance. Phenylephrine increased spontaneous and intracellular current pulse-evoked spiking. In many cases, subthreshold membrane potential responses to current injections were converted to suprathreshold spiking responses after phenylephrine application. Thus, a1 receptor activation may increase granule cell-mediated lateral inhibition. In voltage clamp recordings, phenylephrine induced an inward current that appeared to be mediated by inhibition of potassium channels. By contrast, the a2 receptor agonist clonidine hyperpolarized and

reduced granule cell spiking. These results indicate that a1 and a2 receptor activation exert opposing effects on granule cell excitability. a1 and a2 receptor subtypes have differing affinities for NE. Consequently, granule cell-mediated inhibition may be bi-directionally modulated as a function of extracellular NE levels, which in turn, is dependent on behavioral state-dependent variations in locus coeruleus neuronal firing rates.

#P224 **Poster session V: Chemosensory memory/ Central synaptic physiology/Neurogenesis**

Taurine deficiency causes loss of mitral cells in the olfactory bulb of mice

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Aim: Taurine is, after glutamate, the most abundant free amino acid in the cerebral cortex and in the olfactory bulb (OB). Taurine was found to play an important role in regulating the depolarization-evoked GABA release via GABA receptors. Furthermore, taurine is involved in cell volume homeostasis, antioxidant defense and protein stabilization. Previous studies on taurine transporter knockout mice *(taut -/-)* showed degeneration of retina, skeletal muscle and olfactory epithelium. The aim of this study was to investigate cellular reactions due to taurine deficiency of more central olfactory components, such as mitral cells, which constitute the major output neurons of the OB. Methods: The present study assesses quantitative differences between *taut* -/- mice and controls (on postnatal day 21, 42, 70) concerning the size of the OB as well as numbers and circumference of glomeruli and mitral cells. For histochemical identification of mitral cells in tissue sections we used an antibody against PGP 9.5. *Results and Conclusions: Taut* -/- mice had significantly smaller OBs than controls. Furthermore, the average cell circumference of mitral cells is higher in control mice of every age. After 21d, *taut*-/- animals showed more mitral cells, but later they presented significantly less mitral cells than controls. Also, the OB size of *taut* -/- mice were significantly smaller in *taut* -/- mice. The results suggest that taurine plays an important role in development and maintenance of neurons in the olfactory pathway, especially during embryogenesis. Further, loss of olfactory receptor neurons may lead to a subsequent loss of the secondary relay neurons, namely mitral cells.

#P225 **Poster session V: Chemosensory memory/ Central synaptic physiology/Neurogenesis**

Adult and developmental expression of a GABA transporter by a subset of centrally derived glial cells in the antennal lobe of the moth

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A subset of glial cells in the olfactory (antennal) lobe (AL) of the moth expresses a high-affinity membrane GABA transporter (MsGAT) throughout their extent. Early in development of the AL, GABAergic dendrites extend into the shell of glial cells that surrounds the neuropil and that includes the MsGAT-positive cells. In the adult, GABAergic dendrites form a dense meshwork of processes within the glia-surrounded glomerular neuropil. The juxtaposition of GABAergic dendrites and transporter-expressing glia suggests that the transporter may be important in modulating the GABA levels to which neurons and glia cells are exposed in both developing and adult systems. Using immunocytochemistry, we have shown that the transporter is expressed from the beginning of metamorphic development in certain glia but not in developing neurons. We also have shown that (1) MsGAT continues to be expressed in the adult, in a subset of glia that have the morphological appearance of a cell type we have called "complex" glia, (2) MsGAT is not found in adult AL neurons, and (3) GABA is not detectable in MsGAT-positive glial cells under resting conditions. GABA is, however, detectable in most glial cells after brief incubation in 10-50 uM GABA; the intensity of GABA labeling in the dendrites of GABAergic neurons is greatly enhanced under the same conditions. These data suggest that the kinetics of transporter-mediated GABA uptake into glial cells may be relatively slow or that the transporter has a non-transporter function *in vivo* despite its similarity to rat and human GAT-1 in sequence and in biochemical and pharmacological profile when expressed in *Xenopus* oocytes (Mbungu et al., 1995). The data also suggest that a second, as yet unidentified, form of GABA transporter may be present on *Manduca* neurons.

#P226 **Poster session V: Chemosensory memory/ Central synaptic physiology/Neurogenesis**

Heterogeneous Expression of Pannexin 1 and Pannexin 2 in the Olfactory Epithelium and Olfactory Bulb

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Gap junctions regulate a variety of functions by directly connecting two cells through intercellular channels. Gap junctions are formed by connexins or pannexin gene families. Connexins and Pannexins may form independent gap junction channels in the same tissues. Here, we report expression patterns of pannexin 1 (Px1) and pannexin 2 (Px2) in the olfactory system of adult mice. *In situ* hybridization revealed that mRNAs for Px1 and Px2 were expressed in the olfactory epithelium and olfactory bulb. Cells expressing Px1 and Px2 were distributed in the main olfactory bulb and the accessory olfactory bulb. Although expressed in spatial patterns, many mitral cells, tufted cells, periglomerular cells and granule cells were Px1 and Px2 positive. Expression of Px1 was weak in portions of the dorsal-lateral olfactory bulb, while the medial regions had relatively high expression. In contrast, expression of Px2 was stronger in the dorsal and lateral regions than medial regions of the olfactory bulb. There were more Px2 mRNA positive mitral cells and granule cells compared to those of Px1. Expression of Px1 and Px2 was mainly found in cell bodies below the supporting cell layer in the olfactory epithelium although there might be Px2 positive supporting cells in few areas. A majority of the olfactory epithelium expressed Px1 and Px2 while degrees of expression varied among neighboring cells. In summary, Px1 and Px2 are spatially expressed in neurons in the olfactory epithelium and olfactory bulb. Our findings of expression of pannexins in the olfactory system of adult mice raise the novel possibility that pannexins play a role in information processing in the olfactory sensation. Demonstration of expression patterns of Px1 and Px2 in the olfactory system provides anatomical basis for future functional studies.

#P227 **Poster session V: Chemosensory memory/ Central synaptic physiology/Neurogenesis**

Chloride imaging in trigeminal sensory neurons of mice

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The trigeminal system has a warning function that protects the body from potential noxious stimuli. Receptors located on free trigeminal nerve endings detect different environmental stimuli like temperature, touch and chemicals. Recent work showed the involvement of ligand-gated cation channels in the detection of chemical stimuli. Until now, a potential role of ligand-gated chloride channels in trigeminal chemodetection has not been investigated. In contrast to central neurons, in which the expression of chloride transporters is changed postnatally, trigeminal ganglion (TG) neurons keep high levels of the $Na^+K^+2Cl^-$ cotransporter NKCC1 and thereby probably accumulate chloride intracellularly. Thus, opening of a chloride conductance would lead to a chloride efflux and thereby depolarizing the neuron. Indeed, current work in our lab shows Ca^{2+} transients upon stimulation with γ-aminobutyric acid (GABA) in TG neurons of mice in Ca^{2+} imaging experiments. Here, we use the chloride imaging technique to investigate changes of intracellular chloride concentration upon GABA application on dissociated TG neurons. We show that GABA stimulation leads to a chloride efflux in soma and neurites. Creation of a higher Cl^- gradient across the cell membrane by removal of extracellular Cl^- enhances the GABA-induced Cl^- efflux. In further experiments we will characterize this Cl^- efflux using pharmacological tools. Our data show that opening of a chloride conductance by GABA leads to an efflux of Cl^- and thus to a depolarization of trigeminal sensory neurons. Neuronal excitation by activation of GABA receptors makes them potential targets for the trigeminal perception of toxic chemicals.

#P228 **Poster session V: Chemosensory memory/ Central synaptic physiology/Neurogenesis**

Odor discrimination by mice with long-term unilateral naris occlusion and contralateral bulbectomy

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Unilateral naris occlusion (UNO) has been the most common method of effecting stimulus deprivation in studies of olfactory plasticity. However, despite the large corpus on this manipulation, dating back to the 19th century, there is almost nothing known about the behavioral capabilities of animals raised with UNO. Here we report the results of olfactory habituation studies on two groups (n = 38) of control and perinatally UNO adult mice before and after unilateral bulbectomy (bulb-x). Control and UNO mice formed two groups termed young (1-3 months) and old (>6 months). For UNO mice, the bulb-x was opposite the side of occlusion. Olfactory discrimination was tested using a two-odor habituation paradigm in which investigation times to six consecutive presentation of one odor were compared to that for a novel odor presented in a seventh trial. The odors were 0.1% v/v ethyl butyrate or isoamyl acetate in mineral oil or mixtures of these two solutions. If mice showed habituation-dishabituation to the pure odors they were tested with decreasing dilutions of test odor mixed in the habituation odor (1:10, 1:50, 1:250). For untreated mice neither age nor bulb-x significantly influenced the ability to discriminate between the habituating odor and the test odor down to a dilution mixture of 1:50. Also, discrimination was unaffected by UNO prior to bulb-x. Surprisingly, after bulb-x, young and old UNO mice were still able to discriminate the habituation and test odor down to a mixture of 1:50. Young bulb-x mice in the control and UNO group failed to discriminate the habituation and test odor at a dilution of 1:250. These counterintuitive results suggest that UNO is neither an absolute method of deprivation nor does its diminish odor discrimination.

#P229 **Poster session V: Chemosensory memory/ Central synaptic physiology/Neurogenesis**

Characterization of GABA-Induced Responses of Trigeminal Sensory Neurons

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In the adult central nervous system opening of chloride conductances leads to neuronal inhibition. Different from central neurons, some populations of peripheral neurons namely olfactory receptor neurons (OSNs) and neurons of the dorsal root ganglia (DRG) maintain high levels of the $Na^+K^+2Cl^-$-cotransporter (NKCC1) at adulthood. NKCC1 accumulates chloride in OSNs and DRG neurons which results in cellular depolarization upon opening of chloride channels. As a consequence, DRG neurons are excited by the inhibitory neurotransmitter γ-aminobutyric acid (GABA). Although proposed, there is no experimental evidence showing that the same logic applies to neurons of the trigeminal ganglia (TG) to date. Here, we examine the effect of GABA on primary sensory neurons isolated from murine trigeminal ganglia. In whole-cell patch-clamp experiments GABA elicited responses in all TGNs in a dose dependent manner. Furthermore, GABA stimulation lead to a quick and robust increase of intracellular calcium in TG neurons observable in calcium-imaging measurements. GABA-induced excitation could be seen in neurons of different developmental stages and was independent of culturing conditions. Preincubation with the NKCC1 blocker bumetanide inhibited the calcium rise in TG neurons from early postnatal as well as adult animals suggesting that a high intracellular Cl^- concentration is essential for the response. Pharmacological characterizations showed that the responses are mediated by $GABA_A$ receptors and involve an influx of extracellular calcium via voltage gated calcium channels (VGCCs). In summary, we suggest intracellular Cl^- accumulation in TG neurons produced by NKCC1 leading to a depolarizing efflux of chloride upon $GABA_A$ receptor opening which in turn is followed by an influx of extracellular Ca^{2+} through VGCCs.

#P230 **Poster session V: Chemosensory memory/ Central synaptic physiology/Neurogenesis**

Properties of rostral nucleus of the solitary tract (rNST) GABAergic interneurons

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Inhibition has been shown to play a significant role in rNST sensory processing mediated by GABAergic interneurons. However, the properties of these inhibitory interneurons have not been systematically investigated. We have used transgenic mice in which enhanced green fluorescent protein (EGFP) expression is linked to glutamic acid decarboxylase expression (GAD^+) to identify and characterize the rNST GABAergic interneurons. In coronal brainstem sections GABAergic interneurons were localized to the ventral subdivision of rNST. The GAD-EGFP neurons also immunoreact with GABA and NeuN antibodies and different subpopulations of these neurons also immunostain with somastatin and parvalbumin. In whole cell brain slice recordings GAD-EGFP neurons could be grouped based on their firing and morphologic properties. In response to a depolarizing current pulse 71% of the GAD-EGFP neurons responded with a short initial burst of action potentials, 13% responded with a stutter firing pattern and 16% responded with a tonic firing pattern. The burst firing neurons had a small round soma area. Stutter firing neurons had larger soma areas and round cell bodies and tonic firing neurons had large fusiform or multipolar somas. In conclusion, the rNST GAD-EGFP interneurons differ in peptide expression and have heterogeneous physiological and morphological properties. These differences may indicate that these inhibitory interneurons may have different roles in rNST sensory processing of information derived from taste receptors in the oral cavity.

#P231 **Poster session V: Chemosensory memory/ Central synaptic physiology/Neurogenesis**

Sox2 Regulation of Neurogenesis in the Adult Olfactory Epithelium

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Sox2 is expressed in multiple cell types in the adult olfactory epithelium (OE), including horizontal basal cells, globose basal cells and sustentacular cells. During lesion-induced regeneration of the OE, residual activated stem and progenitor cells express Sox2 robustly. The absence of Sox2 from neurons suggests that it may function to suppress neuronal differentiation in both the normal and regenerative settings. To test this we used a replication-incompetent retrovirus to drive expression of *Sox2-IRES-eGFP* in the regenerating mouse OE. The composition of the clones derived from the infected progenitors was characterized using cell type-specific antibodies. *Sox2*-infected clones have a greater number of cells on average compared with the control vector (*eGFP*-only). Surprisingly, *Sox2*-infected clones did contain numerous neurons as shown by co-expression of GFP with Tuj1 and/or PGP9.5. Thus, *Sox2* seems to be incapable of blocking the differentiation of olfactory neurons on its own. However, immunoreactive Sox2 cannot be detected in a considerable percentage of GFP-labeled neurons, despite enhanced levels of Sox2 expression by infected sustentacular and basal cells. Moreover, those neurons in which Sox2 expression is detectible by immunostaining were more lightly labeled than other infected cells. This constellation of findings suggests that Sox2 is being degraded in neurons infected with the *Sox2* vector. Of note, no clone-derived, *Sox2*-expressing neurons co-express Pax6, a known binding partner, indicating that Sox2 may need a co-factor to suppress neuronal differentiation in the OE. In summary, selective over-expression of *Sox2* is not able to suppress olfactory neuronal differentiation by itself, which may reflect a need to partner with Pax6, as in other tissues, to accomplish any inhibition.

#P232 **Poster session V: Chemosensory memory/ Central synaptic physiology/Neurogenesis**

PACAP Enhances Cell Survival in Cultured Slices of Mouse Olfactory Bulb

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Our lab has previously demonstrated the importance of the neuropeptide PACAP in protection of neurons of the olfactory epithelium against both axotomy-induced and cytokine-induced apoptosis. Expression of PACAP and its receptor PAC1 is widespread in the olfactory bulb (OB). In this study, we therefore examined whether PACAP enhances cell survival in the OB. We cut 300 micron thick live slices of the OB on a vibratome. Slices were immediately transferred into tissue culture media (DMEM/F12, 0.5% BSA) containing PACAP at 4 nM or 40 nM, or vehicle alone. Slices were cultured in a tissue culture incubator at 37°C for 21 hours, and then treated with 0.5% propidium iodide to label dying cells. Slices were then fixed and all cells labeled with sytox-green. Slices were imaged on a Zeiss LSM500 confocal microscope, PI-labeled cells and total cells were counted, and the percent of dying /total cells calculated. After 21 hrs in culture, the percentage of dying cells in the 4 nM PACAP group was similar to control: 90 ± 4% that of control. The 40 nM PACAP group however showed significantly lower percentages of dying cells than both control and 4 nM PACAP (One Way ANOVA, $p<0.01$, $N = 3$). Adding 40 nM PACAP to the cultures decreased the percentage of dying cells to 42 ± 4% of control. These data show that PACAP functions to enhance cell survival in cultured OB slices. Further studies will be conducted to identify the mechanism by which PACAP functions in its protection of cells of the OB.

#P233 **Poster session V: Chemosensory memory/ Central synaptic physiology/Neurogenesis**

Cytoarchitecture of Neuroblasts and their Stem Cell Niche Maintaining Adult Neurogenesis in the Olfactory Midbrain of Spiny Lobsters, *Panulirus argus*

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Adult neurogenesis persists in the olfactory midbrain of spiny lobsters, *Panulirus argus*. Neuronal precursor cells are located in a small proliferation zone (PZ) in each of the four soma clusters of local and projection neurons. One neuronal stem cell – a large adult neuroblast (aNB) - is located close to each PZ and is itself associated with a unique clump of cells constituting a putative stem cell niche (Schmidt, *J. Comp. Neurol*. 503:64-84, 2007). We analyzed the cytoarchitecture of the aNB and the clump of cells with immunocytochemistry and TEM. These analyses showed that the clump is comprised of cells (clump cells) whose small somata form a dense mantle around a nucleus-free core and that the aNB has a unique hourglass-like shape. The peripheral part of the aNB contains a large nucleus and is connected via a thin cytoplasmic bridge to a bulb-shaped 'foot' extending into core of the clump of cells. The clump cells are bipolar with a long outward-facing process and a shorter process reaching into the core of the clump. The outward-facing processes form a strand that surrounds the peripheral part of the aNB and projects further to the PZ. The shorter processes are convoluted and completely cover the bulbous foot of the aNB. Processes of multipolar, soma-associated glial cells envelope clump and strand in several layers and separate them from neighboring neuronal somata and arterioles. We conclude that the clump of cells has morphological features of a protected stem cell niche, in which clump cells constitute the microenvironment of the aNB and insulate it from the surrounding tissue. Since the clump cells differ from glial cells in immunocytochemical properties and in overt morphology, we hypothesize that they maintain embryonic characters, a common feature of stem cell niches in adult tissues.

#P234 **Poster session V: Chemosensory memory/ Central synaptic physiology/Neurogenesis**

Ectopic gene expression by postnatal electroporation during olfactory interneurons neurogenesis

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Neurogenesis persists in the olfactory system throughout life. The mechanisms of how new neurons are generated, how they integrate into circuits, and their role in coding remain mysteries. Here we report a technique that will greatly facilitate research into these questions. We found that electroporation can be used to robustly and selectively label progenitors in the Subventicular Zone. The approach was performed postnatally, without surgery, and with near 100% success rates. Labeling was found in all classes of interneurons in the olfactory bulb, persisted to adulthood and had no adverse effects. The broad utility of electroporation was demonstrated by encoding a calcium sensor and markers of intracellular organelles. The approach was found to be effective in wildtype and transgenic mice as well as rats. Given its versatility, robustness, and both time and cost effectiveness, this method offers a powerful new way to use genetic manipulation to understand adult neurogenesis.

#P235 **Poster session V: Chemosensory memory/ Central synaptic physiology/Neurogenesis**

Deafferentation affects cell genesis and neuron survival in the olfactory bulb of adult zebrafish

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The potential effects of afferent innervation on adult neurogenesis in the olfactory bulb were examined with deafferentation. Olfactory organs of adult zebrafish were completely ablated by cautery to cause permanent denervation of the olfactory bulb. Animals were exposed to bromodeoxyuridine then examined using immunocytochemistry following short (4 hour) or long (3 week) survival periods. Short survival times allowed analysis of cell proliferation in the bulb and long survival times permitted investigation of survival of adult-formed cells. When examined immediately after deafferentation, no effect on cell proliferation was observed. However, there was an effect on the number of adult-formed cells present in the bulb three weeks later suggesting that afferent removal influenced the fate of newly formed cells by impacting subsequent divisions, maturation, or survival of those cells. One week of deafferentation altered the pattern of cell genesis, with a significant increase in the number of dividing cells located in the olfactory bulb. Survival also was impacted by one week of deafferentation since there was an elevation in the number of adult-formed cells in the glomerular layer of the bulb. Sham surgery and longer deafferentation times did not impact either proliferation or survival of adult-formed cells. Thus, afferent innervation is necessary for normal cell proliferation and maintenance of the olfactory bulb in adult zebrafish. The mechanisms by which olfactory axons exert an influence on the process of adult neurogenesis will be the subject of future studies.

#P236 **Poster session V: Chemosensory memory/ Central synaptic physiology/Neurogenesis**

Cell specific deletion of BDNF leads to impairments in murine adult olfactory neurogenesis

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Neurotrophins are a class of molecules known to influence the development and survival of cells of the nervous system. BDNF signaling through TrkB receptors is important for postnatal modifications of the nervous system such as synaptic remodeling, dendritic outgrowth, and cell survival. In adult animals, the application or overexpression of exogenous BDNF has been shown to impact neurogenesis. We have recently shown, through the use of several genetically engineered lines of mice and novel reagents that endogenously produced BDNF plays a significant role in regulating adult olfactory bulb (OB) neurogenesis. Furthermore, we have shown that adult OB neurogenesis relies heavily upon the regulated release of BDNF and signaling through TrkB and not p75 receptors. However, the source of the endogenously released BDNF has remained elusive. We present

data here from mouse studies using Cre/LoxP site-specific recombination in which we genetically remove BDNF from discrete populations of cells. To our knowledge, these studies are the first to attempt to localize the source of BDNF controlling adult neurogenesis.

#P237 **Poster session V: Chemosensory memory/ Central synaptic physiology/Neurogenesis**

***In vivo* optical imaging of experience-induced olfactory bulb glomerular plasticity**

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While the olfactory system has been shown to have a remarkable capability for undergoing experience-dependent plasticity, how such odor memories are imprinted in the adult olfactory neuronal circuits remains unclear. This process most likely involves changes at multiple stages along the central olfactory processing pathways. One interesting site for plasticity is the olfactory glomerular layer. Within this layer, the organization of receptor neuron inputs allows odorant information to be transformed into an odorant-specific spatial map of glomerular activity. We have visualized such activity patterns in vivo by using a recently-developed transgenic mouse with a GFP-based calcium indicator (G-CaMP2) expressed in output neurons postsynaptic to olfactory nerve inputs. Unlike previously applied optical imaging methods, this mouse allowed us to observed purely postsynaptic odor maps in the glomerular layer. Using this mouse, we tested the hypothesis that the olfactory learning process can significantly alter olfactory bulb postsynaptic glomerular odorant representations for the trained odorant. This was carried out by comparing the odorant-evoked glomerular activity patterns in the same animal before and after olfactory associative conditioning with foot shock. Preliminary data suggests that conditioning with a given odorant significantly alters glomerular responses to that odorant following training. These results suggest that simple forms of olfactory experience can have a significant impact on olfactory odor coding even at the earliest stages of central processing.

#P238 **Poster session V: Chemosensory memory/ Central synaptic physiology/Neurogenesis**

Maternal Modulation of the Functional Emergence of the Hippocampus in Context Fear Learning in Infant Rats

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The hippocampus is important for the formation of associative memories, such as acquiring information about context (i.e. the place where an experience occurred) during emotional learning (i.e. fear conditioning). Previous work suggests that context learning ontogenetically emerges at weaning. First, we assess whether the hippocampus is responsible for pups' newly emerging context learning. In all experiments, postnatal day (PN) 21 and PN24 rat pups received 10 pairings of odor-0.5mA shock or control unpaired odor-shock or odor only. Some pups were used for context, cue or odor avoidance tests, while the remaining pups were used for c-Fos immunohistochemistry to assess hippocampal activity during acquisition. Our results show that cue and odor avoidance learning were similar at both ages, while contextual fear learning and learning associated hippocampal (CA1, CA3 and dentate gyrus) activity only occurred in PN24 paired pups. To assess a causal relationship between the hippocampus and context conditioning, we infused muscimol into the hippocampus, which blocked acquisition of context fear learning in PN24 pups, but did not affect cue learning or aversion to the odor at PN21 or PN24. Secondly, we assess whether the emergence of the context fear learning is modulated by the maternal presence. PN24 rat pups were odor-shock conditioned with or without maternal presence. Similarly to muscimol infusion into the hippocampus result, maternal presence prevents context fear learning at PN24 but do not affect cue learning and odor aversion. The results suggest that maternal presence modulates the newly emerging contextual fear learning exhibited by PN24 pups that is supported by the hippocampus.

#P239 **Poster session V: Chemosensory memory/ Central synaptic physiology/Neurogenesis**

Rethinking statistical analysis of associative learning in an olfactometer

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Statistical analyses of performance in olfactory learning tasks typically focus on confirming that the association has been learned rather than investigating the process through which it is established. In the former approach, the animal needs to be proficient in the task before a particular association can be demonstrated. Because the animal may make the association and thus treat the stimuli differently before reaching the performance standard, the statistical analysis of the acquisition process should not be constrained by a set criterion. Here I present a new method to calculate when the subject's behavior departs from randomness. This is done by comparing the probability value associated with the animal's performance with a spectrum of probability values generated from multiple simulations of random behavior in the same task. These probability values are incremented on a single-event basis to detect more precise timing of the learning process. This new approach is also more flexible in handling variability within and across individuals and sessions. The efficacy of this method is evident in the reported results from a pilot study using natural and artificial odors. Applying our method provides evidence that mice can achieve better than chance performance considerably sooner than previously demonstrated with the standard statistical analysis.

#P240 Poster session V: Chemosensory memory/ Central synaptic physiology/Neurogenesis

Changes in Sniffing Patterns During Learning of the Association of Odor with Reward

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Active sniffing has been hypothesized to provide a filter for input to the olfactory system, and firing of mitral cells in the olfactory bulb has been demonstrated to be coupled with respiration. Our goal was to investigate changes in sniffing as mice learn to associate a novel odor with reward. Mice were implanted with a nasal cannula that allowed measurements of changes in intranasal pressure and they were exposed to novel odors in a go-no go task. Odors were associated with either a water reward delivered through a tube or no reward, and the mouse responded to the odor stimuli by licking on a tube for the rewarded odor or not licking for the unrewarded odor. Sniff frequency and amplitude of intranasal pressure changes were recorded, along with lick behavior. Our preliminary data indicates that variability in sniffing frequency and amplitude decreases as the mouse learns the association during the go-no go session. The data also reveals that sniffing frequency increases in anticipation of odors, and amplitude changes as odor associations are learned.

#P241 Poster session V: Chemosensory memory/ Central synaptic physiology/Neurogenesis

Long-term reductions of olfactory sensitivity due to short-term exposures to a peri-threshold odorant

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Adaptation refers to reduced sensitivity to a stimulus due to exposure to the stimulus. It is standard to classify olfactory adaptation as short-term versus long-term, with the assumption that rapid exposures (seconds to minutes) lead to quick recoveries from the odorant (within minutes) while prolonged exposures (hours to days and weeks) yield slow recoveries (weeks or longer). In the current study, we assessed the olfactory threshold of phenylethyl alcohol (PEA) of 5 female subjects once every three days over a course of two months. Each time, the subjects were exposed to peri-threshold concentrations of PEA for 2s per minute for a total of under 40s of PEA exposure time. We showed a significant monotonic reduction of PEA sensitivity in the course of testing in all 5 subjects and large individual variations in the severity of the reduction. Our findings suggest that mere short-term exposures to a peri-threshold odorant can amount to progressive long-term adaptation, and provide new insights in the interplay between the receptor and higher cognitive levels of olfactory adaptation.

#P242 Poster session V: Chemosensory memory/ Central synaptic physiology/Neurogenesis

Learned preferences for odours determined by individual variations in taste intensity and hedonics

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Repeated co-exposure of novel odors with sweet or bitter tastes changes their hedonic and sensory characteristics. However, individual variations in responses to tastes could influence these conditioned effects. Thus, tasters (T) of 6-n-propylthiouracil (PROP) find saccharin (Sacc) both more bitter and more sweet than do PROP non-tasters (NTs). We therefore predicted that acquired liking for odors conditioned by pairing with Sacc would vary with PROP taster status. Since odor perceptual characteristics, e.g., smelled sweetness and bitterness, arise via association with tastes, we tested if PROP taster status would influence such learned effects. We also examined the impact of individual variations in hedonic responses to sweet tastes (the sweet liker/disliker dichotomy). Eighty-seven volunteers evaluated two novel odors before and after co-exposure of one odor with 0.0004M Sacc and one with water. PROP taster status was based on ratings of 3.2mM PROP, and sweet-liker status from hedonic ratings of sucrose and Sacc solutions. Liking for the Sacc-paired odor increased in sweet likers but decreased in sweet-dislikers. Both odors were less pleasant post-exposure in the PROP T but not NT groups. The Sacc-paired odor was sweeter smelling post-exposure, regardless of PROP taster or sweet-liker status. PROP super-tasters rated the Sacc-paired odor as more bitter smelling post-exposure, in-line with their higher ratings of Sacc bitterness. These data show that acquired liking for, and sensory characteristics of, odors (and hence flavors) is at least partly determined by individual differences in perceptual and hedonic responses to bitter and sweet stimuli. Moreover, at least some of the PROP group differences in food preferences are probably due to differential learning processes.

#P243 Poster session VI: Chemosensory development and Psychophysics I

Sonic hedgehog and Sox2 expression in taste cell progenitors in genetic mouse models of gustatory nerve transection

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Our previous studies, using double neurotrophin knock out (double-KO) mice in combination ($BDNF^{-/-}xNT4^{-/-}$ or $BDNF^{-/-}xNT3^{-/-}$), showed severe deficits in the gustatory innervation of taste buds. The number of taste buds increased in double-KO and wild type (WT) mice during the early stages of taste bud development, as indicated by Troma-1 positive cells. There are no nerve fibers in the proximity of the taste bud progenitors at early stages of development. Interestingly, after nerve connection stage in taste bud development, fungiform taste bud number decreased significantly in the double-KO compared to WT. While taste placode/taste bud induction and initial stages of taste bud development are nerve independent, full maturation and function are not. To further study the role of early pioneering nerve fibers

in taste bud induction and development, we utilized our double-KO genetic nerve transection mouse models in combination with immunohistochemistry for two early taste placode/taste bud markers; Sonic hedgehog (Shh) and Sox2. Embryonic and neonatal double-KO and WT tongues were dissected, fixed and processed for immunohistochemistry. Shh positive placodes were observed in the lingual epithelium of both double-KO and WT prior to innervation. Shh positive placodes had a similar morphological appearance in all mice. Sox2 positive cells were also observed in all mice from E13.5 to P0. Null mutation of neurotrophins and the subsequent influence on the innervation did not affect early expression of Shh and Sox2, forwarding our hypothesis that the initial stages of taste bud development are independent from innervation.

#P244 **Poster session VI: Chemosensory development and Psychophysics I**

Expression of Stem Cell Factor and Kit receptor during development of the main and accessory olfactory systems

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Stem Cell Factor (SCF; also known as steel factor or Kit ligand) is a growth factor that signals through the Kit receptor, a type III receptor tyrosine kinase family member. Upon binding by its ligand, Kit receptor dimerizes and autophosphorylates to activate downstream signaling pathways. Kit signaling is important in several processes during nervous system development, including migration of neural crest cells. Kit+ cells are expressed in the dorsomedial neural tube and migrate exclusively into the dermis where they express markers of the melanocyte lineage. Recently SCF was identified as a guidance cue for commissural axons. It is required to stimulate the outgrowth of axons that enables midline crossing in the developing spinal cord. We have begun to investigate the expression of SCF and Kit in the developing olfactory system of mice using immunocytochemical and in situ hybridization techniques. Kit is expressed in neurons in the developing olfactory epithelium (OE) and in the vomeronasal organ (VNO) as early as embryonic day 11 (E11). In contrast, SCF is expressed in the rostral forebrain of early embryonic mice but is largely absent from developing olfactory and vomeronasal neurons. As the olfactory bulb (OB) emerges from the rostral telencephalon at E12-13, SCF expression is heavily expressed in he ventral OB adjacent to the cribriform plate where axons from the OE and VNO converge following their extension through the nasal mesenchyme. It is also where migrating GnRH neurons exit the nasal mesenchyme in transit towards the basal forebrain. Studies in mutant mice should allow us to decipher the role of SCF and Kit in cell migration and axon guidance in the embryonic mouse olfactory system.

#P245 **Poster session VI: Chemosensory development and Psychophysics I**

Differentiation and migration of neurons derived from the olfactory placode

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The mechanisms that influence the differentiation of olfactory sensory neurons (OSNs) during development are not fully understood. OSNs appear in the olfactory placode (OP) prior to embryonic day 10 (E10) and the first axons penetrate the telencephalic vesicle at E11 (Hinds, 1971). In addition, a population of GnRH+ cells, migrate along the nerve from the OP beginning at ~E11.5 (Wray, 1989). We noticed, beginning around E9.5, a population of cells emerged from the OP, into the mesenchyme, between the OP and cerebral vesicle. To characterize these cells, which we have tentatively termed Migrating Olfactory Placode Cells (MOPCs) we used a panel of neuronal and developmental markers from E9.5 through E13. The data reported here establish the neuronal identity of the MOPCs, their migration from the OP and the spatio-temporal framework of their appearance. Of interest, the MOPCs create a pathway between the developing olfactory epithelium and the bulb in the precise location where the olfactory nerve develops beginning around E11. Thus, this raises the possibility that the MOPCs may create a "scaffold" along which the axons can travel as they move toward the bulb. The data suggest strongly that these are the first cells to exit the placode and may be distinct from others previously associated with the concurrent migration of OSN axons, such as the GnRH cells and ensheathing glia that occur at a later time (de Carlos, 1995). Of interest, cells within the mesenchyme have recently been described which express odor receptors and may be involved in the targeting of OSN axons (Conzelmann, 2002). It appears the OR-expressing cells and the MOPCs likely represent two distinct neuronal populations emerging from the placode because of their morphological appearance and differences in their temporal framework.

#P246 **Poster session VI: Chemosensory development and Psychophysics I**

Early GABAergic Specification of Subventricular Derived Progenitors

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Olfactory bulb interneurons are continuously generated throughout development and in adulthood. These neurons are born in the subventricular zone (SVZ) and migrate along the rostral migratory stream (RMS) into the olfactory bulb (OB) where the majority become local GABAergic interneurons. To investigate the differentiation of these GABAergic interneurons we examined migration in a transgenic mouse which expresses green fluorescent protein (GFP) under the control of the glutamic acid decarboxylase 67 kDa (GAD67) promoter. During development, and in adult, GFP was expressed by a subpopulation of migratory cells along the rostral migratory

stream and in the olfactory bulb. Doublecortin (DCX) and Polysialic acid neural cell adhesion molecule (PSA-NCAM), both markers of migrating neuroblasts, were found co-expressed by the majority of the GAD67-GFP-positive SVZ-derived progenitor cells. This observation is similar to our previous work showing that SVZ-derived progenitor cells expressed GAD-65 very early in the migratory pathway, and in contrast to tyrosine hydroxylase-GFP phenotype which is acquired only when the cells are in the olfactory bulb. Although the GAD65/67 genes are expressed early in migration, there is minimal protein production in the cells within the RMS prior to the olfactory bulb. Taken together, our results suggest that the SVZ-derived neuroblasts during migration acquire GABAergic identify (GAD65 or GAD67 gene expression) very early in the SVZ-RMS-OB pathway. This suggests that at least some SVZ progenitors differentiate prior to reaching their final location in the olfactory bulb.

#P247 **Poster session VI: Chemosensory development and Psychophysics I**

The positional variability of the P2, M72, and MOR23 glomeruli in the mouse main olfactory bulb in young and adult animals

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The surface of the main olfactory bulb (MOB) contains a topographical map of olfactory sensory neuron activation known as an odor map. Odor maps in genetically inbred animals exposed to the same odorant contain both global similarities and regional differences. A major step towards understanding odor coding in the olfactory bulb is to understand the source of the variation in these maps. We have developed an accurate and sensitive method to map the location of glomeruli on the surface of the olfactory bulb to within biological variability. We have incorporated into our mapping technique a three-dimensional, digital representation of an average adult mouse olfactory bulb (called a standard bulb). This standard bulb allows us to address alignment issues that can result from individual differences in bulb size or from technical errors during the surgical preparation of the bulbs. To test these new mapping tools, our lab has generated a new strain of transgenic mice which coexpress the green fluorescent protein (GFP) with three different odorant receptors. Each of these transgenic animals contains on average six different GFP-labeled glomeruli located in distinct locations on the surface of the MOB. Previous investigation has shown that any given glomerulus occupies a region of statistical probability on the surface of the bulb. We have mapped the number and location of these glomeruli across different developmental stages in order to determine the extent of their positional variability in reference to each other as the bulb develops. By mapping these three different glomeruli, we can estimate the extent by which the positional permutations of a given glomerulus occurs in reference to other glomerular positions in a given bulb across several developmental stages.

#P248 **Poster session VI: Chemosensory development and Psychophysics I**

Sensory inputs modulate olfactory receptor expression patterns in the mouse olfactory epithelium

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Postnatal experience plays critical roles in normal development of the olfactory system, which undergoes considerable neurogenesis throughout life. Odor stimulation is an essential element for cell survival, dendritic and axonal refinement, and synaptic plasticity of olfactory sensory neurons (OSNs). Each OSN stably expresses one odorant receptor (OR) from a repertoire of ~1300 and all OSNs with the same OR are distributed within one of the few broadly-defined zones. However, it is not clear how such expression patterns are shaped by the sensory inputs. Here we investigated how sensory deprivation modulates OR expression patterns by performing unilateral naris closure on newborn mice. After four-week occlusion, we compared the cell densities of 14 selected OR genes in the closed and open side using in situ hybridization. The cell density was significantly higher in the open side for two ORs (MOR174-13 and 40-12) in zone 1 (dorsal) and two ORs (K20 and P2) in zone 2-3 (middle), suggesting that odor stimulation may promote cell survival or regeneration of these OSNs. No differences between the closed and open side were observed for three other ORs in the dorsal or middle zone. This may reflect activity-independent survival of these neurons or lack of appropriate ligands in the environment. Surprisingly, all seven ORs (MOR0-2, 160-5, 232-2, 235-1, 236-1, 244-3 and 270-1) from zone 4 (ventral) had a significantly higher cell density in the deprived side than the open side. Whether this is due to better protected OSNs on the closed side or increased cell death/compromised neurogenesis in the ventral portion of the open side remains to be determined. Our results indicate that sensory inputs have differential effects on OSNs expressing different ORs.

#P249 **Poster session VI: Chemosensory development and Psychophysics I**

Influences of p53 gene in the development of olfactory neurons

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The p53 tumor suppressor protein plays an essential role in regulating neuronal apoptosis and survival in the developing nervous system. It mediates neural differentiation and maturation. To examine the role of p53 in the olfactory development and neurogenesis, we studied expression of a few maker proteins of the olfactory epithelium in p53 knock out mice. Among them, growth associated protein (GAP-43) is a protein uniquely expressed in immature olfactory neurons and olfactory marker protein (OMP) is expressed in mature olfactory neurons. Olfactory G protein ($G_{a/o}$) transmits information from cell surface receptors to intracellular effectors. Western analysis in postnatal 3 day p53 heterozygous ($p53^{+/-}$) mice showed that expression of GAP-43 was 69% ($p < 0.02$) of that observed in wildtype (WT). Expression of OMP and $G_{a/o}$ was also reduced to 59% ($p < 0.01$) and 47% ($p < 0.01$), respectively, in $p53^{+/-}$. Homozygous p53 ($p53^{-/-}$) mice exhibited the same reduction

trends in expression of GAP-43, OMP and $G_{a/o}$. Expression was reduced to 58%, 60%, and 43%, respectively, but was not significantly different from WT, probably due to a small sample size (n = 3 for $p53^{-/-}$). We observed similar results of GAP-43 and OMP ($p < 0.05$) between young adult $p53^{+/-}$ and WT. However, it appears that adults showed more individual variability, especially in $p53^{-/-}$. Our study suggests that p53 influences olfactory neuron development and functions. The mechanism that affects olfactory neuron differentiation and maturation warrants further study.

#P250 **Poster session VI: Chemosensory development and Psychophysics I**

How Does Adding Cocoa to Sucrose Affect Pain Tolerance?

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The sweet taste of sucrose is known to act as an analgesic, increasing pain tolerance in both infants (Blass & Hoffmeyer, 1991) and adults. In contrast, the taste of quinine, a bitter substance, decreases pain tolerance (Lewkowski, Ditto, Roussos & Young, 2003). The mechanisms underlying this effect may include the physiological properties of sugars, experiential associations including hedonic tone, or some combination of the two (Pepino & Mennella, 2005). Regardless, it is clear that sucrose may impart its analgesic properties to other stimuli through associations, as odors paired with the sweet taste exhibit analgesic qualities (Prescott & Wilkie, 2007). The present experiment asks how adding cocoa, a substance that is often associated with a sweet taste (Small, Zatorre, Dagher, Evans, & Jones-Gotman, 2001) but is itself bitter, would affect pain tolerance. The 19 male participants were exposed to four cold-pressor tests over two days and were asked in each test to hold their hand in 4° C water for as long as they could tolerate the pain while holding a tastant in their mouths. The tastants were either water (presented each day) or a tastant [either 24% (w/v) sucrose alone or 24% (w/v) sucrose with 12% (w/v) cocoa] and were presented in a counterbalanced order. After each test, participants rated pain intensity and tastant qualities. PROP status was evaluated at experiment's end. When effects of testing order were taken into account, sucrose clearly showed analgesic properties, $t(15) = 2.19$, $p < 0.05$, and increased the amount of time that participants held their hands in the cold water significantly more than cocoa, $t(15) = -2.08$, $p < 0.05$. Thus, rather than enhancing the effect of sucrose, the cocoa decreased the sweet analgesic effect, due to cocoa's bitter taste.

#P251 **Poster session VI: Chemosensory development and Psychophysics I**

Effects of Chocolate Consumption on Pain Perception and Tolerance

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Previous research has shown the benefits of sweet substance consumption on pain tolerance. The current study used 30 participants to compare pain tolerance, mood, and perceived task load in chocolate consumption. In a within-subjects design, participants completed four randomized consumption conditions (milk chocolate, dark chocolate, carob, and control) as well as a cold pressor task. The results show that participants had greater pain tolerance when consuming sweetened chocolate substances as compared to consuming an unsweetened substance or the control condition. These results provide additional evidence for sweet taste analgesia.

#P252 **Poster session VI: Chemosensory development and Psychophysics I**

Is perception of nasal patency a function of air temperature, humidity, mucosal heat loss, nasal resistance or trigeminal sensitivity?

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The lack of perceived nasal airflow (i.e. nasal patency) is the primary cause that drives patients with nasal sinus diseases to seek medical treatment. However, to objectively assess the degree of nasal patency remains a challenge to clinicians because objective measurements such as rhinomanometry or acoustic rhinometry do not correlate well with perceived patency. The current study examined other factors that may influence the perception of patency, including air temperature, humidity, nasal resistance, and trigeminal sensitivity. Forty-four healthy participants unilaterally rated patency under different air environment conditions using a visual analogue scale. In one condition, participants sampled untreated air in a test room. In three other conditions (cold air, untreated room air, and dry air), air was pumped into a face box. In all conditions, temperature and relative humidity were recorded. Nasal resistance and airway cross sectional area were measured unilaterally using rhinomanometry and acoustic rhinometry, respectively. Trigeminal sensitivity was assessed by measuring lateralization thresholds for butanol. No significant correlation was found between perceived patency and nasal resistance, although, within participants there was a significant difference in perceived patency between the high and low resistance nostrils. In contrast, air temperature, humidity and butanol thresholds taken together, could significantly predict ratings of patency ($R^2=0.145$, $p<0.000$), with temperature being the most heavily weighted predictor. Considering the significance of both temperature and humidity in patency rating, heat loss in the nasal mucosa and the trigeminal feedback may likely play a central role in individual's perception of patency.

#P253 **Poster session VI: Chemosensory development and Psychophysics I**

Retronasal and Oral-Cavity-Only Responses to TRPM8 Odorants

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Six TRPM8 agonists were presented as vapor-phase odorants retronasally and oral-cavity-only. Twenty-four participants selected, on a digital computer display under forced-choice conditions, 1 of 6 previously practiced identifications (ID).

Across all odorants and the 2 presentation conditions, as well as across the odorants for just retronasal and for just the oral-cavity-only (OCO) presentations, there were significant differences in % correct ID, $p < 0.001$. Retronasal median correct ID of eucalyptol (modal ID = ointment) was 100%, significantly different from the other odorants, $p < 0.05$, Bonferroni corrected. For the other 5 odorants, median % correct retronasal ID and modal ID were dl-menthol 17% (toothpaste), L-carvone 67% (spearmint), isopulegol 33% (toothpaste), linalool 67% (cleaner), geraniol 67% (lemon). OCO median % correct ID and modal ID were: dl-menthol 17% (peppermint), L-carvone 33% (spearmint), eucalyptol 84% (ointment), isopulegol 33% (toothpaste), linalool 33% (cleaner), geraniol 0% (peppermint). Correct ID for linalool and geraniol differed significantly between retronasal and OCO, $p \leq 0.001$, Bonferroni corrected. SUMMARY: Correct ID for eucalyptol > 80% occurred for both retronasal and OCO. L-carvone, linalool, and geraniol were readily identified retronasally (> 65%) but not OCO (≤ 33%), with OCO geraniol ID at 0%. For 4 of the 6 odorants, retronasal median % correct ID were larger than corresponding OCO median % correct ID. Within the studied set of odorants, ID of dl-menthol was difficult. CONCLUSIONS: TRPM8 agonists do not share a common odorant ID and are responded to differently both between retronasal versus OCO presentations and for OCO per se. The OCO differences suggest that TRM8 classification is not sufficient to predict trigeminal responses to odorants. Support from the Food Science Summer Scholars Program, the Biological Sciences Honors Program, and a Susan Lynn Sage Professorship

#P254 **Poster session VI: Chemosensory development and Psychophysics I**

Eph/Ephrin Expression in the Developing and Adult Taste System

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We are profiling the expression of Eph receptors and their Ephrin ligands in the taste system of embryonic to adult mouse and rat. Both classes of protein can initiate signaling cascades that underlie contact dependent repulsion or adhesion, thereby influencing axon pathfinding, target selection, and epithelial cell arrangement. "Forward" signaling refers to signaling within the Eph-bearing cell; "reverse" signaling is for Ephrin-bearing cells. In E11.5 and 14.5 mouse, EphrinB1 is detected in mandibular and chorda tympani axons that are bordered by EphB2-positive and EphB3-positive cells, respectively. Thus, reverse signaling could constrict these axons to their paths. In the tongues of embryonic mouse/rat and postnatal rat, however, anti-EphB1,2,3 labels geniculate and trigeminal axons; and Ephrins B1 and B2 are most concentrated in the non-gustatory epithelium avoided by these axons (mouse only). These data are consistent with a role for forward signaling in restricting the exploration of sensory axons in the target. EphA4, EphA7, and EphrinA2 are also present in embryonic mouse geniculate and trigeminal nerves. We have not detected Eph receptors or ephrins in adult mouse/rat sensory nerves, but both classes are detected in other adult tissues: In mouse, EphrinB1 and B2 staining of non-gustatory epithelium persists, and EphrinB1 is also detected at low levels within taste buds. In rat fungiform papillae, anti-EphA4 labels taste buds and anti-EphA7 appears to label microvasculature. These results are consistent with a role for Eph/ephrin signaling in axon guidance and targeting during development and plasticity, and perhaps during recovery following injury. In vitro functional assays are underway to determine if Eph/ephrin signaling supports or inhibits sensory axons throughout development.

#P255 **Poster session VI: Chemosensory development and Psychophysics I**

Investigation of detection and pain thresholds at different sites at the human nasal mucosa in response to electrical stimuli

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Background: Previous investigations in humans suggest differences in the distribution of intranasal trigeminal chemosensitivity. The aim of the present study was to investigate these topographical differences in the human nasal mucosa using unspecific electrical stimuli. *Material and Methods:* A total of 27 young, healthy volunteers participated (11 men, 16 women; age 22-30 years). Detection and pain threshold of trigeminal stimuli were investigated at 5 different sites at the nasal mucosa: anterior septum, posterior septum, lower turbinate, middle turbinate and anterior lateral nasal wall. Electrical stimuli were applied with a spherical electrode. *Results:* At the anterior parts of the nose significantly higher trigeminal sensitivity was found than at the posterior part. There was a similar distribution pattern of the sensitivity for detection and pain thresholds. *Conclusions:* The present data suggest that there are topographical differences in the distribution of trigeminal receptors at the human nasal cavity. Thereby it seems that the highest sensitivity is located in the anterior part. This finding is compatible with the idea that the trigeminal system acts as a sentinel of the human airways with regard to toxic agents.

#P256 **Poster session VI: Chemosensory development and Psychophysics I**

Time-Intensity ratings of nasal irritation from pulsed homologous alcohols

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Relatively few studies have focused on how nasal irritation changes over time. To simulate the rhythm of natural respiration, subjects received 3-sec pulses of VOC (volatile organic compound) interspersed with 3-sec pulses of clean air. Each trial, subjects received nine VOC pulses over 51 sec. Subjects rated nasal irritation from each pulse using magnitude estimation. Within a trial, compound and concentration were fixed. Compound (n-ethanol, n-butanol, and n-hexanol) and concentration (four levels for each compound) varied across trials. Findings: 1) For all stimuli, rated irritation decreased over time (adaptation). Plots of log rated intensity versus elapsed time were approximately linear (intensity decreased by a fixed ratio per unit time). Interestingly, the slopes of intensity versus time functions differed very little: Regardless of concentration and compound, rated irritation decreased by about 30 or 40% over the nine pulses. 2) For a given compound, the slopes of intensity versus concentration (psychophysical) functions remained constant across pulses. Overall intensity decreased over time, but the

relationship between concentration and intensity remained the same. 3) Across compounds, slopes of psychophysical functions differed greatly. Differences among compounds in the slope of psychophysical functions are unsurprising, but serve as a positive control of sorts for the lack of differences among compounds in dynamics. In short, some aspects of the dynamics of nasal irritation may differ very little across VOCs. The basic mechanism of short-term adaptation may be the same for the compounds studied. Regardless, these and other recent data suggest that very simple models can describe some aspects of perceptual dynamics quite well.

#P257 **Poster session VI: Chemosensory development and Psychophysics I**

Nasal biopsy assessment of veterinary students exposed to formaldehyde in anatomy class

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Inhaled formaldehyde has been linked to nasal irritation, inflammation and olfactory dysfunction and at high concentrations to nasal tumors in animal and human epidemiological studies. Occupational exposure to formaldehyde in gross anatomy class from formalin-preserved cadavers is a health concern to both staffs and students. As part of our on-going studies to understand mechanisms underlying chemical induced chemosensory dysfunction, we examined nasal biopsies, taken from the upper aspect of the middle turbinate, from 15 (5M, 10F) veterinary students (mean age = 24) at pre-exposure and after 20 weeks of formaldehyde exposure in anatomy class (approximately 4hrs/day, 4 days/week) and from 13 (5M, 8F) age-matched controls. Formaldehyde exposures (range 0.15-2.15 ppm) of the veterinary students were monitored with formaldehyde-specific badges (3M). Histopathology of the sensory epithelium (i.e, pseudostratified, hyperplasia, squamous metaplasia and erosion) was evaluated. In preliminary findings, biopsies of 5 veterinary students (33.5%) showed an increase in erosion after post-exposure compared to their pre-exposure biopsies. In comparison, just 2 control biopsies (15.4%) exhibited an increase in erosion compared to their initial biopsies. The degree of inflammatory infiltration will be analyzed to determine if histopathological changes correlates with inflammation and formaldehyde exposure levels. We also collected chemosensory thresholds, nasal nitric oxide levels, nasal cytology and nasal lavage fluids, which will allow us to examine correlations between psychophysics and neuropathology/inflammation. Assessment of nasal biopsies provides an opportunity into understanding how changes at the cellular/tissue level can manifest into chemosensory dysfunctions after chemical exposure.

#P258 **Poster session VI: Chemosensory development and Psychophysics I**

Functional and Inflammatory Consequences of Veterinary Gross Anatomy Lab Enrollment: Effects of Formaldehyde on Chemosensation

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Occupational exposure to pollutants and other toxic substances has been associated with impairment in human chemosensory function. Formaldehyde, a ubiquitous air contaminant that occurs from natural as well as man-made sources, is known to have adverse health effects, including upper airway irritation. The objective of this study was to evaluate the chemosensory impact of repetitive exposure to formaldehyde. The experimental cohort for this research was veterinary students working in a gross anatomy laboratory. Individual formaldehyde exposure was recorded through the use of passive dosimeters worn while students were in the laboratory. Chemosensory function was evaluated using a standard battery of tests prior to and throughout the duration of the three month exposure. Tests included odor identification test along with odor detection thresholds for formalin, PEA, and l-carvone. Lateralization (irritation) thresholds were collected for formalin and a control irritant, n-butanol. Upper airway inflammation was evaluated using nasal nitric oxide (nNO) concentrations and analysis of inflammatory mediators from nasal lavage. All results were compared to a non-exposed control group matched on age and gender. Exposure-associated increases in inflammatory biomarkers from nasal lavage fluid and nNO were correlated with significant alterations in chemosensory performance. Although the changes in these young, healthy participants are likely reversible, these results emphasize the need for continued monitoring of individuals with long-term employment in occupations where these types of exposures will be incurred.

#P259 **Poster session VI: Chemosensory development and Psychophysics I**

Development and Evaluation of the Monell Odor Identification Task for the NIH Toolbox

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The National Institutes of Health (NIH) Toolbox initiative seeks to assemble brief, comprehensive assessment tools that will be useful to clinicians and researchers in a variety of settings, with a particular emphasis on measuring outcomes in longitudinal epidemiologic studies and prevention or intervention trials across the lifespan. Monell's involvement in the NIH Toolbox is to develop tools to assess olfactory functioning in individuals between the ages of 3 and 85 years. We reported here on the development and preliminary analysis of the Monell Odor Identification Task that was designed to be sensitive to the cognitive limitations of children. We tested 368 subjects who were between the ages of 3 and 17 years; 186 were tested at the Monell Center and 182 at Nemours/A.I. DuPont Hospital. Subjects were presented with 6 cards, each of which was affixed with a scratch-

and-sniff sticker. For 55% of the children the odors on the cards were lemon, coffee, Play-Doh™, floral, bubble gum and peanut butter; whereas for the remaining children (particularly those whose mothers reported they had peanut allergies), the odors were the same with the exception that peanut butter was replaced by cinnamon. After smelling each card, the subject was then asked to identify the odor by pointing to 1 of 4 pictures. All but one child (3 years) completed the task which took less than 5 minutes. Preliminary analyses revealed that there were significant effects of both age group and odor on identification ($p<0.05$). In brief, we found children as young as 4 years of age understood the task but identification of some of the odors was difficult for all age groups, thus leading us to conclude that the quality of some of the fragrances may not be adequate. In conclusion, this method is fast and reliable for detecting odor identification performance across the ages, but investigations using other fragrances from different manufacturers are necessary to determine which odors yield consistent results across all ages.

#P260 **Poster session VI: Chemosensory development and Psychophysics I**

Musk odorants – a useful tool for the study of olfactory genetics

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The objective of this study was to estimate the frequency of specific anosmias and the heritability of odor perception in response to the National Geographic Smell Survey, a scratch and sniff panel of six odorants [androstenone (musk or urine), isoamyl acetate (banana), Galaxolide (musk), eugenol (cloves), mercaptans (natural gas), and a synthetic fragrance (rose)]. Subjects reported whether they could detect the odor, and if so, they rated its quality, intensity and pleasantness. Data were collected from 992 Australian twins/siblings [436 males and 556 females, mean age 17.8 years (SD 3.1 years); 110 monozygotic (45M, 65F) and 228 dizygotic (56M, 64F, 108MF) twin pairs, 155 siblings of twins, 30 sibling pairs, and 101 unpaired twins/siblings]. The rate of specific anosmias was higher for the musks (androstenone and Galaxolide) and natural gas odorant (18, 12, and 20%, respectively) than for the food and perfume odors (<1%). To determine whether these individual differences in detection, as well as intensity and pleasantness ratings might be genetic in origin, the variation in these responses was decomposed into additive genetic, common (shared), and individual (nonshared) environmental effects by genetic modeling using the *Mx* software. Heritability (additive genetic effects) were significant only for the detection and intensity of androstenone (54% and 56%, $p<0.05$) and the detection of Galaxolide (63%, $p<0.05$). The variance for all other responses was entirely due to common and/or individual environmental influences. Because many people are blind to the odor of some musks and this trait is highly heritable, musk odorants are a useful model system for the study of olfactory genetics.

#P261 **Poster session VI: Chemosensory development and Psychophysics I**

Evaluation of a Forced-Choice, Paired-Comparison Tracking Procedure Method for Determining Taste Preferences Across the Lifespan

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In 1990, Cowart and Beauchamp developed a method to measure taste preferences that was sensitive to the cognitive limitations of pediatric populations. The method consists of two series in which pairs of solutions of different concentrations of the tastant are presented to subjects, who then indicate which of the pair they prefer. The weaker solution of the pair is presented first in series 1 whereas the stronger solution is presented first in series 2. The order in which stronger and weaker stimuli are presented prevents children from reaching criterion responding if they chose on the basis of first or second position bias. Between 2003 and 2007, this method was used to measure sucrose preferences in 434 children (5-9.9 yrs), 93 adolescents (10-19.9 yrs) and 327 adults (20-55 yrs). Data are now being analyzed for the NIH Toolbox Initiative which aims to develop brief, yet comprehensive, tools to assess taste preferences for people between the ages of 3 and 85 years. First, we determined whether there were age-related differences in the reliability of the data obtained in the two series. Second, we assessed whether the data obtained from series 1 were in agreement with data obtained from series 2 because the task must be brief. Four children refused to participate and 15 completed one series only. As expected, adults had lower sucrose preference than adolescents and children, both t's > 4.76, both Cohen's d >= .42, p's <.05). Intraclass correlations showed that reliability between the two series was adequate for children (ICC = .42, N = 415), adolescents (ICC = .52, N = 93), and adults (ICC = .64, N = 327). The agreement varied with age (2 [2] = 14.22, p <.05); 87% of adults, 82% of teenagers and 75% percent of children chose the same or the closest concentration of sucrose in the two series. Although sucrose preferences obtained in series 2 were higher than that obtained in series 1 for 38% of children, 33% of adolescents, and 32% of adults (F [1, 832] = 20.14, Cohen's d = .18, p <.05), no interaction effect was found between age group and trial. In conclusion, this method is fast and reliable for detecting age-related changes in sucrose preferences.

#P262 **Poster session VI: Chemosensory development and Psychophysics I**

OLFACT-K™: A Test for Assessing Olfactory Function in Young Children

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Testing olfactory function in young children presents many challenges. Nevertheless, several tests to assess the sense of smell in children have been developed, e.g., Laing et al, 2008; Murphy et al, 1994. We describe a new test based upon the OLFACT™ olfactometer which is suitable for testing young children. The test is based upon an odor identification paradigm and consists of 20 common odors presented by the olfactometer. For each odor stimulus, four choices (in both lexical and pictorial format) are presented on a LCD. The participant must select one from the

four (4 AFC) that they believe is most representative of the stimulus. Prior to testing the subject is presented with a picture of each of the odor stimuli on the LCD and they must name the object. A correct or incorrect response is recorded by the examiner. In order to keep the participants engaged in the task, one additional modification was implemented. Before testing began, each participant was allowed to choose their form of reinforcement for correctly identifying an odor stimulus. A correct response would either 1) "virtually" burst one balloon from an array of 20 displayed on the LCD; or 2) add one "virtual balloon" to a bouquet of balloons on the screen. To evaluate the utility of this test, two groups of children 6-8 years old were tested as part of a larger battery of tests that included measures of postural sway and auditory function. The groups were part of a larger study examining the effects of environmental manganese exposure on childhood development. Collection of the data is ongoing; results of the study will be described in detail at the meeting. Supported by NIDCD grant DC6369.

#P263 Poster session VI: Chemosensory development and Psychophysics I

Comparison of two different olfactory detection threshold tests of the Sniffin' Sticks

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Objectives: The olfactory test battery Sniffin' Sticks is a test of nasal chemosensory function which is based on pen-like devices for odor presentation. It consists of three subtests: odor threshold, odor discrimination and odor identification. The detection threshold can be measured using two different odorants – *n*-butanol or PEA (phenylethyl alcohol). Both test batteries are commonly employed in published studies, but there has never been a formal comparison of values obtained using them. The purpose of this study was to compare two different olfactory detection threshold tests (*n*-butanol and PEA). **Methods:** Both tests were applied to a group of 78 healthy, normosmic subjects (41 male, 37 female). The experiment was divided into two sessions performed on two different days. The order of both tests was pseudo randomized. After each threshold test a discrimination and identification test was conducted. **Results:** There were significant differences in odor detection thresholds of PEA and *n*-butanol. The mean score of PEA detection threshold and PEA TDI (threshold discrimination identification) was significantly higher compared to *n*-butanol. Participants detected PEA at a lower concentration than *n*-butanol. No significant correlation between individual PEA and *n*-butanol thresholds was detected. **Conclusion:** Previous work regarding the test-retest reliability and validity of the Sniffin' Sticks was performed using the *n*-butanol threshold test only. The differences between both olfactory test batteries indicate that a formal validation of Sniffin' Sticks test with PEA as odorant for detecting the olfactory threshold may be required.

#P264 Poster session VI: Chemosensory development and Psychophysics I

Longitudinal study of olfactory preferences during childhood

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Pleasantness is a prominent facet to the olfactory world. Whereas some aspects of odor hedonics are innate, others are formed during development. One question that is still debated in the current literature is the early stages of development whereby such formation of odor preferences occurs. In the present study we hypothesized that a critical time-window may be between 3 and 5 years old, a period of life whereby the ability to detect, name and memorize odors significantly improves. The present study was aimed at testing this hypothesis through a 3-years long longitudinal experiment. Fifteen 3-years old children participated to 3 experimental sessions from year 1 (2006) to year 3 (2008). Participants were first asked to complete a standardized French test of language and were then exposed to 12 odors (presented for around 2 sec in a random order). After smelling each compound, they were asked to answer two questions: 1) Do you like or dislike this odor? and 2) Can you tell me what it is? Children were filmed during the session, and both verbal and behavioral responses were analyzed to give the most reliable measure of hedonic responses. In line with previous findings, we observed that general language abilities (production ($p<.003$) and comprehension ($p<.0001$)) improve from 3 to 5 years old. Moreover, verbal descriptions about odors were more frequent at 4 and 5 years (vs. 3 years) (chi-square, $p<.0001$). Finally, and more importantly, an interaction was seen between hedonic categorization and age: the number of odors perceived as pleasant increased significantly from 3 to 5 years of age whereas the opposite pattern was seen for unpleasant odors (chi-square, $p<.0001$). Taken together, these results suggest that the 3-to-5-year age range may be a turning point in the development of olfactory preferences during childhood.

#P265 Poster session VI: Chemosensory development and Psychophysics I

Neuronal and neural crest cell markers identify specific cell types in developing tongue and taste papillae

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The field of taste biology lacks a clear understanding of defined taste cell precursors in developing papillae and there is no detailed information about cell specification of the early lingual epithelium. With immunohistochemistry we characterized and localized neuronal cell phenotypes in embryonic tongue epithelium and mesenchyme. We found that early neuronal markers, III-tubulin and doublecortin, label a restricted set of apical epithelial cells in embryonic fungiform papillae. These neuronal cell types develop and are sustained in papillae in tongue cultures with no intact sensory innervation. Identification of early neuronal markers suggests the possibility of neuronal cell differentiation in the early fungiform papilla epithelium. Further, to explore potential contributions of neural crest derived cells (NCDCs) to development of tongue, taste papillae and taste buds,

we used two transgenic mouse lines, with Wnt1- and P0-Cre driven reporters. A progressive developmental association of NCDCs with taste papillae is observed in tongues of Wnt1-Cre reporter mice (E12.5, 16.5, P1). Labeled cells are intensely distributed in fungiform, foliate and circumvallate papillae. Brightly labeled cells are within the mesenchymal core of papillae and in the basal lamina region underlying early taste buds. A few labeled cells are also seen in the epithelium of taste papillae at P1. In P0-Cre reporter tongue sections (E14.5, 16.5 and P10), numerous labeled cells are seen in clusters in the tongue epithelium (within papillae or between papillae) and mesenchyme. At P10, intensely labeled cells are observed in early taste buds. The distribution of labels for NCDCs in the developing tongue, taste papillae and early taste buds strongly implicates a neural crest contribution to papilla and taste bud development.

#P266 **Poster session VI: Chemosensory development and Psychophysics I**

Impact of Proportion on Configural Perception of Odor Mixtures in a Newborn Mammal

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Configural perception of odor mixtures appears functional early in life. Recent results underline that after the learning of a binary mixture (AB) that blends in humans, newborn rabbits respond both to the mixture and to its components. However, after the learning of a single component they do not generalize to the mixture. This suggests that they perceive more in the mixture than the odor of each constituent (Coureaud et al., Physiol. Behav. 2008). Here, we pursued the assessment of their configural perception of AB, with the aim to determine whether specific component proportions of A and B elicit the perceptual emergence of an additional odor in AB. Starting from the initial composition of the AB mixture, we tested whether pups perceived mixtures with weakly different proportions of A and B (A^-B, A^+B) either elementally of configurally. In Exp. 1, 17 pups (age: 2d, 4 litters) were conditioned to A, and tested for their oral response to AB, A^-B, and A^+B. In Exp. 2, 2x20 pups (2d, 8 litters) were enforced to learn A^- or A^+, and respectively tested with A^-, A^-B, AB, and A^+, A^+B, AB. In Exp. 3, 18 and 20 pups (2d, 8 litters) were conditioned to A^+B and tested for their response to A^+, B or to A^+B and AB. As expected, pups conditioned to A did not respond to AB, but interestingly they responded to A^-B and A^+B (>45%; Exp. 1). Additionally, after learning of A^- or A^+, newborns strongly responded to A^-B or A^+B (> 70%) but their response remained poor to AB (<20%). Finally, following the learning of A^+B, pups well responded to A^+ and B (> 90%) but significantly less to AB (50%). These results highlight the rabbit newborn ability to discriminate between odor mixtures presenting slightly different proportions of odorants, and confirm the configural perception of certain odor mixtures by the young animal.

#P267 **Poster session VI: Chemosensory development and Psychophysics I**

Correlation between olfactory bulb volume and olfactory function in children

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The olfactory bulb (OB) is considered to be the first important relay station in odor processing. The aim of the present study was to investigate whether and how the human bulb increases during childhood and youth. Involving a large number of subjects the present study also aimed to investigate a possible correlation between the OB volume and specific olfactory functions including odor threshold, odor discrimination, and odor identification. A total of 87 randomly selected subjects (46 men, 41 women), aged 1 to 17 years (mean age 8 years), participated in this study. None of them reported olfactory dysfunction. All participants received an otolaryngological investigation including a volumetric scan of the brain (MRI), and lateralized olfactory tests. The history of all participants was taken in great detail to exclude possible causes of smell dysfunction. Volumetric measurements of the right and left OB were performed by two independent observers by manual segmentation of the coronal slices through the OBs using the AMIRA 3D visualization and modeling system (Visage Imaging, Carlsbad, USA). Significant correlations between left and right OB volumes in relation to odor thresholds (left: r_{51}=0.56; right: r_{53}=0.63; p<0.001) as well as left and right OB volumes in relation to odor identification (left: r_{51}=0.46; right: r_{53}=0.50; p<0.001) were observed. Also, significant correlation were found between OB volumes and odor discrimination results (left: r_{51}=0.58; right: r_{53}=0.58; p<0.001). In addition, absolute OB volume increased with age (left: r_{87}=0.37; right: r_{87}=0.34; p<0.001). However the relative OB volume decreased with age (r_{87}: -0,92; p <0,05). Furthermore, although men exhibited larger OB volumes than women on average (men: 70 mm^3; women: 66 mm^3), the increase of OB volume with age was similar for men and women. In addition the olfactory function (SDI-result) increased with age (men: r_{30} = 0,85; p <0,001; women: r_{21} = 0,83; p <0,001) on both sides (left: r_{51} = 0,76; p <0,001; right: r_{51} = 0,84; p <0,001). Using "age" as a control variable for partial correlations, olfactory bulb volume correlated significantly with odor thresholds (left: r_{51}=0.74; right: r_{53}=0.81; p<0.001). By using "age" again as a control variable for partial correlations, correlational analyses between right and left OB volumes and odor identification test results were still significant (left: r_{51}=0.55; right: r_{53}=0.59; p<0.001). Significant correlations were also found between OB volumes and odor discrimination, when using "age" as a control variable for partial correlations (left: r_{51}=0.73; right: r_{53}=0.84; p<0.001). The present study confirmed the correlation between OB volume and specific olfactory functions. Furthermore, the correlation between OB volume and olfactory function is not mediated by the subjects' age. It is also remarkable, that Children at the age of 1 already showed a large average OB volume of 61,3 mm^3.

#P268 **Poster session VI: Chemosensory development and Psychophysics I**

Pregnancy and Olfactory Sensitivity

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INTRODUCTION The purported role of hormones in olfaction has led to the hypothesis that women should outperform men in olfactory tests and that olfactory changes should occur across the menstrual cycle and pregnancy. Although women do outperform men on many tasks and there is evidence for changes in olfactory sensitivity across the menstrual cycle, the literature on pregnancy does not provide strong support. A careful review of the literature reveals that when present differences are often very small and depend on many factors. This on-going study assesses olfactory function in women across pregnancy. METHODS Sixteen pregnant women and 10 non–pregnant controls were each tested 3 times. Women rated their "sense of smell" and pregnant women reported odors to which they were particularly sensitive. Thresholds for phenyl ethyl alcohol were established using a standard staircase procedure, followed by 75 signal detection (SD) trials. RESULTS Pregnant women rated their sense of smell significantly higher during the first trimester compared to pre-pregnancy and the third trimester, but thresholds did not change across pregnancy nor differ from controls. Preliminary analyses revealed no difference on SD trials across trimester nor pregnancy status. However, early in pregnancy ~75% of women identified at least one odor to which they were more sensitive, but none mentioned "rose". CONCLUSIONS These results are consistent with previous pregnancy studies demonstrating more consistent and larger changes in self-rating/report than in behavioral measures. Thus, although there are fluctuating levels of hormones across pregnancy, systematic changes in olfaction across pregnancy were not observed. The relationship between hormones and olfactory function and the role of odor on task performance is discussed.

#P269 **Poster session VI: Chemosensory development and Psychophysics I**

Beliefs About Health Effects from an Odor Alter Sniffing of that Odor

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Just as eye tracking has been used to monitor shifts in attention to visual input, sniffing can be tracked to monitor attentional shifts to olfactory input. We investigated whether a cover story about health effects from an odor altered sniffing of that odor. Methods: 63 Subjects were asked to smell the odors vanilla, citrus, ethylbutyrate, and the target odor Orange Flower Ether in random order and match each to one of four previously provided cover stories. There were three different groups based on cover story of the target odor only: negative bias (NB: n= 22, "odor is chemical rest product"), positive bias (PB: n=20, " aromatherapy product"), and the neutral group (N; n=21, "standard odor"). Odors were rated on attributes such as pleasantness, intensity, and dangerousness on 7-point scales. Sniffing was measured via a nasal air pressure transducer. Results: Effects of bias manipulation on sniffing parameters were not significant (p >.05). What turned out to be important was whether subjects, after smelling the odor, *believed* the odor to be dangerous. A comparison between "odor believed to be dangerous" versus "odor believed to be safe" groups based on median split of the target odor danger ratings revealed a smaller area under the curve (p=.02) and shorter duration (p=.02) from the first sniff of the target odor in the former relative to the latter group (with bias group as covariate). Conclusion: Odor sampling behavior or sniffing is not affected by a cover story about the odor's possible health effects, only by whether people believe it to be dangerous after smelling it, regardless of the cover story.

#P270 **Poster session VI: Chemosensory development and Psychophysics I**

Relationship between Odor Properties for Pleasant and Unpleasant Odors

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Relationships between different odor properties have been studied extensively, but whether different patterns characterize pleasant and unpleasant odors is unknown. We examined whether the relationships between perceived odor intensity, pleasantness, familiarity, and irritability differ for pleasant and unpleasant odors. Thirty healthy participants rated a set of 24 odors (half pleasant, half unpleasant) on four odor properties. Each odor was presented in three concentrations (weak, intermediate, and strong) for a total of 72 odors, and ratings were completed using a visual analogue rating scale. Correlation between intensity and pleasantness was stronger for unpleasant than for pleasant odors, in addition to being in the opposite direction. Pleasantness was strongly correlated with familiarity for pleasant but not for unpleasant odors. Finally, irritability was strongly correlated with intensity for both categories of odors, whereas it was more strongly correlated with pleasantness for unpleasant than for pleasant odors. These findings demonstrate that pleasant and unpleasant odors represent fundamentally different odor categories and corroborate previous claims that pleasantness is a primary dimension in olfaction.

#P271 **Poster session VI: Chemosensory development and Psychophysics I**

Slight Variations in Components Ratio affect Odor Pleasantness of a Blending Mixture

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Odors rely mainly on the perception of odorants mixtures but are commonly perceived as single undivided entities; nevertheless, the processes involved remain poorly explored. It has been reported that perceptual blending, based on configural olfactory processing, can lead odorant mixtures to give rise to an emergent

odor quality not present in the components. Furthermore, very slight variations (just noticeable differences, jnd) in components concentrations were shown to be sufficient to modify the odor quality of a blending mixture. In the present study, we set out to examine whether jnd in components concentrations could also affect the odor pleasantness of a blending mixture. We started from the composition of a ternary target mixture of odorants in which an emergent pineapple odor has been evidenced for a given concentration of each component. Then, we set 4 concentration levels of each component that elicit jnd. Each combination of levels was used to design sample mixtures that were delivered using a dynamic air-dilution olfactometer. Fifteen subjects (3 men, 19 to 26 years old) compared the intensity, typicality, and pleasantness of each sample mixture against the target mixture in a paired-comparison protocol. Statistical modeling of the results showed that slight variations in components ratio did affect significantly odor pleasantness of the ternary mixtures. When the concentration of one of the components increased, the mixture pleasantness decreased whereas an interaction between the two other components was observed. These results underlined (i) the key role of odorants concentrations ratio on the pleasantness of a mixture odor and (ii) the human ability to discriminate between odor percepts induced by mixtures including even close odorant proportions.

#P272 **Poster session VI: Chemosensory development and Psychophysics I**

Identification of odorants induced by stress and deception in humans

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Behavioral studies and anecdotal reports suggest that stressful situations create recognizable changes to human body odors. The odor change may be related to increased levels of stress-related hormones. However, the identity of any stress-induced odorants has not been reported. In this study, we recruited volunteers who then went through a standardized mental stress procedure (Trier Social Stress Test). The six most stressed individuals out of 18 volunteers were selected based on their monitored heart rate, respiration, salivary cortisol and self-reported stress ratings. Axillary sweat samples were collected on cotton pads from each underarm area during stress and non-stress condition. These were extracted, evaluated by sensory panels and analyzed by gas chromatography/mass spectrometry in order to identify the stress-associated odorants. Overall, the sensory panelists discriminated the odor differences above chance (with 63% accuracy) between the samples collected during the stress session and the samples in the pre-stress session. The chemical analyses showed that the levels of several endogenously produced odorants were increased after the stress exposure.

#P273 **Poster session VI: Chemosensory development and Psychophysics I**

The Recognition Point in Odor Detection

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If an investigator measures a threshold for odor detection, someone will ask, "What is the recognition threshold?" No one has seriously measured that point, though some have polled subjects and ventured estimates of when quality begins to emerge over mere detection. Some have figured it to occur at low multiples of the detection threshold, such as 2x or 5x. Though reasonable, such estimates need an operational definition. The present research affords a way to extract information on recognition from 1000's of confidence judgments per odorant gathered during forced-choice testing of detection in many subjects. The ratings range from 1 (very low confidence) to 5 (very high confidence). One can chart the distributions of the ratings, i.e., frequency of each level vs. concentration, as iso-confidence contours. The distributions have surprisingly uniform variance within odorants. The data encourage a definition: The recognition point corresponds to the concentration at the median of the distribution for "high confidence." For some odorants, the point occurs when performance in detection has begun to converge on perfection. For others, it occurs at a lower point on the detection function. Differences will depend upon the distinctiveness ("brightness") of quality. For the fruity odorants d-limonene (orange rind), n-butyl acetate (banana), and t-butyl alcohol (blueberry), the point occurs just twofold above the detection threshold. For the less bright material, ozone (electric arc odor), it occurs sixfold above. As long as confidence ratings co-exist with measures of detection, one can actually use any desired level of confidence to specify recognition points. Distributions of high and very high confidence within and across odorants can serve as iso-recognition contours.

#P274 **Poster session VI: Chemosensory development and Psychophysics I**

Time encoded in smell

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Odor perception is bound by the primary dimensions of odor intensity and odor pleasantness, that have both been systematically linked to odorant molecular structure. However, higher-order dimensions of olfactory perception remain largely unexplored. Here we set out to ask whether humans can put a time label on an odor ("how long has this milk been open?"), or in other words, ask whether "time" is a dimension in smell. Considering that materials break-down over time, we hypothesized that as more kinds of molecules are added to an odor mixture, it will be perceived as older. Using a visual-analogue scale (VAS), 20 subjects (age <40)rated the intensity (high-low), pleasantness (good-bad), and time (old-new) of 40 odorants (ISI = 30s) at 5 levels of molecular mixture complexity (8 monomolecular odors, 8 bimolecular, etc., up to 8 pentamolecular mixtures). Rating of "time" was unrelated to rating of intensity, but significantly correlated with rating of

pleasantness (r=.275 p=.006), whereby worse smells smelled older. Repeated measures regression analysis, with "time" as a dependent and "intensity" and "pleasantness" as covariates, provided no significant results. However, the means of mixture levels 1 to 4 was in the expected direction, as revealed by a trend in the regression analysis excluding level 5 (F=3.28, p<.08). This trend in the expected direction merits an increase in sample size, combined with theory-based reconsideration of the odorant molecules selected.

#P275 **Poster session VI: Chemosensory development and Psychophysics I**

A view of the world through the nose

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Olfaction differs from other distal senses in its pronounced quantal temporal sampling. Whereas audition is continuous, and vision may be broken into saccades, olfaction consists of pockets of information gathering at the frequency of sniffing, typically 3 to 7 Hz in mammals. Since humans are primarily visual animals, and our cognition is dominated by visual perception, we have found it difficult to consider the implications of this temporal sampling dynamic. To overcome this obstacle to introspection, we have built a device that links visual input to sniffing. The device consists of goggles that use liquid crystal light shutters as lenses (Balder optoelectronic elements, Slovenia). These lenses are linked to airflow sensors placed at the entrance of the nostrils. The goggles are opaque at rest, but increase in transparency as a function of nasal inhalation. Put simply, the device enables seeing as a function of sniffing. The device is equipped with dual video cameras, one pointed at the direction of gaze, and one pointed at the users own eyes. All components feed into a backpack mounted logging device that records the nasal airflow parameters and collects the video images. The backpack provides sufficient storage and power for ~12 hours of continuous use. We will present data from one week of continuous uninterrupted use. Analysis will include daily fMRI sessions devised at identifying any reorganization of olfactory-visual connectivity.

#P276 **Poster session VI: Chemosensory development and Psychophysics I**

***Wnt5a* has stage and location specific effects in embryonic tongue epithelium and mesenchyme**

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Wnt5a is a developmental regulator of cell polarity, migration, differentiation and proliferation. Previously we reported a role for *Wnt5a* in regulating embryonic tongue outgrowth but not fungiform papilla number. Continuing studies focus on cell and tissue attributes of epithelium and mesenchyme in the truncated tongues of embryonic (E)12.5 - 18.5 *Wnt5a* null mutant mice, compared with wild type littermates. With Western blots on separated epithelium and mesenchyme, we surveyed Wnt5a and found expression in tongue through E15.5, localized principally in mesenchyme of anterior tongue only. In serial tongue sections, filiform papilla are absent or delayed in development on tip of E18.5 mutant tongues and subepithelial mesenchyme is highly disorganized and reduced in depth. Mutant fungiform papillae do contain distinctive collections of apical cells that presumably are taste bud precursors. Immunoreactions for BrdU (S phase cell labeling) and Ki67 (labels all proliferating cells) were examined in sections from E13.5 and E16.5 tongues, harvested 2 hours after a single dose of BrdU via intraperitoneal injection to the pregnant dam. In preliminary results, at E13.5 BrdU and Ki67 labelled cells are extensive in tongue epithelium and mesenchyme of *Wnt5a* -/- and wild type littermates. However, at E16.5, numbers of BrdU labeled cells are reduced in epithelium and mesenchyme of mutant compared to wild type tongues. Our data demonstrate regulatory roles for *Wnt5a* in anterior tongue tissue integrity and suggest that *Wnt5a* participates in regulation of cell proliferation. Effects are primarily in anterior tongue epithelium and mesenchyme, are more pronounced in later stage embryos and likely contribute to embryonic arrest of tongue outgrowth in Wnt5a mutants.

#P277 **Poster session VI: Chemosensory development and Psychophysics I**

Odor Discrimination Is Influenced By Odor Naming Ability

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The ability to discriminate between odors of different quality is a seemingly simple sensory task that appears to rely minimally on memory or previous experience. However, it has been demonstrated that performance on an odor quality discrimination task can be improved by previous experience or providing information about the stimuli, for example, a list of odor names (de WijK & Cain, 1994). The current study examined the influence of odor naming ability on odor discrimination performance. Study participants completed a 15-trial odor discrimination test composed of 30 common odors delivered using a computer-controlled olfactometer (Osmic Enterprises, Inc.). On each trial the participant was presented with three odor stimuli. Two of the odors were the same and one was different. The participant's task was to pick the "odd" odor. An odor identification task also was completed using the same stimuli presented one at a time with odor quality labels in a typical four alternative, forced choice paradigm. Odor discrimination performance improved significantly when a participant could correctly identify both of the odors being discriminated as compared to when only one or no odors could be identified. These findings support the idea that access to knowledge about an odor has a significant impact on odor discrimination performance.

#P278 **Poster session VI: Chemosensory development and Psychophysics I**

Crossmodal interactions between odors and abstract symbols
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So far, in many crossmodal studies between vision and olfaction, verbal labels, colors, or pictures have been used as visual cues, all of which elicit strong contextual backgrounds, whereas little is known about the relationship between abstract symbols and odors. The aim of this study was to investigate the crossmodal association of an "abstract symbol," designed for representation of an odor, with its corresponding odor. In other words, we explored the application of an abstract symbol for expression of an odor via an extension of the Stroop effect (congruence/incongruence paradigm). Based on the result of an odor-symbol association test, two abstract symbols associated with rose or cheese odor were selected. Two different odors representing "rose" and "cheese" were applied with three types of visual cues (blank screen, and two abstract symbols for rose and cheese odors, respectively), followed by psychometric ratings (olfactory intensity and hedonic response) and recording of event-related potentials. When the odors were presented with their congruent abstract symbols, the hedonic responses of rose and cheese odors were most pleasant for rose odor and most unpleasant for cheese odor. The event-related potential latencies of peaks were also the shortest at all electrode sites tested on condition that the cheese odor was provided with its congruent symbol. In conclusion, our findings demonstrate that an abstract symbol may affect olfactory perception, and indirectly suggest that an abstract symbol could represent its corresponding odor.

#P279 **Poster session VI: Chemosensory development and Psychophysics I**

How big is the gap between detection and recognition of aliphatic aldehydes?
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It is widely agreed that two different measures of olfactory sensitivity can be distinguished: a detection threshold, defined as the lowest concentration at which an odorant can be detected or discriminated from a blank stimulus, and a recognition threshold, defined as the lowest concentration at which an odorant can be assigned a recognizable quality or discriminated from another odorant. It is further widely agreed that the detection threshold is lower than the recognition threshold. Surprisingly few studies, however, have investigated the magnitude of the difference in concentration between olfactory detection and recognition thresholds. It was therefore the aim of the present study to determine olfactory detection thresholds for five aliphatic aldehydes (C4-C8) in a group of 16 human subjects, and to assess the ability of the same subjects to discriminate between the same odorants presented at different concentrations above their individual detection thresholds. We found that as a group the subjects significantly discriminated between 4 of the 10 odorant pairs when presented at a factor of 100, and 7 of the 10 odorant pairs when presented at a factor of 1000 above the individual detection thresholds. The 3 remaining odorant pairs were not discriminated above chance level even when presented at a factor of 1000 above detection threshold. However, single subjects successfully discriminated between certain aldehyde pairs presented at a factor as low as 3 above detection threshold. Further, a significant negative correlation between discrimination performance and structural similarity of the aldehydes tested was found. The results demonstrate that the gap between detection and recognition of aliphatic aldehydes is odorant pair-dependent but – at the group level – spans at least a factor of 100.

#P280 **Poster session VI: Chemosensory development and Psychophysics I**

Comparison of odor threshold for phenylethylalcohol and butanol
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Aim of the study was to compare the results of odor threshold test using different number of dilution steps, separately for butanol and phenylethylalcohol (PEA). A total of 125 subjects participated (30 patients with olfactory dysfunction, 95 normosmic controls). In 2 sessions the olfactory threshold for butanol and PEA was examined with 8 (wide step method) and 16 (narrow step method) dilutions using felt tip pens. Test time was shortened by approximately 2 minutes and remained more constant when using the wide step method. Butanol and the PEA threshold were not significantly different; in addition, a significant correlation was found between thresholds for the two odors ($r=0.57$, $p<0.01$). PEA thresholds appeared to be slightly more reproducible than butanol thresholds (test-retest). The data of narrow and wide step method correlated, but the narrow step method appeared to have a higher reliability. Patients with olfactory dysfunction could be distinguished from normosmic subjects using all 4 different tests. The results indicate that threshold testing with PEA is an alternative to butanol. Concerning the reproducibility PEA seems to have an advantage compared to butanol. The wide step method provided similar results as the narrow step method, but required less time.

#P281 **Poster session VI: Chemosensory development and Psychophysics I**

Human Olfactory Detectability of Homologous 2-Ketones and n-Alkylbenzenes
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The study is part of a project that aims to quantify and model the human olfactory detectability of a variety of volatile organic compounds (VOCs), using carbon chain length and chemical functional group as units of chemical change to develop quantitative structure-activity relationships (QSARs) for odor potency. Stimuli comprised 2-ketones (acetone, pentanone, heptanone, and nonanone) and n-alkylbenzenes (toluene, ethyl,

butyl, hexyl, and octyl benzene). We employed dynamic olfactometry, natural odor sampling (sniffing), a three-alternative forced-choice procedure against carbon-filtered air blanks, and a large, tightly controlled, vapor stimulus source quantified via gas chromatography. Concentration-detection, i.e., psychometric, functions, from which odor detection thresholds (ODTs) can be determined, constituted the outcome of interest. Among ketones, ODTs decreased from acetone to 2-heptanone, remaining constant for 2-nonanone. Among alkylbenzenes, ODTs decreased from toluene to butyl benzene, then increased for hexyl and further for octyl benzene. These ODT trends resembled those found in the literature but the absolute ODTs were consistently among the lowest previously reported. Interindividual variability of ODTs amounted to about one order of magnitude. The outcome provides additional support to the key role played in odor potency by physicochemical parameters governing the transfer of the odorant from the vapor phase (where it enters the nose) through the various biophases (from the nasal mucus to the olfactory receptor environment), but it also indicates that, at some point, molecular dimensions begin to put a limit, and even reverse, the gain in olfactory potency observed with increasing carbon chain length. Supported by grant number R01 DC002741 form the NIDCD, NIH.

#P282 **Poster session VI: Chemosensory development and Psychophysics I**

Cigarette Smoking and the Olfactory Detection of Cyanide

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Some organic chemists of a bygone era claimed smoking was linked to an increased sensitivity to cyanide and so they justified smoking because cyanide exposure was a profession hazard. The purpose of this study was to text this claim. Smokers and nonsmokers between the ages of 18 and 22 were ask to rate the intensity of a series of odors using the Green Scale (*Chemical Senses 21*:323-334, 1996). Olfactory threshold measurements were also determined for four odorants. Included in the odorants used for the intensity ratings were almond oil, ground-up peach pits and trans-2-hexenal. Almond was included because it has a cyanide-like smell, peach pits because they contain cyanide compounds and trans-2-hexenal because it closely resembles the molecular structure of cyanide. Two of the odors used for the threshold measurements were almond oil and trans-2-hexenal. Confirming what has been reported in many other studies, on the average, smokers gave lower intensity ratings and had higher thresholds compared to nonsmokers. However the intensity ratings for trans-2-hexenal were not as reduced when compared to the ratings for the other odorants and its threshold was not reduced by smoking. As a possible explanation for this observation, perhaps the cyanide like compounds found in some cigarettes are facilitating olfactory detection for this compound and this facilitation offsets the overall decrease in olfactory ability that often accompanies smoking. In conclusion, although there may be some validity in the claims of the organic chemists, these results suggest they would have been even better at detecting cyanide if they had not smoked at all.

#P283 **Poster session VI: Chemosensory development and Psychophysics I**

Retronasal olfaction influences swallowing

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Objectives: Identical ortho- and retronasal stimuli are processed differently. Since retronasal odorant perception is strongly associated with food intake and flavour, an influence of retronasal stimulation on swallowing can be assumed. It was the aim of our study to evaluate the impact of retronasal olfaction on swallowing. **Subjects and Methods:** Fifty normosmic and normogeusic subjects (23 male, 24 female, mean age: 24 .2 years, range: 18-42) took part in the study. Olfactory stimuli (vanillin, 40 % vol/vol) were presented randomized ortho- and retronasally (8 stimuli each) using a computer-controlled olfactometer. Simultaneously a sweet taste (glucose) was continuously delivered through a taste dispenser which was kept within the mouth. Ultrasound examination of the mouth floor was continuously recorded on a videotape to monitor swallowing activity. The videotapes were evaluated by one of the authors´ blinded concerning place of stimulation (ortho- vs. retro). **Results:** After retronasal stimulation swallowing not only occurred faster (7.49 vs. 9.42s; $p < 0.001$) but also took place more often compared to orthonasal stimulation (1.38 times vs. 1.14 times; $p<0.001$). **Conclusion:** These results show that retronasal olfaction differentially affects process related to food intake and flavor perception, such as swallowing, compared to orthonasal olfaction. From the clinical point of view this knowledge might be used to support swallowing training in patients having swallowing disorders.

#P284 **Poster session VI: Chemosensory development and Psychophysics I**

Rapid plasticity in the olfactory system modulates detection threshold in an odorant-specific manner

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Although the olfactory sense demonstrates a high degree of adult neuronal plasticity, the functional benefits thereof remain a matter of debate. We explored whether aversive conditioning of an odorant may enhance absolute detection threshold in a target-specific manner. Using a between-groups design, participants were exposed to brief pulses of two indiscriminable enantiomer pairs (odorants A1, A2; B1, B2) by means of air dilution olfactometery; a weak electric shock was paired with either odorant A1 (experimental group) or B1 (control group). Olfactory detection threshold for A1, as well as skin conductance responses (SCR) for all odorants, was measured before and after conditioning in both groups. In each group, elevated SCR were observed only in response to conditioned stimuli, thus demonstrating successful conditioning. Neither the pre-conditioning A1 detection threshold nor ratings of shock intensity during conditioning differed between groups. A repeated

measures ANOVA demonstrated a significant interaction between groups and pre-/post-conditioning A1 detection thresholds; the Experimental group demonstrated a significant increase and the Control group a significant post-conditioning sensitivity decrease, relative to pre-conditioning. Re-testing of olfactory sensitivity to A1 two months post-conditioning indicated no difference between the two groups. Taken together, these data demonstrate that the olfactory system exhibits rapid plasticity with the functional benefit of a sharp and odorant specific increase in absolute detection threshold.

#P285 **Poster session VI: Chemosensory development and Psychophysics I**

Effects of sub-threshold odorants on rapid olfactory adaptation in human observers

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In previous presentations we have described use of a novel psychophysical technique for estimating the onset time course of perceptual odor adaptation in humans. The premise of the technique is that extended presentation of an odorant will produce adaptation, decreasing the sensitivity of the receptor and increasing thresholds for a brief, simultaneous target odorant presented at various time-points after the adapting stimulus onset; where both the adapting odorant and the target odorant are the same (i.e., self adapting). Those results suggested that perceptual odorant adaptation was measureable within 100-200 ms after stimulus onset. In those initial studies, the adapting odorant concentration for each subject was set to twice the baseline threshold for the 600-ms target (i.e., the same level relative to threshold). In this presentation, using the same stimulus paradigm, we report changes in perceptual adaptation collected with the adapting odorant set to different *subthreshold* concentrations levels. To characterize the effect of adapting odorant level we used a liquid-dilution olfactometer to estimate two-bottle discrimination thresholds for brief (600 ms) presentations of vanilla odor; 13 volunteers (ages 18-21) served as subjects. Adapting odorant concentration levels were then set to 0.25, 0.5, 0.75 and 1 times threshold concentration for each subject and threshold for the 600-ms target was measured as a function of the relative delay between the onset of the adapting stimulus and the onset of the target. The results demonstrate that significant increases in odorant sensitivity were evident even at subthreshold adapting odorant levels. The time course of this subthreshold adaptation was similar to that measured with suprathreshold adapting odorants.

#P286 **Poster session VI: Chemosensory development and Psychophysics I**

Update on Racial and Gender Differences in Odor Perception

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For the past four years we have been screening African-American and Caucasian men and women for sensitivity to a select group of odorants, viz., 5-a-androst-16-en-3-one (androstenone; having a urinous or sandalwood odor), amyl acetate (banana- or pear-like odor), Galaxolide (musky), isovaleric acid (sweaty), 3-methyl-2-hexenoic acid (3M2H; sweaty), pentadecalactone (musky), benzyl salicylate (balsamic), muscone (musky), Jeger's Ketel (woody, amber), 2-nonenal (unpleasant, fatty), skatole (fecal) and geosmin (earthy), selected because some people have a specific anosmia to the odorant. Psychophysical testing is performed in a single session and DNA is obtained for subsequent analysis. Phenotypic differences between the two racial groups and between men and women have been observed. Caucasians have a significantly greater rate of specific anosmia to androstenone (7%) than do African-Americans (0%). The opposite is true for benzyl salycilate (Caucasians, 22%; African-Americans, 35%). Disregarding race, men exhibit higher rates of specific anosmias for amyl acetate (3% vs 0%), Jeger's Ketel (7% vs. 2%) and androstenone (10% vs. 3%) than do women. Among individuals who can smell the odorants, Caucasians have higher olfactory detection thresholds for 3M2H (0.0058 w/v), Galaxolide (0.0407% v/v) and isovaleric acid (0.0020% w/v) than do African-Americans (respectively, 0.0024%; 0.0092%; and 0.0006%). Data collection continues and the above is subject to change.

#P287 **Poster session VI: Chemosensory development and Psychophysics I**

Taste cell specific over-expression of BDNF leads to multiple fold increase of expression levels of BDNF and increased size and number of taste buds

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Brain-derived neurotrophic factor (BDNF) is the most potent neurotrophic factor in the taste system during development, and is also expressed in taste cells of adult mice. We over-expressed BDNF using a promoter for alpha-gustducin to study the roles of BDNF in the adult taste system. Gustducin is normally expressed in type II receptor cells that also express G-protein coupled taste receptors. Taste buds were isolated using laser capture microdissection from circumvallate papillae of six weeks old gustducin-BDNF taster mouse lines. RNA was isolated and real time PCR analysis revealed a six-fold increase of BDNF in two transgenic mouse lines. We also employed immunohistochemistry on taste papillae of gustducin-BDNF mice using Troma-1 antibody which identifies taste buds. In addition, we analyzed tongue surface and papillae morphology using scanning electron microscopy. Circumvallate papillae are wider in gustducin-BDNF compared to wild-type mice. Additionally, circumvallate taste buds in high BDNF expressing transgenic mice are wider and

taller compared to wild-type mice. Fungiform taste bud size and papillae number are also significantly higher in high BDNF expressing transgenic mice compared to wild-type mice. We are currently performing microarray analysis to examine gene expression profile in Gust-BDNF mice.

#P288 **Poster session VI: Chemosensory development and Psychophysics I**

Dreams and Smell - The Impact of Nocturnal Olfactory Stimulation on Dreams

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Only a limited number of trials is available regarding the impact of external stimulation on dreams. An incorporation of stimuli into dreams is depending on the intensity and significance of the stimulus. Olfactory stimuli have hardly been investigated in this context. Aim of the study was to assess whether olfactory stimuli are incorporated into dreams and whether they influence dream emotions. 15 female volunteers were investigated during 30 nights (first night for adaptation). Standardized awakenings were performed during REM sleep under constant monitoring of sleep stages. Subjects were exposed to non-odorous control, a positive (PEA, 20%) and a negative odor (H2S, 4 ppm) for 10 seconds each during REM sleep in a randomized fashion via a computer olfactometer. After awakening, subjects were advised to report dream content in a standardized fashion and to rate dream emotions (positive and negative) on a four digit scale (0 to 3: no to strong feelings). Dreams were analyzed by an independent investigator for potential incorporations. The overall emotional coloration was calculated. Direct incorporation was detected in one dream only (neutral condition), indirect incorporation (contents associated with smelling) was detected in four dreams without relation to stimulus condition. Mean emotional coloration after control stimulation was slightly positive (+0.5). After negative stimulation, it was shifted to negative values (-0.4) while the mean emotional coloration was more positive after positive stimulation (+1.2), the difference reaching statistical significance. With olfactory stimulation direct incorporation of olfactory stimuli into dreams does not seem to appear. In contrast, the emotional coloration of dreams can be significantly influenced according to the hedonic aspect of the stimulus.

#P289 **Poster session VI: Chemosensory development and Psychophysics I**

Effects of Septoplasty — A Pre- and Postoperative Study on Trigeminal Sensitivity and Olfactory Performance

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Surgery of the nose may have an impact on olfactory performance and trigeminal sensitivity. Aim of the present study was to investigate the actual extent of these effects in clinical practice. Normosmic healthy subjects (n=43) and patients who underwent septoplasty (n=38) were tested immediately before and up to three months after surgery, this interval was matched in the control group. In both sessions olfactory performance and trigeminal sensitivity were assessed using electrophysiological (event-related potentials [oERP], [tERP], stimuli: H_2S and CO_2) as well as psychophysical methods (odor identification test, CO_2 detection threshold, CO_2 pain threshold, detection of stimulus duration for CO_2). Ability to identify odors was superior in the control group ($p=0.026$). There was no significant group effect regarding oERP, furthermore no interaction "pre/post" * "group" ($p=0.60$). Concerning trigeminal detection threshold and pain threshold no differences were observed among groups. In addition, a relative decrease in N1 amplitudes ($p=0.004$) combined with a relative increase in P2 latencies ($p=0.088$) was present in the patient group after septoplasty. However, no significant difference between the first and second session could be detected. Trigeminal sensitivity does not seem to differ among the more and less obstructed side of nose. Furthermore the results give reason to presume that there is a difference in trigeminal sensitivity between patients and controls. Even though septoplasty is a matter of massive manipulation of nasal mucosa, these findings indicate no major effect of surgery on olfactory performance or intranasal trigeminal sensitivity.

#P290 **Poster session VI: Chemosensory development and Psychophysics I**

Putative Human Pheromones Increase Women's Observed Flirtatious Behaviors and Ratings of Attraction

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Mammalian conditioning paradigms suggest that androstenol conditions hormonal effects in women that are unconsciously associated with the potential behavioral affects of androsterone. We evaluated individual video-taped fifteen-minute interactions fourteen ovulatory-phase women during a cooperative task. During the task, our male accomplice wore either a standardized androstenol / androsterone mixture diluted in propylene glycol, or he wore the diluent (i.e., propylene glycol). Sandalwood odor was added to the mixture and to the propylene glycol to keep our accomplice blind to his condition. Women were more likely to display flirtatious behaviors when our accomplice was wearing the mixture than when he wore the diluent ($t(12) = 4.38$, $p < .01$; IRR: $r = .914$, $p = .01$). Specifically, they were more likely to make eye contact with our accomplice ($t(12) = 3.43$, $p = .01$; IRR: $r = .964$, $p = .01$) and they laughed more during the interaction ($t(12) = 5.20$, $p < .01$; IRR: $r = .810$, $p = .01$). There was no significant effect of the mixture in the women's rating of our accomplice as being more intelligent, more comfortable to be around, funnier, more "good-looking," or in having our accomplice as a task partner again. However, when our accomplice was wearing the mixture, the women rated themselves as being more attracted to him ($t(12) = 2.786$, $p = .016$). Our results suggest that combining the known hormonal effects of androstenol (e.g., on luteinizing hormone) and the possible behavioral affects of androsterone extends non-human animal models of olfactory/pheromonal communication to humans. Our disclosed mixture may help to better characterize species-specific human pheromones.

#P291 **Poster session VI: Chemosensory development and Psychophysics I**

Effects of Video Game Console and Snack Type on Snack Consumption During Play
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Past research has shown the effects of distraction on food intake. The present study examined the effects of snack type (healthy, unhealthy, and neutral) on snack consumption while playing a variety of video games. Participants wore a device to measure the amount of their movement during the session. For one of the conditions, the participants played the Nintendo Wii's boxing game that comes equipped on *Wii* Sports. The participants warmed-up for five minutes and continued to play the game for the duration of the fifteen-minute session. For another condition, the participants played the Xbox version of *Rocky Legends* on exhibition mode. The participant warmed-up for five minutes and then continued to play for the duration of the fifteen-minute session. The third condition was used as a control, and the participants sat in an empty room for the duration of fifteen minutes. Before and after the video game play or the control session, the participants' physiological measurements were taken. Three different snack types (healthy, unhealthy, and neutral) were left in the room. For each participant 38 grams of pretzels, 160 grams of carrots, and 100 grams of M&M's were presented. Overall activity level was greatest in the Wii condition indicating significantly more calories burned, and participants ate less in that condition. Although both the Wii and X-Box conditions showed less snack consumption in general, participants ate more healthy snacks in the Wii condition.

#P292 **Poster session VI: Chemosensory development and Psychophysics I**

Effects of Peppermint Scent Administration on Increasing Nintendo Wii Guitar Hero Performance
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Previous research has shown the benefits of peppermint in a variety of situations. The current study used 60 participants to compare performance, mood, and perceived task load in video game play. Participants were randomly assigned to either the experimental (peppermint scent administration) condition or control (no scent) condition. Each participant played 5 songs on Guitar Hero III on the Nintendo Wii game system on 3 occasions. Analyses were performed to determine effects of peppermint scent administration on learning, attention, mood, and perceived workload using appropriate ANOVA techniques. The results show that participants performed significantly better in the peppermint scent administration condition and felt their mental demand and effort throughout the songs were less than the control group. These results provide further evidence of pepperment scent administration to enhance learning and performance.

#P293 **Poster session VI: Chemosensory development and Psychophysics I**

Deposition of inhaled particles in the olfactory region in rat and human nasal cavities during breathing
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Inhalation of airborne particles represents another route of mass transport that may directly expose olfactory neurons to aerosolized chemicals: odorants or toxic agents. Furthermore, insoluble nano-particles deposited in the rat olfactory region can translocate to the brain via the olfactory nerve. Although there have been abundant in-vivo, in-vitro experiments and numerical study of aerosol deposition in the nasal cavity of laboratory animals, there is limited information about the effects of anatomical difference on the aerosol deposition and to which extent animal data can be extrapolated to humans. In this study, the deposition of nano to coarse particles (1nm~10um) in human and rat nasal cavity was numerically investigated and compared under steady inspiratory flows ranging from restful breathing to strong sniffing. The results showed that the deposition rates and patterns strongly depended on particle size, flow rates and the anatomical differences between the species. With the increase of flow rate, the deposition rate decreases for nano-particles, however increases for coarser particles. In general, the rat nasal cavity has much higher deposition efficiency than human. At restful breathing, the deposition rate of nano-particle (3nm) was 82%(25%) and 31%(2%) in the rat and human nasal cavities (olfactory region) respectively. For coarse particle (4um), the deposition rate in rat was 79% and reduced to 6% in human. We further perturbed the nasal valve and olfaction region in our human model and demonstrated more significant impact on the deposition rate and pattern in the olfactory region than in the whole nasal cavity. These results warrant the caution when translating aerosol deposition data derived from laboratory animals to human or to a specific individual.

#P294 **Poster session VI: Chemosensory development and Psychophysics I**

The Effects of Orally Administered Capsaicin on Rat Taste Bud Volume and Papillae Morphology Throughout Development
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After chorda tympani nerve transection, there is a permanent and drastic reduction in the number of taste buds when rats receive the surgery as neonates, but not as adults (Sollars, 2005). Additionally, neonatal rats experience a greater decrease in taste bud volumes than adults after lingual nerve transection, however after 50 days, neonates' taste buds regenerate to their normal size (Gomez & Sollars, 2006). Because capsaicin is known to be a neurotoxin to lingual fibers in fungiform papillae (Nagy, et al., 1982), this chemical was used to determine if orally administered capsaicin yields similar effects on the tongue as those observed with lingual transection. Female Sprague-Dawley rats were given 2% of their body weight of either a 15% sucrose solution (SUC) or a capsaicin (.0033 mM) in 15% sucrose solution (CAP) every day for 36 days. The neonatal group began treatment on postnatal day 5 (P5), while the adult group began treatment on P45. Neonates were administered solutions by hand using a cotton-tipped applicator until P12, and then via a pipet until P24. All

animals over the age of P24 were given solutions using a drinking tube. Animals were sacrificed 2 days after their last treatment to observe immediate effects of the treatment. Contrary to what might be expected given previous research, adult rats' taste buds were more susceptible to capsaicin than neonates. Specifically, adults treated with CAP had significantly smaller taste buds 2 days post treatment than adults treated with SUC. Such an effect was not observed in neonates. Tongues were also analyzed 50 days post treatment to identify any possible regeneration of volumes, and surface analyses were performed at both 2 and 50 day time points to determine papillae count and morphology.

#P295 **Poster session VI: Chemosensory development and Psychophysics I**

Competitive Effects of GSP and IX Nerve Transection on the Maturation of CT Terminal Field Volume in the Nucleus of the Solitary Tract

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Neural competition among multiple inputs can affect the organization of circuits in many sensory systems; however, this has not been examined in the developing gustatory system. The degree of overlap among the greater superficial petrosal (GSP) nerve, the glossopharyngeal (IX) nerve, and the chorda tympani (CT) nerve terminal fields in the rostral nucleus of the solitary tract (NTS) is greatest early in development and decreases as animals age to adulthood. To assess the effects of lack of competition from the GSP and IX on the development of the CT terminal field, we sectioned the GSP and the IX at postnatal day 15 (P15), P25 or P65. At 35 days post nerve section, the CT was labeled and its terminal field was subsequently imaged and analyzed. The results show that sectioning GSP and IX at P15 results in a CT terminal field volume at adulthood that is similar to P15 controls and much greater than that of adult, uncut animals. Therefore, the CT terminal field does not reorganize and prune back as it does in normal development when competitive influences from GSP and IX are removed. Animals in which GSP and IX were cut at P25 or P65 also have CT terminal field volumes comparable to P15 controls. This indicates that the CT nerve remains plastic into adulthood and is still subject to the effects of competition. Preliminary evidence shows no changes in peripheral function of the CT nerve prior to GSP and IX regeneration, and significant loss (or absence) of palatal and posterior tongue taste buds at 30 days post nerve section. These studies provide evidence that competition between individual inputs to the gustatory system plays a role in organizing the terminal fields.

#P296 **Poster session VI: Chemosensory development and Psychophysics I**

Postnatal development of trigeminal neuronal sensitivity to capsaicin, nicotine, and innocuous cooling

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Changes in behavioral repertoire that occur over the course of maturation may reflect changes in peripheral neuronal populations. We examined trigeminal neuronal sensitivity in neonatal (2-3 day) and adult (5 week) rats to capsaicin (CAP, 200nM), nicotine (NIC,100uM) and cooling (31.5 to 18C) using intracellular calcium responses as an index of neuronal sensitivity. The overall proportions of neurons sensitive to each of the stimuli remained the same over 5 weeks of development. However, the proportion of different subtypes of neonatal and adult neurons sensitive to 1, 2 or 3 of the stimuli changed. Percentages were calculated on the basis of total number of neonate or adult neurons tested (n=304 and 580) and sorted into subtypes responsive to none,1,2, or 3 stimuli. In nicotine-sensitive neurons, the proportion of CAP-sensitive cells was similar in neonates and adults. However, the proportion of cells sensitive to both cooling and nicotine was higher in neonates than adults. This difference between neonates and adults was most pronounced in the proportion of cool-sensitive neurons also responsive to capsaicin (8.5% vs 4.4%, respectively). Consistent with neonatal behavioral aversion to cooling, these neurons are likely nociceptors that are also sensitive to innocuous cooling. The capsaicin dose-response function differed insignificantly (t-test, p=0.12) in terms of EC50 in the two age groups (adult, 72.5±4nM, n=137; neonate, 55.6±10.9nM, n=112), while the S-nicotine concentration (0.1-1000uM) response function was nearly identical in neonatal and adult trigeminal neurons. Our data suggest that changes in neuronal subtypes may account for changes in behavioral phenotype and for some purposes, neonatal trigeminal neurons may not be a good model for neuronal response patterns of the adult.

#P297 **Poster session VI: Chemosensory development and Psychophysics I**

Development of the olfactory organ in fish: a comparison

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Location and shape of the olfactory organ as well as structure and development of the olfactory epithelium (OE) vary greatly in ray-finned fish (Actinopterygii). There is still considerable lack of information regarding the development of the olfactory organ in many basal taxa. This information is crucial for reconstruction of the evolution of this sensory system and the phylogeny of osteognathostomes (the monophyletic group of osteichthyan fishes and tetrapods). In this study we examined the development of the olfactory organ in "primitive" groups that are close to the dichotomy of ray-finned fishes and lobe-finned fishes and tetrapods. We employed LM, TEM and SEM methods and compared the development of the olfactory organ in *Polypterus* (bichir) to that of *Acipenser* (sturgeon) and teleosts. Our results indicate the following plesiomorphic (primitive) character states in bichirs: The primary olfactory pit is formed by invagination of

the epidermal and the subepidermal layer (as in *Acipenser* and *Xenopus*). The incurrent and excurrent nostrils derive from a single primary opening (as in *Acipenser* and many teleosts). The OE derives from an epidermal and a subepidermal layer (as in *Acipenser* and *Xenopus*). Apomorphic (derived) features are an internal lumen as primordium of the future olfactory chamber. A subepidermal layer gives rise to the OE (as in teleosts). As to the origin of the supporting cells in *Polypterus* we assume a combination of plesiomorphic and apomorphic characters. We conclude that *Acipenser* and *Xenopus* exhibit the most widely distributed features among basal gnathostomes and thus ancestral osteichthyan character states in the development of the olfactory organ. *Polypterus* and teleosts both show a mixture of plesiomorphic, apomorphic, and probably convergently derived features.

#P298 Poster session VII: Chemosensory Psychophysics II

The influence of suprathreshold gustatory stimuli on resting respiration in humans

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Until now there is no widespread method for objectivying taste perception. Such a method would be useful for expert opinions in the appraisal of gustatory disorders. In dysosmia an objectivization with olfactory evoked alteration of breathing patterns is already established. The aim of our study was to evaluate an analogous method for the gustatory sense. A continuously flow of water was presented to the tongue of 34 subjects. In this stream 2 seconds lasting gustatory (sweat, sour, salty, bitter) and control stimuli were inserted without additional tactile stimulation. Regulary resting respiration was required for at least 5 breaths before the stimulus was applicated. With a differential pressure transducer nasal respiration was unilaterally measured and analyzed with Labview. In the result gustatory stimuli lead significantly more often to changes breathing pattern than the control stimuli. No difference between the sweet, sour, salty and bitter taste could be measured. The first breath after the stimulus showed a more often prolonged ex- and inspiration. The second and third stimuli lead to a more often shortening of ex- and inspiration. Maybe this is due to an orienting reaction. This new method could provide benefit in medical reports of gustatory dysfunction.

#P299 Poster session VII: Chemosensory Psychophysics II

Selective Inhibition of Taste in Humans by Cathodal Current

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Electrical taste effects in humans have been known since the time of Volta. The positive anode when applied to the tongue produces a metallic or battery sensation at a 10-30 μA threshold that is used to provide an objective measure of taste performance. The normal threshold for the negative cathode is 10 times higher and produces a prickling sensation. Any accumulation of electrolysis products (acid and base) on the tongue's surface is excluded by use of an intervening conducting solution. The role of ions in human salt tastes was addressed by applying cathodal currents of 40 to 80 μA to the tongue tip through supra-threshold solutions of sodium chloride, sodium bromide, potassium chloride, ammonium chloride, calcium chloride, sodium nitrate, sodium sulfate, sodium saccharin, sodium acetate and sodium benzoate; which together encompass *salty*, *bitter*, *sour* and *sweet* taste qualities. With this arrangement, we demonstrate that <100 μA cathodal currents selectively block tastes of halide salts ($p = 0.001$-0.0001) and the cathode "off" taste increment is equivalent to anode "on" and independent of the conducting solution ($p = 0.03$-0.0001). We conclude that (1) metallic anodal taste involves stimulation via ion channels that are independent of apical membrane taste receptors, (2) cathodal current withdraws stimulatory cations from the taste receptors, (3) ionic taste stimuli with tastes due primarily to the anion component are not inhibited by cathodal current and (4) neither anodal "on" taste nor cathodal "off" taste is caused by electrolysis products.

#P300 Poster session VII: Chemosensory Psychophysics II

Rare Subjects with the TAS2R38 AVI/AVI "Non-Taster" Diplotype Perceive Propylthiouracil as Bitter: The Quest for the Rescue

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The bitter taste receptor TAS2R38 accounts for the majority of perceptual variation to the thyroid drug 6-*n*-**prop**ylthiouracil (PROP). The two most common alleles of TAS2R38 are AVI and PAV, after the Alanine/Proline, Valine/Alanine, Isoleucine/Valine polymorphisms at residue positions 49, 262, and 296, respectively. The AVI receptor is weakly activated by PROP and the PAV receptor is strongly activated. However, while most subjects with the AVI/AVI diplotype are either very weakly or un-responsive to PROP, occasionally these subjects find PROP very bitter. Thus, there are clearly other factors in addition to TAS2R38 polymorphisms that account for individual differences in PROP perception. We hypothesize that another hTAS2R receptor is rescuing this function. hTAS2R3, hTAS2R4, and hTAS2R5, are related to hTAS2R38 both by sequence similarity and proximity on chromosome 7 and appear to be recent repeats of TAS2R38. We investigate SNPs in hTAS2R1 as well, since is closely related to hTAS2R38 but resides on chromosome 5. We are testing whether any rare alleles of these genes are associated with sensitivity to PROP.

#P301 Poster session VII: Chemosensory Psychophysics II

Examination of PROP Taste Recognition Thresholds and Suprathresholds with Edible Taste Strips

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The goal of this study was to examine taste recognition thresholds and suprathresholds for 6-propyl-2-thiouracil (PROP). Taste intensities and hedonics values were examined by presenting each subject with edible strips that contained varying amounts of PROP. Normalized taste intensity values with the gLMS reached maximal values with strips that contained 400 nmoles of PROP. Taste strips with 400 or 600 nmoles of PROP were then used to

classify subjects as PROP non-tasters, moderate tasters, or supertasters. The classification of PROP tasters was determined by comparing PROP taste intensity to edible taste strips that contained 140 nmoles of NaCl. Next, taste recognition thresholds for PROP were examined by the ascending limits method, and the method of reversals. In our subject population (n = 100), approximately 80 percent of subjects could detect PROP as bitter. For PROP tasters, taste recognition thresholds spanned two log units with an upper limit of 130 nmoles. These taste recognition thresholds were over one order of magnitude lower than those reported with aqueous tests. Subjects were then tested for their ability to detect PROP with their nasal passages occluded. Taste recognition thresholds for half of the subject population were identical in both the absence and presence of nose clamps. The remaining subjects detected PROP at the next higher or next lower amount. Edible taste strip technology is a highly sensitive and promising method for examining bitter taste function. These strips readily identify taste blindness to PROP, and can identify moderate tasters and supertasters. Future studies will correlate PROP taster status with genotype analysis of the *TAS2R38* gene, and how the ability to detect PROP may affect overall bitter taste function.

#P302 Poster session VII: Chemosensory Psychophysics II

B6 mice display confusion in behaviorally discriminating between quinine and citric acid

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In rodents, some taste-responsive neurons respond to both quinine and acid stimuli. There is also evidence in the literature suggesting that, under certain circumstances, rodents display some degree of difficulty in discriminating quinine and acid stimuli. Here, male C57BL/6J mice (n=10) were explicitly tested in a quinine vs. citric acid discrimination task. Water-restricted mice were first trained in a two response-operant discrimination procedure to lick a response spout upon sampling from an array of sucrose concentrations and to lick another response spout upon sampling from an array of citric acid concentrations. Correct responses were reinforced with water and incorrect responses were punished with a time-out. The mice were then tested for their ability to discriminate between other pairs of taste stimuli. Mice had severe difficulty discriminating citric acid from quinine and 6-*n*-propylthiouracil (PROP) with performance slightly, but significantly, above chance. In contrast, mice were able to competently discriminate sucrose from citric acid, NaCl, quinine and PROP. In another experiment, mice that were conditioned to avoid quinine by pairings with LiCl injections (n=8), subsequently suppressed licking responses to quinine and citric acid and, to a lesser extent, NaCl, but not to sucrose in a brief-access test (25 min session, 5 s trials) relative to NaCl-injected control animals (n=7). However, mice that were conditioned to avoid citric acid significantly suppressed licking only to that stimulus compared with controls (n=8/group). Collectively, the findings from these experiments suggest that in mice, citric acid and quinine substantially share chemosensory features making discrimination difficult but are not perceptually identical.

#P303 Poster session VII: Chemosensory Psychophysics II

Non synomymous SNPs in human tas1r1, tas1r3, mGluR1 and individual taste sensitivity to glutamate

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Human subjects show different taste sensitivities to monosodium glutamate (MSG), some of them being unable to detect the presence of glutamate. Our objective was to study possible relationships between non-synonymous single nucleotide polymorphisms (nsSNP) in human *tas1r1, tas1r3*, as well as other putative receptor coding genes (mGluR4 and mGluR1), and the taste sensitivity of each subject to glutamate. The sensitivity was measured using a battery of tests to distinguish the effect of sodium ions from the effect of glutamate ions in monosodium glutamate. A total of 142 genetically unrelated Caucasian French subjects were considered: 27 non tasters to glutamate (specific ageusia), 21 hypo-tasters and 94 tasters. Expression of *tas1r1* and *tas1r3* was tested on fungiform papillae by RT-PCR on a sample of subjects from all groups. nsSNPs were determined by genomic DNA sequencing. The frequency of the mutations was compared across subject groups. All subjects, including those presenting a specific ageusia for glutamate**,** expressed the candidate heterodimeric receptor Tas1R1-Tas1R3**.** Ten nsSNPs were identified in *tas1r1* (n=3), *tas1r3* (n=3) and *mGluR1* (n=4) genes**.** *mGluR4* only showed 3 silent SNPs. Two mutations -C329T in *tas1r1* and C2269T in *tas1r3*- were significantly more frequent in non tasters compared to tasters whereas G1114A in *tas1r1* was more frequent in tasters. Other nsSNPs including T2977C in mGluR1 and more rare nsSNPs are also involved but, using these three genes, a part of the interindividual variance remains unexplained. Some of the nsSNPs reported here can partly explain the observed individual differences of sensitivity to glutamate and the taste of glutamate may involve also other receptors than those considered here.

#P304 Poster session VII: Chemosensory Psychophysics II

Understanding the Relationship Between Saltiness and Umami

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NaCl and MSG evoke sensations that humans recognize as salty and umami, respectively. However, sensorially the relationship between salt and umami is complex as most foods contain both NaCl and umami compounds and the prototypical umami stimulus, MSG, includes Na+. Herein we sought to determine how perceived salt and umami intensities change as (A) NaCl is held constant and MSG concentrations vary and (B) MSG is held constant and NaCl concentrations vary. In each experiment, panelists were asked to rank aqueous mixtures of NaCl and MSG at varying concentrations according to perceived saltiness and/or umami intensity. As expected in exp 1, increasing MSG while holding the NaCl concentration constant resulted in greater perceived umami intensity. Similarly, as MSG levels increased, perceived saltiness increased. In exp 2, adding salt to a constant level of MSG increased perceived saltiness but had no effect on

umami intensity. To ascertain the mechanism by which MSG increased perceived saltiness of NaCl solutions (see exp 1), NaCl-MSG mixtures were compared to NaCl only solutions matched for total Na+ concentration using a 2-AFC methodology. When test solutions (MSG+NaCl and NaCl only) contained eqimolar concentrations of Na, observed saltiness enhancement by MSG was eliminated. This suggests that Na+ from MSG is responsible for the perceived saltiness enhancement observed in exp 1. Finally, advances in flavor chemistry have identified Na-free compounds that evoke umami sensations (Na-FUs). In exp 4 we investigated if perceived salt and umami intensities change if NaCl levels are held constant and a Na-FU compound is added. Similar to exp 1, adding Na-FU to NaCl increased perceived umami sensation but only marginally increased perceived saltiness at low concentration.

#P305 Poster session VII: Chemosensory Psychophysics II

Perceptual variation in umami taste and polymorphisms in *TAS1R* taste receptor genes

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The TAS1R1 and TAS1R3 G-protein coupled receptors (GPCRs) are believed to function in combination as a heteromeric glutamate taste receptor in humans. In this study, we first characterized the general sensitivity to glutamate in a sample population of 242 subjects using discrimination task and extensively tested a subset of ten subjects at extremes of sensitivity. We followed these experiments by sequencing the coding regions of the genomic TAS1R1 and TAS1R3 genes for a separate set of 87 individuals who were tested repeatedly with monopotassium glutamate solutions and asked to rate umami taste on a general Labeled Magnitude Scale. We established that there is considerable variation in umami taste perception. A subset of subjects at the extremes of sensitivity was subsequently studied in a battery of psychophysical tests validated the observation. In genetic sequencing experiment we revealed that TAS1R3, the shared subunit of the TAS1R sweet and umami taste heteromer receptors, contained more variations than did TAS1R1, contrary to earlier reports. We identified one rare nonsynonymous single nucleotide polymorphism (SNP) and four synonymous SNPs in TAS1R3 that have not been previously reported. Statistical analysis showed that the rare 'T' allele of SNP R757C in TAS1R3 led to a doubling of umami ratings of 25 mM MPG. Other suggestive SNPs of TAS1R3 include the 'A' allele of A5T and the 'A' allele of R247H, which both resulted in an almost doubling of umami ratings of 200 mM MPG. In conclusion, there is reliable and valid variation in human umami taste from L-glutamate. Variations in perception of umami taste correlated with variations in the human TAS1R3 gene. Thus, this receptor is likely contributing to human umami taste perception.

#P306 Poster session VII: Chemosensory Psychophysics II

Monosodium Glutamate Taste Recognition Thresholds are not Affected by Modulation of Serotonin or Noradrenaline Levels in Healthy Humans

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This study was designed to determine the effect of altering serotonin and noradrenaline levels on monosodium glutamate (MSG) taste recognition thresholds in humans. We have previously shown that manipulation of these neurotransmitters lowers taste thresholds of specific taste modalities (Heath et al 2006). A preliminary tongue map for MSG taste was generated in 34 healthy adults (6 male, 28 female, age range 19-71) to determine the most sensitive area of the tongue for further testing. In a further 26 adults (13 male, 13 female, age range 19-48) a series of MSG and sodium chloride solutions were presented either to the back or the tip of the tongue in a pseudorandom order. Recognition thresholds were calculated from psychophysical function curves before and 2 hours after a single acute dose of either paroxetine (serotonin selective reuptake inhibitor), reboxetine (noradrenaline reuptake inhibitor), caffeine (active placebo) or placebo (lactose) in a double blind cross-over design. MSG taste recognition thresholds were significantly lower at the back of the tongue compared to the tip (back 14±3mM, tip, 60±13mM, $p<0.01$). Comparison of thresholds at either the back or the tip of the tongue showed MSG recognition thresholds were not affected by any drug, or by placebo, in either region. Sodium chloride thresholds were also unaffected by any intervention, as previously shown. Thus, pharmacological modulation of serotonin or noradrenaline levels in humans has no effect on glutamate taste. These findings, together with our previous study showing that the same interventions modulate bitter and sweet taste, support a modality specific neuromodulatory role for serotonin and noradrenaline in human taste perception. Studies were approved by Bath LREC, UK.

#P307 Poster session VII: Chemosensory Psychophysics II

A receptor focused analysis of experience induced changes in glucose and monosodium glutamate (MSG) taste sensitivity

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Brief treatment with fructose induces increased taste discrimination ability (sensitivity) for glucose, and MSG treatment increases MSG sensitivity in humans. Both induction effects reverse after treatment is stopped (Kobayashi & Kennedy, 2002; Kobayashi *et al.*, 2006, Gonzalez *et al.*, 2008). Hamster chorda tympani and *Drosophila* receptor cell neurophysiological data suggest a peripheral mechanism (Berteretche et al., 2005, Gonzalez et al., 2009). We conducted further human studies with various sweeteners and umami stimuli. The tests were designed according to current knowledge of the binding of stimuli to the sweet and umami receptors, to test hypotheses about the receptors' function in the induction. All sweetener concentrations were isosweet, and the umami concentrations isointense, to the original fructose treatment and glucose or MSG test concentrations, as determined by a gLMS scale and magnitude matching. Treatment and testing paradigms were as in Gonzalez *et*

al 2008. Increases in glucose sensitivity were induced by treatment with glucose, Na-cyclamate and MSG, but not by water or acesulfame-K. Treatment with MSG, glucose or fructose increased sensitivity to MSG, while water and acesulfame-K did not, and Na-cyclamate decreased MSG sensitivity. Treatment with IMP increased MSG sensitivity, and that increase was reversed by adding Na-cyclamate to the IMP treatment solution. IMP treatment had no effect on glucose sensitivity, and adding IMP to a Na-cyclamate treatment solution did not reverse the increased glucose sensitivity produced by Na-cyclamate. The overall data support a peripheral mechanism and a role for T1R3 in the induction. KMG is supported by NSF Graduate Fellowship. We thank Bio 040 students for assistance.

#P308 Poster session VII: Chemosensory Psychophysics II

The Effect of Amiloride on the Taste Quality of Salty Solutions
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Salt perception in humans may be partially mediated by the activation of the epithelial sodium channel (ENaC), a selective ion channel present in taste buds. Amiloride, an ENaC channel blocker, has been shown to affect salt taste perception in rodents; however its effect on human taste is not fully understood. While amiloride does not block salt taste in humans, it may affect the quality. In this study we investigated individual differences in amiloride sensitivity in humans, in particular the effect of amiloride on the taste quality of salt. Subjects were asked to rate the intensity and quality of salt solutions tasted with and without amiloride. Our results suggest an effect of amiloride on some humans and may help to futher elucidate the role of ENaC in human salt taste.

#P309 Poster session VII: Chemosensory Psychophysics II

Synchronicity Judgement of Gustation and Olfaction
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Synchronicity of gustation and olfaction in food intake seriously will affect recognition of food perception. The synchronicity, however, is rarely investigated for integration process of gustation and olfaction. In this study, therefore, we focused on the synchronicity perception between gustation and olfaction. We used taste stimulator which was able to present pure gustation without tactile stimuli, and the timing of taste stimuli to tip of participants' tongues was measured in real time by three optical sensors. We also used olfactometer equivalent Burghart's OM4, with original developed real time stimulus monitoring using ultrasonic sensor. We used vision stimuli using LED in control condition. The distribution of synchronicity for "olfaction and vision", and "gustation and vision" was almost equivalent as "vision and audition". These results mean the perception processes for olfaction and gustation have equivalent temporal resolution compared to visual perception. The distribution of synchronicity for "olfaction and gustation" was quite different from others. In other words, the synchronicity distribution of olfaction and gustation was found to be much broader than others. This result might imply that the relation between olfaction and gustation is much closer than that between other sensations.

#P310 Poster session VII: Chemosensory Psychophysics II

The Effect of pH on Arginine Enhancement of Salty Taste
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We have previously shown that L-arginine enhances salty taste. The mechanism for this remains unclear. In this study, in order to help elucidate the phenomenon further, we s determined the effects of pH on L-arginine's enhancement of salty taste in humans. Subjects were presented with mixtures of L-arginine and NaCl solutions of different pHs and asked to rate perceived saltiness using a general labeled magnitude scale. We replicated our previous findings and showed that L-arginine does enhance salty taste of salts. We further show that manipulations of pH affect L-arginine's ability to do so.

#P311 Poster session VII: Chemosensory Psychophysics II

Synergistic responses to L-glutamine and IMP in brief access preference testing in C57BL mice
Benjamin K. Eschle, Meghan C. Eddy, J. Tyler Van Backer, Eugene R. Delay
Department of Biology & Vermont Chemical Senses Group, University of Vermont Burlington, VT, USA

Synergy between 5'-inosine monophosphate (IMP) and L-glutamate is a defining characteristic of umami taste. Molecular expression studies have shown that IMP may also interact synergistically with other L-amino acids and, in some cases, IMP was necessary for cellular activation by the amino acid. These data suggest that IMP-induced synergy may extend to the tastes of other L-amino acids. Synergy is revealed when the response to a binary mixture is greater than the sum of the responses to the components of the mixture. We examined behavioral synergy between glutamine and IMP in C57BL mice. Mice were studied in a non-deprived state in a brief access preference test paradigm. Lick rates were used to measure preferences for a range of concentrations of both glutamine (10, 30, 100, and 200 mM) and IMP (0.3, 1, 3, and 10 mM) alone, as well as 16 binary combinations of the two. A predicted value for each of the binary mixtures was generated by summing the lick rates for each the components presented along. These predicted values are what would be expected in the absence of any synergistic interaction. Actual lick rates to the mixtures were compared to the predicted values to determine whether or not synergy was present. Synergy was observed in mixtures with concentrations of glutamine 30 mM and higher, and IMP 3 mM and higher. Testing will be continued with T1R3 KO mice to examine what, if any, effect the absence of this receptor has on synergistic interactions between L-glutamine and IMP.

#P312 Poster session VII: Chemosensory Psychophysics II

Taste Recognition Thresholds for Human Sweet Taste Function

Sahbina A. Ebba[1], Bradford Speck[1], Lloyd Hastings[2], Gregory Smutzer[1,3]
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Edible taste strips composed of pullulan-hydroxypropyl methylcellulose polymers allow the delivery of precise amounts of tastants to the oral cavity. Edible taste strips are being used to identify norms for human sweet taste perception as a function of age in decades and sex. Taste recognition thresholds for sucrose were examined by the ascending limits method and by the method of reversals. Both approaches yielded nearly identical taste recognition thresholds for sucrose. With edible strips, taste recognition thresholds for sucrose ranged from 0.8 to 7.8 micromoles (n = 60). The variability in thresholds was considerably smaller with edible taste strips than with the use of solutions to deliver tastants. Overall, taste recognition thresholds for sucrose increased with subject age, and recognition thresholds were similar for both males and females. Taste recognition thresholds were also identified for the artificial sweetener sucralose. When sucralose was incorporated into edible strips, taste recognition thresholds averaged 4.9 nanomoles (n = 30), which was approximately 700 times lower than the average taste recognition threshold for sucrose. Lactisole (sodium 2-(4-methoxyphenoxy) propanoate) is a compound that inhibits sweet taste perception in humans. An oral rise with a 0.23 millimolar solution of lactisole failed to block the sweet taste of sucralose when this tastant was incorporated into taste strips, and presented to subjects. Our threshold values for sucrose and sucralose are nearly two orders of magnitude lower when compared to results obtained from traditional aqueous taste tests. These results indicate that edible taste strips are a highly sensitive method for examining sweet taste function in humans.

#P313 Poster session VII: Chemosensory Psychophysics II

Sweetness Resists Suppression in Complex Mixtures

Juyun Lim[1], Floor Oosterhoff [2], Barry Green[2, 3]
[1]Oregon State University Corvallis, OR, USA, [2]The John B. Pierce Laboratory New Haven, CT, USA, [3]Yale School of Medicine New Haven, CT, USA

Little is known about the suppression of individual taste qualities in complex mixtures. The present study investigated taste suppression in all possible binary, ternary and quaternary mixtures of sucrose (0.56M), NaCl (0.32M), citric acid (10mM) and QSO_4 (0.18mM). In exp 1, aqueous solutions were warmed in a 37°C water bath and presented in 5-ml samples in a sip-and-spit procedure. On each trial Ss sequentially rated sweetness, sourness, saltiness, bitterness and 'other' using the gLMS. The results showed that although all taste qualities exhibited suppression, sweetness was more resistant to suppression than the other taste qualities. Sweetness was not significantly suppressed in any binary mixture, and in quaternary mixture it was reduced by just 58% compared to 87%, 82% and 78% for the tastes of QSO_4, NaCl and citric acid, respectively. In addition, it was found that suppression in ternary and quaternary mixtures was driven primarily by specific taste-quality interactions, e.g., sucrose alone suppressed NaCl saltiness by 83%. The unexpected finding that sweetness was less vulnerable to suppression than other tastes led to a second experiment to rule out the possibility that this finding was an artifact of rating sweetness first on every trial, or from the use of 37°C solutions rather than typical room temperature solutions. The results confirmed the findings of exp. 1 and found no significant effect of solution temperature or the order of taste quality ratings. From a functional standpoint, the resistance of sucrose sweetness to suppression may be important for motivating the consumption of foods that are rich in carbohydrates. From a practical standpoint, the pronounced suppression of saltiness by sucrose may explain why the high salt content of some processed foods goes undetected.

#P314 Poster session VII: Chemosensory Psychophysics II

Sucroseoctaacetate aversion: preliminary evaluation of MSM consomic mouse strains for gene-mapping

David A Blizard[1], Tsuyoshi Koide[2], Toshihiko Shiroishi[2], Thomas P Hettinger[3], Marion E Frank[3], Ayako Ishii[2]
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For several decades, it had been assumed that aversion for the bitter tastant sucroseoctacetate (SOA) was under the control of a single gene on mouse chromosome (Chr) 6. However, a recent QTL study reported that additional variants on mouse Chr 2, 11 and 19 also influenced aversion for either 0.1 mM or 1 mM concentrations of SOA in a cross of C57BL/6By and NZB/BINJ strains (Le Roy, Pager and Roubertoux, 1999). The creation of a new set of consomic strains by transferring M. m. molossinus genetic material from the MSM wild-derived strain onto a C57BL/6 background presented the opportunity to screen for variants that affect SOA aversion. A preliminary screen of male mice (N =5-10 per group) of C57BL/6 (B6: the host strain) and MSM (the donor strain) and 6 consomic strains was carried out using 48 h 2-bottle tests in which water was paired with either 0.1 mM or 1 mM SOA and preference/aversion ratios calculated. MSM strongly (aversion ratio ~10%) and B6 weakly (~30%) avoided both concentrations of SOA. No consomic strain avoided 0.1 mM SOA but two (B6-6CMSM and B6-2CMSM) containing, respectively, chromosomal inserts from Chr 6 and Chr 2 avoided 1 mM SOA (aversion ~10%). These findings confirm that SOA aversion is determined by more than one gene in the mouse. Completion of a screen of all of the MSM consomic strains may unearth more variants that influence SOA aversion. The tools available for leveraging MSM/B6 comparisons (e.g. congenic strains, availability of genomic sequence) make the MSM/B6 model system an attractive resource for gene/gustation investigations. Reference Le Roy I, Pager J, Roubertoux PL. (1999) Genetic dissection of gustatory sensitivity to bitterness (sucrose octaacetate) in mice. C R Acad Sci III. 322(10):831-6.

#P315 Poster session VII: Chemosensory Psychophysics II

Measurements of stimulus preference vs. stimulus pleasantness give rise to different optimally liked concentrations of sucrose

Kristin J Rudenga[1], Wambura Fobbs[1], Dana M Small[1, 2]
[1]Yale University New Haven, CT, USA, [2]John B Pierce Laboratory New Haven, CT, USA

The pleasantness of a sweet taste has been described as a single-peaked function that increases with intensity up to an optimally liked concentration and then declines (Moskowitz et al. 1975 and others). Pepino and Menella (2007) used a 2-alternative forced choice test (2-AFC) to show that subjects with a family history of alcoholism prefer higher sucrose concentrations than those without. The goal of the current study was to determine if the 2-AFC test identifies the same concentration of sucrose as the psychohedonic curve (PHC). 29 healthy subjects were recruited and their optimally liked concentration of sucrose was determined by the 2-AFC and the PHC (conducted on separate days and counterbalanced). The PHC was generated by asking subjects to rate the intensity (using the gLMS) and pleasantness (using the VAS) of repeated presentations of 0.09, 0.18, 0.32, 0.56, and 1.00M sucrose. The 2-AFC consisted of subjects choosing their preferred sample from a pair of sucrose solutions of different strengths (selected from 0.09, 0.18, 0.32, 0.56, 0.70, and 1.00M sucrose). This identifies the concentration that is preferred more than those immediately weaker or stronger. We found that the 2-AFC and PHC identify different concentrations of sucrose as maximally liked (PHC =0.70; 2-AFC =0.62; r^2=0.096]. Moreover, the concentration determined by 2-AFC, but not the concentration determined by the PHC, was significantly correlated negatively with a subject's BMI and positively with alcohol use. This suggests that PHC and 2-AFC methods measure different processes. Future work is needed to determine the nature of the difference but an intriguing possibility is that judging pleasantness vs. making a choice about preference represent different dimensions of taste hedonics. Supported by RO1 DC006706.

#P316 Poster session VII: Chemosensory Psychophysics II

Taste damage associated with otitis media

Linda M. Bartoshuk[1], Frank A. Catalanotto[1], Valerie B. Duffy[2], Miriam Grushka[1], Vicki D. Mayo[1], Monica C. Skarulis[3], Derek J. Snyder[1,4]
[1]University of Florida Center for Smell and Taste Gainesville, FL, USA, [2]University of Connecticut Storrs, CT, USA, [3]National Institutes of Health Bethesda, MD, USA, [4]Yale University New Haven, CT, USA

A history of otitis media (middle ear infection) is associated with enhanced liking for energy dense foods and higher body mass index (Snyder et al, 2008). Since the chorda tympani (CN VII, taste, anterior tongue) passes through the middle ear on its way to the brain, it is vulnerable to damage from otitis media. We confirm this damage with a dataset (N=736) of spatial taste tests done for a variety of studies. NaCl, sucrose, citric acid and quinine were swabbed on loci innervated by the chorda tympani and glossopharyngeal (CN IX) nerves; whole mouth taste was also tested. Two psychophysical scales were used, both designed to provide valid comparisons across subjects/groups. The first varied from 0-100 where 0=no sensation and 100=the most intense sensation of any kind imaginable. For the second, 100=the most intense sensation of any kind ever experienced. The change in labels reflects data showing that "imaginable" offers no advantages and can produce confusion in some subjects. Both scales showed significant losses for bitter on the anterior tongue (chorda tympani) but not the posterior tongue (glossopharyngeal). Whole mouth taste intensities were not reduced; in fact, taste intensities for some stimuli were intensified. This provides support for our hypothesis that inhibitory connections among taste nerve projection sites in the brain result in release of inhibition when one nerve is damaged. Thus, paradoxically, localized taste damage can intensify whole mouth taste. Localized taste damage can also intensify non-taste sensations like the tactile sensations evoked by high fat foods (see Catalanotto poster). We suggest that these sensory/hedonic changes lead to the weight gain observed in individuals with histories of otitis media.

#P317 Poster session VII: Chemosensory Psychophysics II

Otitis Media and intensification of non-taste oral sensations

Frank A Catalanotto[1,2], Eric T Broe[1], Linda M Bartoshuk[1,2], Vicki D Mayo[1,2], Derek J Snyder[1,2]
[1]University of Florida College of Dentistry, Department of Community Dentistry and Behavioral Science Gainesville, FL, USA, [2]Center for Smell and Taste Gainesville, FL, USA

A history of otitis media (OM) is associated with enhanced liking for energy dense foods and higher body mass index (Snyder et al, 2008). The chorda tympani nerve (CN VII, carries taste from the anterior tongue) passes through the middle ear on its way to the brain; thus it is vulnerable to damage from OM. Paradoxically, localized damage can intensify whole mouth taste (see evidence at the Bartoshuk poster) because of inhibitory connections between taste nerve projection sites in the brain (Catalanotto et al, 1993). Evidence also suggests that central inhibitory connections exist between taste and oral pain leading to an association between intensified oral pain and OM (Bartoshuk et al, AChems 2007). Similarly, damage to the glossopharyngeal taste nerve (CN IX) has recently been associated with increased oral pain (Logan et al, 2008). The present study was conducted to test another non-taste oral sensation: tactile sensations evoked by fats. Subjects rated the thickness of 5 dairy products: nonfat milk, whole milk, half&half, heavy cream, heavy cream+oil on a scale from 0 to 100 where 0=no sensation and 100=strongest sensation of any kind ever experienced. ANOVA with follow-up t-tests were conducted for subjects with no history of OM vs those with histories of OM. The 2 groups were not significantly different for nonfat milk; subjects with histories of OM rated 3 of the 4 stimuli containing fat as significantly "thicker" than did those without this history (the difference for half&half did not reach statistical significance but was in the same direction). We suggest that oral thickness is a cue for energy density; this cue is likely to be associated with food liking through conditioning. Intensification of this cue would presumably be associated with even greater food liking leading to weight gain.

#P318 Poster session VII: Chemosensory Psychophysics II

Oral irritation elicited by menthol and cinnamaldehyde (CA): self- and cross-desensitization
E. Carstens, Mirela Iodi Carstens, Karen Zanotto
University of California, Davis Davis, CA, USA

Menthol and CA are used in oral hygiene products for their refreshing sensory properties, but are irritating at higher concentrations. We investigated if they exhibit self- and cross-desensitization of their oral irritancy. Using a 2-alternative forced-choice (2-AFC) paradigm, either menthol (19.2 , 28.8 mM) or CA (15.2, 30.4 mM) was briefly applied by filter paper to one side of the dorsal tongue, with vehicle similarly applied to the other side. Following variable intervals, either menthol or CA was applied bilaterally and subjects stated on which side they experienced greater irritation, followed by ratings of irritant intensity on each side using a labeled magnitude scale. Desensitization was manifest by a significant proportion of subjects choosing the vehicle-treated side as having stronger irritation, and assigning significantly higher ratings to that side. Menthol 19.2 mM exhibited significant self-desensitization and cross-desensitized irritation elicited by 15.2 mM CA applied 5 but not 30 min later. CA 30.4 mM (but not 15.2 mM) cross-desensitized irritation evoked by 28.8 mM menthol applied 5 and 30 min later, and exhibited self-desensitization. These data are consistent with self- and mutual cross-desensitization of menthol and CA-evoked responses of neurons in superficial layers of trigeminal subnucleus caudalis. Using calcium imaging of cultured rat trigeminal ganglion neurons, we observed CA suppression of menthol-evoked responses, suggesting a peripheral site of interaction of these compounds on trigeminal nerve endings.

#P319 Poster session VII: Chemosensory Psychophysics II

Evidence that repeated threshold testing can alter the perceived intensity of taste
Barry Green[1,2], Juyun Lim[3]
[1]The John B. Pierce Laboratory New Haven, CT, USA, [2]Dept. of Surgery (Otolaryngology), Yale University School of Medicine New Haven, CT, USA, [3]Dept. of Food Science and Technology, Oregon State University Corvallis, OR, USA

Context effects in taste have generally been attributed to response biases rather than to sensory mechanisms intrinsic to taste perception. Here we report evidence that extensive exposure to threshold-level tastes appears to heighten the perception of suprathreshold tastes. We discovered the effect in a study of individual differences in threshold and suprathreshold sensitivity to sucrose and NaCl that involved measurement of 6, 2AFC detection thresholds over 3 sessions, followed by a 4th session in which intensity ratings were obtained for 12 suprathreshold stimuli using the gLMS. Under these conditions intensity ratings were 2-3 times higher than in a preceding experiment that included some of the same stimuli but no detection task. Analysis of data from 7 Ss who participated in both experiments confirmed the differences were statistically significant ($p<0.05$). We then asked 12 Ss who had participated in the study of individual differences to return to the lab and rate the same sucrose and NaCl stimuli 4 more times over two additional sessions. Intensity ratings trended progressively lower across replicates and sessions, i.e., the context effect decayed over time, particularly for the weakest stimuli ($p's<0.001$). Subsequently, an experiment in which taste intensity was rated before as well as after measurement of only two, 2AFC detection thresholds yielded no apparent context effect. The latter result implies that merely switching the experimental context from a threshold task to a suprathreshold task cannot account for the observed effect. Experiments are ongoing to test the hypothesis that repeated exposure to threshold-level taste stimulation can intensify suprathreshold taste perception.

#P320 Poster session VII: Chemosensory Psychophysics II

Recognizing Taste Stimuli below the Detection Threshold
Timothy G. Shepard[1], Miao-Fen Wang[1,2], Maria G. Veldhuizen[1,2], Lawrence E. Marks[1,2]
[1]John B. Pierce Laboratory New Haven, CT, USA, [2]Yale University School of Medicine New Haven, CT, USA

Recognition thresholds for gustatory stimuli are greater than the corresponding detection thresholds. Nevertheless, even just-detectable taste stimuli may contain usable information about gustatory quality. In a set of six experiments, we asked whether subjects obtain substantial information about the quality of a taste stimulus that is near or even below the traditional detection threshold (detection d' = 0.95, equivalent to 75% correct in two-alternative forced choice). All experiments used a sip method: On each trial, subjects received 5 ml of deionized water, sucrose, or citric acid (three experiments) or these three plus a sucrose-citric acid mixture (three other experiments). Subjects were instructed to (a) identify the stimulus, (b) rate the confidence of the identification, and (c) make a second choice (on all trials, regardless of whether the first response was correct). The experiments used overlapping groups of 12-16 subjects, testing different stimulus concentrations producing different levels of detection, d', greater or smaller than the nominal threshold of 0.95. In all experiments, subjects correctly recognized the stimulus (except the mixture) on most of the trials on which it was detected, even when detection fell well below traditional threshold. This was true both when the experiment included the sucrose-acid mixture (e.g., in Experiment 5, d'=0.38 0.29, and 0.60 for detection of sucrose, acid, and mixture, and correct recognition = 52%, 59%, and 25%, respectively, chance = 33%) and when the mixture was excluded (e.g., in Experiment 6, d'=0.38 and 0.41 for sucrose and acid, and correct recognition = 68% and 71%, chance = 50%) These results imply that some information about the quality or identity of gustatory stimuli is available even when the stimuli are barely detectable.

#P321 Poster session VII: Chemosensory Psychophysics II

Comparing the Distributions of human TAS2R38 taste reseptor Genotypes in Philadelphia and in Southern Finland
Mari A Sandell[1,2], Saila KE Mattila[1], Suzanne M Alarcon[2], Paul AS Breslin[2]
[1]University of Turku Turku, Finland, [2]Monell Chemical Senses Center Philadelphia, PA, USA

Based on our previous studies the variations in human TAS2R38 receptor genotypes determine individual differences in bitterness perception of glucosinolate generating vegetables, such as mustard greens, kale, and brussel sprouts (Sandell and Breslin, 2006, Current Biology 16: R792-R794.) Understanding genetic

differences in taste receptors is necessary for the study of food and specific food preferences. The aim of this study was to screen the *hTAS2R38* taste receptor gene for variation in Finland (North Europe) and to compare the distribution of Finnish genotypes to those of people in Philadelphia, PA. A total of 155 volunteer adults (114 females and 41 males) were recruited around South Finland (Fi), and in Philadelphia (Ph) a total of 75 subjects were recruited (39 females and 35 males). All subjects were genotyped at three variable locations: AA 49, 262, 296 (Bufe et al, Current Biology 22: 322-327). A total of four alleles were observed in both samples. Ph was comprised of eight different diplotypes whereas Fi was comprised of six. The PAV/AVI heterozygous subjects were the predominant genotype group in both samples (37 %). The difference in "sensitive" PAV (Fi = 13 %, Ph = 24 %) and "insensitive" AVI (Fi = 39 %, Ph = 24 %) homozygous subject groups was significant between the samples. Our results indicate the influence of wider gene pool on distribution and show the conservation of genes in isolated populations. As Ph has a significant African American and African population segment, these data support the idea that genetic variation is maximized when the population under study is heterogeneous and contains a large portion of the population of African extraction.

#P322 Poster session VII: Chemosensory Psychophysics II

Development of Multichannel Taste Stimulator System

Hideki Toda, Tatsu Kobayakawa
National Institute of Advanced Industrial Science and Technology
Tsukuba, Japan

It is strongly required high performance taste stimulator that is able to present various kinds of tastants without any tactile stimulus for complicated psychophysical experiment, for example, taste detection or interaction between tastants or cross modal experiment. Tastant, water, bubble were driven by positive air pressure in single channel type taste stimulator previously proposed. In this case, positive air pressure worked enough. It would be very difficult to present various tastats by adding parts into original one, because this addition leads instability. In this study, we develop a new negative pressure type gustatory stimulator system instead of previous proposed positive pressure type for driving water and tastants. We can simplify whole mechanism of creating the gustatory stimulation compared with the previous proposed system, especially, using siphon effect for creating the gustatory stimulus section helps us improving the gustatory stimulus timing control stability. For example, our system can create 714 +- 8.7msec (mean +- S.D., N=60) gustatory stimulation if we set the gustatory width set to 700 msec. In addition, maximum pressure change of the mouth position is very small compared to detection threshold (264 Pa). This controllability of the gustatory stimulation will be useful for precise analysis of the signal processing mechanism of taste response in the field of (neuro-) physiology or human psychophysical study and apply for the study field of biology or medical examination.

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Bold indicates first/presenting author

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AChemS
Association for Chemoreception Sciences

PROGRAM AT A GLANCE

Registration
3:30 pm to 8:00 pm

Registration
7:00 am to 1:00 pm, 6:30 pm to 7:30 pm

Registration
7:30 am to 1:00 pm, 6:30 pm to 7:30 pm

WEDNESDAY, APRIL 22

Welcome Banquet
(Ticketed Event)
6:00 - 8:00 PM
SOUTH BALLROOM

Opening and Awards Ceremony
8:00 - 9:00 PM
SOUTH BALLROOM

Givadaun Lecture
9:00 - 10:00 PM
SOUTH BALLROOM

THURSDAY, APRIL 23

Gustation
8:00 - 10:00 AM
SOUTH BALLROOM

Break
10:00 - 10:30 AM

Gender effects on olfactory processing
10:30 AM - 12:30 PM
SOUTH BALLROOM

Break
2:10 - 2:25 PM

Workshop: NIH
3:00 - 5:00 PM
TROPICS ROOM

Presidential Symposium: On beyond glomeruli
7:00 - 9:05 PM
SOUTH BALLROOM

POSTER SESSION I: Chemosensory disorders, models and aging/Central chemosensory circuits
8:00 AM - 12:30 PM
NORTH BALLROOM

Industry Symposium
(Ticketed Event)
1:00 - 4:00 PM
SOUTH BALLROOM

Industry Reception
(Ticketed Event)
4:15 - 6:00 PM
THE BOATHOUSE

POSTER SESSION II: Chemosensory response to, and control of, feeding/ Neuroethology
7:00 - 11:00 PM
NORTH BALLROOM

FRIDAY, APRIL 24

Development and Plasticity: First Central Chemosensory Relays
8:00 - 10:30 AM
SOUTH BALLROOM

Break
10:30 - 11:00 AM

Polak Young Investigator Award Winners
11:00 AM - 12:30 PM
SOUTH BALLROOM

AChemS Business Meeting
12:45 - 2:45 PM
SOUTH BALLROOM

Workshop: NIH
3:00 - 4:00 PM
FLORIDA ROOM

ChEMA Social
5:00 - 7:00 PM
TROPICS ROOM

IFF Special Lecture
7:00 - 8:00 PM
SOUTH BALLROOM

Break
8:00 - 8:15 PM

Reciprocal interactions between primary taste and olfactory processing networks and higher cognition
8:15 - 10:15 PM
SOUTH BALLROOM

POSTER SESSION III: Cortical chemosensory processing/ Receptor genomics and molecular biology
8:00 AM - 12:30 PM
NORTH BALLROOM

POSTER SESSION IV: Chemosensory transduction and perireceptor events
7:00 - 11:00 PM
NORTH BALLROOM

Registration
7:30 am to 1:00 pm, 6:30 pm to 7:30 pm

Registration
7:30 am to 11:00 am

SATURDAY, APRIL 25

Functional evolution of chemosensory receptors
8:00 - 10:05 AM
SOUTH BALLROOM

Break
10:05 - 10:30 AM

Making sense of fat taste
10:30 AM - 12:30 PM
SOUTH BALLROOM

POSTER SESSION V: Chemosensory memory/ Central synaptic physiology/ Neurogenesis
8:00 AM - 12:30 PM
NORTH BALLROOM

Clinical Luncheon
(Ticketed Event)
12:45 - 2:45 PM
THE KEYS ROOM

Workshop: Computational problems in sequential stages of odor processing
3:00 - 5:30 PM
SOUTH BALLROOM

Follow the head, not only the nose: Top-down influences on olfactory perception
7:00 - 9:05 PM
SOUTH BALLROOM

POSTER SESSION VI: Chemosensory development and Psychophysics I
7:00 - 11:00 PM
NORTH BALLROOM

SUNDAY, APRIL 26

GABA in the developing olfactory system: From generation to differentiation
8:00 - 10:05 AM
SOUTH BALLROOM

Break
10:05 - 10:30 AM

Olfactory and Vomeronasal Systems
10:30 AM - 12:30 PM
SOUTH BALLROOM

POSTER SESSION VII: Chemosensory Psychophysics II
8:00 AM - 12:30 PM
NORTH BALLROOM

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See you next year at our new venue!

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AChemS 32nd Annual Meeting
April 21-25, 2010

More space, more rooms, more fun!