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ABSTRACTS

Abstracts from the Thirty-second Annual Meeting of the Association for Chemoreception Sciences

GIVAUDAN LECTURE - NORMAL AND CANCER STEM CELLS AND THE DEVELOPMENT OF MALIGNANCY

1 Normal and Cancer Stem Cells and the Development of Malignancy

Robert A. Weinberg

Member, Whitehead Institute and Director, MIT Ludwig Center for Cancer Research

The progression of carcinoma cells in primary tumors to a state of high-grade malignancy involves the acquisition of a variety of cell traits, including motility, invasiveness, and an increased resistance to apoptosis. These traits are all components of a complex cell-biological program termed the epithelial-mesenchymal transition (EMT), which normally plays a role in various steps of embryonic morphogenesis and in wound healing. By resurrecting this multi-faceted program, cancer cells are able to gain access in a single step to these multiple traits, which in turn empowers them to invade and disseminate. Indeed, the invasive edges of carcinomas often exhibit cells that exhibit signs of having activated an EMT program. Recently it has become apparent that the EMT, rather than creating bona fide mesenchymal cells, actually creates epithelial stem cells that exhibit many mesenchymal traits. Accordingly, the EMT program represents the key to enable the conversion of more differentiated epithelial cells into epithelial stem cells, which holds implications for both normal epithelial cells biology and cancer pathogenesis.

PLATFORM PRESENTATIONS - TIP OF THE TONGUE

2 Analysis of *Drosophila* TRPA1 reveals an ancient origin for human chemical nociception

Paul A Garrity[1], Kyeongjin Kang[1], Stefan R Pulver[1,2], Vincent C Panzano[1], Leslie C Griffith[1], Douglas L Theobald[1]

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Chemical nociception, the detection of tissue-damaging chemicals, is important for animal survival and causes human pain and inflammation, but its evolutionary origins are largely unknown. Reactive electrophiles are a class of noxious compounds humans find pungent and irritating, like allyl isothiocyanate (in wasabi) and acrolein (in cigarette smoke). Insects to humans find reactive electrophiles aversive, but whether this reflects conservation of an ancient sensory modality has been unclear. Using a combination of genetics and physiology, we have determined the molecular basis of reactive electrophile detection in flies. We find that dTRPA1, the Drosophila melanogaster ortholog of the human irritant sensor, acts in gustatory chemosensors to inhibit reactive electrophile ingestion. Both fly and mosquito TRPA1 orthologs act as molecular sensors of electrophiles, and they respond to these chemical via a mechanism conserved with vertebrate TRPA1s. Phylogenetic analyses indicate invertebrate and vertebrate TRPA1s share a common ancestor that possessed critical characteristics required for electrophile detection. These findings support emergence of TRPA1-based electrophile detection in a common bilaterian ancestor, with widespread conservation throughout vertebrate and invertebrate evolution. Such conservation contrasts with the evolutionary divergence of canonical olfactory and gustatory receptors and may relate to electrophile toxicity. These findings suggest that human perceptions of reactive electrophiles rely on an ancient chemical sensor conserved across ~500 million years of animal evolution. Acknowledgements: NIMH (R21 MH080206, P.A.G., RO1 MH067284 , L.C.G.) and NINDS (PO1 NS044232)

3 A subpopulation of mouse Type II taste cells express functional voltage-gated calcium channels

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Type II taste cells contain receptors and signaling components for sweet, bitter, and umami tastants, and are responsible for transducing complex taste stimuli via the phospholipase C (PLC) pathway. Type II cells communicate with the afferent nerve or Type III cells via non-vesicular purinergic signaling. Previous studies concluded that Type II cells lack voltage-gated Ca^{2+} channels (VGCCs), while Type III cells express the VGCCs required for synaptic transmission. However, our functional calcium imaging data in C57Bl/6 mice showed that a small but significant subpopulation of Type II cells, identified by their responses to an established tastant mixture, also responded to high KCl, consistent with the expression of VGCCs in these cells. To explore whether VGCCs are expressed in subpopulations of type II cells, transgenic mice expressing enhanced green fluorescent protein (GFP) under control of the PLCß2 promoter (PLCß2-GFP) were used in both functional calcium imaging and patch clamp recording. Calcium imaging data showed that high KCl elicited a robust intracellular calcium rise in over half of PLCß2-GFP taste cells, consistent with the expression of VGCCs. Patch clamp recording showed that VGCC currents were present in 8 out of 33 PLCß2-GFP taste cells. The VGCC current was large (200~500 pA) when the whole cell configuration was first established, but washed out quickly (τ = 138 s). This rapid washout may partially explain why VGCC current has been rarely seen in type II cells electrophysiologically. Our findings strongly suggest that functional VGCCs are expressed in subpopulations of Type II cells and question the current model of cell signaling within

the taste bud as well as the utility of high KCl to identify unequivocally type III cells within the taste bud. Acknowledgements: Supported by NIH DK059611 and International Flavors & Fragrances.

4 Ryanodine Receptors selectively contribute to the formation of Taste evoked-calcium signals in Mouse taste cells

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While it has been well established that different taste stimuli activate distinct signaling pathways in taste receptor cells, the signal transduction mechanisms associated with these pathways have not been completely characterized. Bitter, umami and sweet taste stimuli activate G-protein coupled receptors (GPCRs) to cause Ca^{2+} release from intracellular stores, which is known to occur in Type II cells via a PLCβ2/IP_3R3 signaling pathway. Sour stimuli depolarize taste cells to cause Ca^{2+} influx through voltage-gated calcium channels (VGCCs), presumably in taste cells with chemical synapses. The transduction pathways of salty stimuli are less well defined. There is also a sub-population of taste cells that express VGCCs and detect bitter taste stimuli but do not express the PLCβ2/IP_3R3 pathway. These cells are termed dual-responsive and appear to express PLCβ3 and IP_3R1. While it is recognized that bitter, sweet and umami taste stimuli activate GPCRs to evoke IP_3-mediated Ca^{2+} release from internal stores, any potential role of ryanodine receptors (RyRs) in this signaling pathway has not been identified in mouse taste cells. Using RT-PCR and immunocytochemistry, we detected the RyR isoform 1 in multiple taste papillae types, while calcium imaging studies revealed that RyRs are physiologically functional in mouse taste cells. We found that RyRs significantly contribute to taste-evoked Ca^{2+} responses in approximately 30% of type II cells but did not significantly contribute to the taste-evoked responses in dual-responsive taste cells. The discovery of RyRs in mouse taste cells indicates that multiple Ca^{2+} release mechanisms contribute to the formation of evoked taste responses and our findings reveal an important role for RyRs in the transduction of these taste-evoked responses. Acknowledgements: This work was supported by NIDCD DC00635801 and NSF 0917893 to KM.

5 Acetylcholine, released from taste buds during gustatory stimulation, enhances taste responses

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Mammalian taste buds possess apical taste receptors as well as basolateral neurotransmitter receptors for synaptic transmission and neuromodulation. One class of basolateral receptors expressed in taste buds are the muscarinic acetylcholine (ACh) receptors (Eguchi et al, 2008), reported to be activated by bath-applied ACh (Ogura, 2002). Acetylcholinesterase has long been known to surround taste buds, suggesting a physiological role for ACh in taste reception. Collectively, these findings implicate a role for ACh in taste bud function. We tested this hypothesis by imaging taste cell responses in lingual slices, and by measuring neurotransmitter secretion from isolated taste buds using cellular biosensors. When we applied ACh or carbachol (1 μM) to a lingual slice, we observed that taste-evoked calcium responses were potentiated. The effects of ACh were blocked by atropine (5 μM), suggesting muscarinic receptor activation. Using ATP biosensors, we measured the effect of bath-applied ACh on ATP secretion from isolated taste buds. ACh augmented taste-evoked ATP release, complementing the ACh potentiation observed in the lingual slice preparation. Moreover, bath-applied atropine itself, in the absence of any added ACh, reduced taste-evoked ATP release from taste buds, suggesting endogenous cholinergic mechanisms are activated during taste stimulation. Finally, we used ACh biosensor cells to test whether taste buds secrete ACh. We observed that ACh is indeed released from isolated taste buds upon tastant stimulation. The source of taste-evoked ACh secretion may be from cholinergic nerve fibers that innervate taste buds or directly from taste cells themselves, as implied by Ogura et al (2007). Our findings strongly implicate ACh as a neurotransmitter that enhances taste responses. Acknowledgements: Supported by NIH/NIDCD grants 5R01DC000374 and 5R01DC007630 to SDR.

6 Epithelial Sodium Channel (ENaC) is Involved in Reception of Sodium Taste: Evidence from Mice with a Tissue-Specific Conditional Targeted Mutation of the ENaCα Gene

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Observations that amiloride and other ENaC blockers alter taste responses to sodium salts generated the hypothesis that ENaC is involved in salt taste reception. To directly test this hypothesis, we have generated mice with the ENaCα subunit selectively eliminated in the lingual epithelium using the Cre-loxP mediated conditional gene deletion technique. Electrophysiological experiments have shown that mice with the tissue-specific conditional ENaCα deletion lacked the amiloride-sensitive component of chorda tympani nerve responses to lingual application of sodium salts. However, the amiloride-insensitive component of the response to sodium salts, responses to non-sodium salts, sweet, bitter and sour taste stimuli, or responses to irritants were not affected in these mice. In brief-access tests, ENaCα knockout mice had an attenuated aversion to higher concentrations of NaCl compared with wild-type mice. These data provide direct evidence that ENaC is involved in detecting sodium taste. Our results further demonstrate that there is a significant ENaC-independent component of taste responses to salts.

7 Novel proteolyzed ENaC isoforms and corresponding salt taste enhancing compounds

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Modulators of novel proteolyzed human ENaC isoforms were identified and confirmed in sensory testing to either potentiate or block human salt taste perception. First, a stable ENaC cell line was produced using Chromovert®, a method that enables testing of

millions of individual cells to rapidly identify and isolate individual clones stably expressing all desired native subunits. Next, a functional 384-well assay specific for the activity of ENaC comprising alpha, beta and gamma subunits was produced. The cell line was treated with limiting proteolysis in conjunction with the functional assay and a series of novel proteolytic ENaC isoforms was defined based on the differing pharmacology of reference compounds. Interestingly, ENaC isoforms that were less sensitive to inhibition by amiloride were identified, providing a cell based platform consistent with both amiloride-sensitive and amiloride-insensitive components of human salt taste perception. High throughput screening of proteolyzed and non-proteolyzed isoforms resulted in at least 12 distinct chemical series with varied activity against the ENaC isoforms. The creation of and access to multiple distinct ENaC isoforms allowed discovery of corresponding compounds for use as research tools to determine which isoforms correlate with *in vivo* ENaC activity. Medicinal chemistry and testing of compounds in Ussing chamber models and against delta ENaC is being pursued to further improve nanomolar active compounds for desired safety and efficacy in taste and ENaC-mediated clinical indications including chronic obstructive pulmonary disease (COPD), Cystic Fibrosis and pulmonary edema. Creation of assays for previously inaccessible native and untagged targets comprising all required subunits can aid research in a broad range of applications. Acknowledgements: None

8 Recovery from Potassium Chloride (KCl) Loading Alters Amiloride-Sensitive Salt Taste in Humans

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An amiloride sensitive pathway is important for humans to describe NaCl as "salty" after adaptation to NaCl. Since amiloride blocks Na transit through the epithelial sodium channel (ENaC) and ENaC activity in most tissues is regulated by aldosterone, we had hypothesized aldosterone stimulates ENaC activity in the human tongue. However, as we reported previously, when aldosterone production was stimulated by diuretic induced volume depletion, amiloride sensitive salt taste was blunted rather than stimulated. We now report on the effect of aldosterone stimulation induced by KCl loading. KCl loading consisted of ingesting 100 mEq in the evening and again the following morning. 19 subjects participated in 3 days of tests, providing blood samples and magnitude estimates of the component taste qualities (salty, sweet, sour and bitter) of 125 mM NaCl after adaptation to 100 mM NaCl. Day 1 testing occurred prior to KCl loading, Day 2 testing occurred after KCl loading, and Day 3 (recovery) testing was performed two days later. KCl loading increased serum potassium concentration from 4.18 ± 0.06 mEq/L to 4.93 ± 0.09 ($p<0.01$), doubled the serum aldosterone concentration ($p<0.01$), but did not affect renin activity. Adapting and test solutions were presented without and with amiloride (10 μM). On Day 1, subjects characterized 125 mM NaCl as predominantly salty and amiloride reduced the saltiness by 54% ($p<0.01$). After KCl loading on Day 2, subjects still characterized 125 mM NaCl as predominantly salty and amiloride reduced saltiness by 53% ($p<0.01$). Two days later (recovery) 125 mM NaCl was still characterized as salty, but amiloride did not affect the estimate of saltiness ($p>0.1$). These data indicate that aldosterone does not directly regulate an amiloride sensitive pathway in the tongue. However, an amiloride sensitive pathway does contribute to human salt taste and this pathway is altered by undefined factors. A clue to the regulation of amiloride sensitive salt taste appears to be weight loss. The loss of amiloride sensitivity occurred on the days subjects lost weight after recovery from KCl loading ($p<0.02$) and after diuretic administration ($p<0.02$). It appears that human salt taste is responsive to regulation and is likely to be important in homeostasis. Acknowledgements: Merit Review Grant from the Department of Veterans Affairs

9 Comparative Analysis of Bitter Taste Receptor Agonist Activation

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Although number and chemical diversity of agonists for individual hTAS2Rs varies considerably, bitter taste receptors in general are broadly tuned. Yet, every single hTAS2R exhibits a unique agonist activation profile indicating that the broad tuning is not simply achieved at the expense of selectivity. This special feature of hTAS2Rs must ultimately be a function of the architecture of agonist binding pockets.By mutagenesis experiments, functional expression studies, and homology modeling we investigated which residues are involved in receptor activation. Focusing first on hTAS2R46, we identified several positions in transmembrane (TM) regions and extracellular loops of hTAS2R46 exhibiting effects on agonist specific receptor activation. To test whether the ligand binding pockets and the positions of residues crucial for agonist activation are conserved within the hTAS2R family, we wanted to compare data obtained for hTAS2R46 with the much less related but equally broadly tuned receptor, hTAS2R10. Although hTAS2R46 and -10 share only 34% amino acid sequence identity, numerous bitter compounds activate both receptors. Starting from a homology model of hTAS2R10, we subjected residues within a distance of 6Å from position N92, which we located as the central bottom of the putative binding pocket to alanine scanning mutagenesis. Most of the 23 mutated residues affected agonist interaction in subsequent functional calcium imaging analyses. In particular position 85 in TM3 was identified to selectively affect activation by some sesquiterpene lactones.We conclude that ligand binding pockets in TAS2Rs are positionally conserved consisting of residues predominantly located in TM2, 3, 5, 6, and 7, but that the relative importance of residues can differ dependent on the receptor and agonist structure.

SYMPOSIUM - GENETICS OF HUMAN OLFACTION

10 Environmental and Genetic Effects on Human Odor Perception

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Human responses to odors show substantial variation. We investigated individuals from Finnish families and Australian, British, Danish, and Finnish twins, who rated intensity and pleasantness of a set of odors and tried to identify the odors and rated their own sense of smell and annoyance experienced with different environmental odors. The odor stimuli of a commercial smell test were presented in the family study. Based on the results of the family study and a literature survey, a new set of odor stimuli was designed for the twin studies. In the family sample, heritabilities of the traits were estimated and underlying genomic regions were searched using a genome-wide linkage scan. We found suggestive evidence for a genetic linkage for pleasantness of cinnamon at a locus on chromosome 4q32.3. High heritability for the pleasantness of cinnamon was found in the family but not the twin study. Heritability of perceived intensity of androstenone odor was determined to be ~30% in the twin sample. A strong genetic correlation between perceived intensity and pleasantness of androstenone, in the absence of any environmental correlation, indicated that only the genetic correlation explained the phenotypic correlation between the traits and that the traits were influenced by an overlapping set of genes. Self-rated olfactory function appeared to reflect the odor annoyance experienced rather than actual olfactory acuity or genetic involvement. Results from nongenetic analyses supported the speculated superiority of females' olfactory abilities, the age-related diminishing of olfactory acuity, and the influences of experience-dependent factors on odor responses. A genetic effect was detected for only a few responses to specific odors, suggesting the predominance of environmental effects in odor perceptions. Acknowledgements: Finnish Academy, Finnish Foundation for Cardiovascular Research

11 Phenotype/Genotype Associations in Human Olfaction

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Humans have ~850 olfactory receptor (OR) genes. Few of the roughly 380 functional OR genes have been deorphanized, i.e., the G-protein coupled receptor coded by the OR gene has been paired with a ligand, although even for those that have it is unknown whether the ligand is the ''best fitting.'' Among the 850 OR genes and pseudogenes is a special subset of segregating pseudogenes (SPGs) wherein some people have an intact OR gene, and others have a pseudogene containing a stop codon or a significant deletion/frame shift. We have focused upon ~60 SPGs and OR7D4 (which has been implicated in the perception of androstenone) in a population of blacks and whites and have obtained, from ~600 people, olfactory detection thresholds, pleasantness ratings and odor quality descriptor for the following odorants: muscone (musky), 3-methyl-2-hexenoic acid (sweaty), benzyl salicylate (balsamic), Galaxolide® (musky), isovaleric acid (sweaty), isoamyl acetate (pear/banana), Jeger's ketal (woody/amber), pentadecalactone (musky) and androstenone (urinous/woody/floral). These odorants were chosen because some people have specific hyposmias or anosmias. Although data are still being collected, to date, more whites (9%) than blacks (2%) have specific anosmia for androstenone; more males than females have specific anosmias for isovlaeric acid (9% vs. 2%), isoamyl acetate (3% vs 0%) and androstenone (11% vs. 4%). Of the 36 pairwise correlations for thresholds, 30 were significant ($p < .001$), suggesting a general factor for olfactory sensitivity. We have identified numerous ($n = 81$) genotype/phenotype associations: More than 50% of the SPGs ($n = 37$) have been implicated; two (OR6J1 and OR8J2p) are associated ($p < .01$) with six phenotypes. These results are in accord with current knowledge of the combinatorial code in olfaction wherein many ORs participate in the perception of a single odorant and a single OR is activated by more than one odorant. OR7D4, previously implicated in the perception of androstenone, was associated with perceived pleasantness of the odorant, but OR7D4 could not account for the specific anosmia to androstenone. These results suggest that focusing upon SPG/phenotype associations can provide useful and promising results. Acknowledgements: Supported by NIH grant RO1 DC00298 to CJW.

12 A genome-wide perspective on the perception of musk-like odorants

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Some people find low concentrations of odorants to be intense whereas others cannot detect them, even at high concentrations. The role of genetic variation in determining these differences is largely unknown but one exception is for androstenone, which is a heritable trait partially determined by alleles of the olfactory receptor gene *OR7D4*. To better understand the genetic architecture of the perception of a range of odorants, Australian twins and their siblings (aged 10 to 25 years, 55% female) rated a panel of odorants for intensity using one of two smell surveys, the *National Geographic Smell Survey* (androstenone, Galaxolide, amyl acetate, eugenol, mercaptans, rose; 110 monozygotic and 228 dizygotic twin pairs) or a survey developed at the University of Helsinki (androstenone, chocolate, cinnamon, isovaleric acid, lemon, turpentine; 65 monozygotic and 170 dizygotic twin pairs). Moderate heritability was observed for the intensity ratings for all musky odorants, androstenone (h^2=0.30, both datasets combined) and Galaxolide (h^2=0.34, *National Geographic Smell Survey* only), but not for the other odorants. A genome-wide association study using 2.5 M single nucleotide polymorphisms indicated, that for androstenone, the genetic variant most associated with its intensity was located on chr 9 (*rs10966900*, p<1.0E-06) and for Galaxolide, it was located on chr 11 within the *INTS4* gene (*rs3819256*, p<1.0E-05). Neither region contains known olfactory receptors nor was an association between androstenone perception and the olfactory receptor gene *OR7D4* detected. Thus the intensity of musk odorants is a heritable trait, probably polygenic, but alleles of known olfactory receptor genes play only a minor role. This genome-wide analysis suggests novel genetic variants on chr 9 and 11 effect on musk perception. Acknowledgements: Funded by the National Institutes of Health [DC 00298 to C.J.W.], the National Health and Medical Research Council (Australia) [241944 to N.G.M.], the Finnish Food Research Foundation [A.K.], and the Academy of Finland [A.K.].

13 Next generation genomics of human olfactory variation

Doron Lancet[1], Yehudit Hasin[1], Sebastian Waszak[2], Ifat Keydar[1], Miriam Khen[1], Charles J. Wysocki[3], Edna Ben-Asher[1], Yoav Gilad[4], Jan O. Korbel[2], Tsviya Olender[1]

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We have previously identified 75 cases of human OR deleterious alleles, stemming from frame-disrupting single nucleotide polymorphisms (SNPs) and null copy number variations (CNVs). This provides a genomic infrastructure for associating OR genotypes to olfactory phenotypes. To augment such a list and extend it beyond OR coding region losses, we now apply the novel Next Generation Sequencing (NGS) method to genome and transcriptome sequences. Test analysis of 100 intact OR genes in 20 individuals indicated a capacity to discover 19 additional disrupting SNPs (segregating pseudogenes, SPGs) with up to 4 bp deletions. More comprehensively, scrutinizing the 1000 Genomes Project (1000GP) data, we have identified ~3500 novel SNPs, including 78 new disrupting ones. In the realm of CNVs, we developed CopySeq, a new algorithm for large-scale determination of accurate copy-number genotypes and applied it to the low-sequence-depth 1000GP data of 150 individuals. We observed a wide range of OR copy-number states (0-9 copies), and are contemplating the possible phenotype at the high copy number end. For OR deletions, we found 95 novel loci and obtained good estimates for their population prevalence, including clear paucity in African-Americans. About 10% of the functional ORs have a deletion allele and ~25% of all individuals show at least one homozygous deletion. In parallel, olfactory epithelial NGS transcriptome analysis is unraveling novel mutation targets, includes upstream splice junctions, as well as a verified and augmented list of human olfactory transduction genes. The emerging variation map of the olfactory gene universe will assist in explaining the full extent of olfactory phenotype variations, covering both odorant-specific differences as well as general chemosensory threshold diversity. Acknowledgements: Supported by NIH grant RO1 DC00298 to CJW and the Crown Human Genome Center at the Weizmann Institute.

14 Genetics of Olfactory Perception in Humans

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Odorant receptors represent the largest gene superfamily in the human genome and. account for the diversity of odors that we can detect, yet it is not known how the olfactory system encodes an olfactory percept. Because humans can sense many more odors that than the number of odorant receptor genes they possess, there must be some overlap in the neuronal substrates processing different odor stimuli. We analyzed correlations between different aspects of odor perception for two concentrations of 66 different odors to study how one aspect of olfactory perception predicts other aspects. We collected olfactory detection thresholds for several odors and subjective assessments of the intensity and pleasantness of more than 100 different odorous stimuli. Strong correlations were found for the perception of different aspects of the same stimulus and for the perception of the same odor at different concentrations. The perception of structurally similar odors like androstenone and androstadienone, showed strong correlations, validating our approach. The same was found for structurally diverse yet perceptually similar odors like the musky odors ethylene brassylate, pentadecalactone, and galaxolide. An unbiased analysis of the correlations also uncovered new connections between odors that are neither perceptually nor structurally similar, but presumably are processed by overlapping neuronal substrates. Taken together, these findings allow us to group human subjects into perceptual phenotypes based on their odor perception. We previously correlated differences in the perceived pleasantness and intensity of androstenone and androstadienone with genetic variation in the OR7D4 gene and suggest that the perceptual phenotypes discovered here may correspond to odorant receptor gene genotypes. Acknowledgements: Work in my laboratory is supported by HHMI and NIH/NIDCD.

PRESIDENTIAL SYMPOSIUM: NEUROTRANSMITTERS AND NEUROMODULATORS IN THE TASTE BUD

15 Cells, signals, and synapses in mammalian taste buds

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A taste bud is a population of 50 to 100 interacting cells that process gustatory stimuli and excite one or more primary sensory afferent fibers that innervate the taste bud. Some taste cells within the population respond to sweet, bitter or umami compounds, others to sour (H+), and yet others to salty (Na+). Fats (as fatty acids) may also stimulate selected subsets of cells within the population. Gustatory stimulation at the taste pore initiates a series of synaptic interactions within the population of taste cells, involving the exchange of excitatory and inhibitory signals, and including feedback signaling. My laboratory has focused on identifying the synaptic transmitters that underlie this exchange of signals in the taste bud. We are investigating which cells release neurotransmitters, what is(are) the transmitter(s), where do those transmitters act within the taste bud, and ultimately, how are the interactions among the different cells orchestrated to produce a meaningful signal output that can be transmitted to the sensory afferent fibers. Our findings to date indicate that considerable synaptic integration takes place during taste reception; taste buds appear to be conducting a remarkable extent of signal processing during gustatory stimulation.

16 Modulation of sweet taste responses by orexigenic and anorexigenic factors

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Sweet taste perception is important for animals to detect external carbohydrate source of calories and has a critical role in the nutritional status of animals. Our previous studies demonstrated that the taste organ is a peripheral target for leptin, an anorexigenic mediator that reduces food intake by acting on hypothalamic receptors. In mice, increase in plasma levels of leptin leads to reduction of sweet taste responses of taste nerve and receptor cells via activation

of leptin receptors. In human, the recognition thresholds for sweet compounds have a diurnal variation that parallels variation for leptin levels, with lowest in the morning and highest in the night. Plasma levels of leptin are shown to inversely correlate with circulating endocannabinonds (anandamide and 2-arachidonoyl glycerol), orexigenic mediators that induce appetite and stimulate food intake via CB1 receptors mainly in hypothalamus. Here, we investigated potential effect of endocannabinoids on sweet taste responses in mice and found that endocannabinoids oppose the action of leptin to act as enhancers of sweet taste. In wild type mice, administration of endocannabinoids enhances behavioral, taste nerve and taste cell responses to sweet taste without affecting responses to bitter, salty, sour and umami stimuli. In contrast, mice genetically lacking CB1 receptors show no such enhancement by endocannabinoids Endocannabinoids, therefore, not only stimulate food intake via central systems but also may increase palatability of foods by enhancing peripheral sweet taste responses. Reciprocal regulation of peripheral sweet taste reception by endocannabinoids and leptin may contribute to their opposing actions on food intake and play an important role in regulating energy homeostasis.

17 Insulin regulates the function of epithelial sodium channels and salt taste preference

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Diabetes is a profound disease that is reflected in an impairment of systemic salt and water balance. We hypothesize that epithelial sodium channels (ENaC) in mouse taste receptor cells (TRCs) play a role in the restoration of salt and water balance by virtue of insulin's effect on the taste system. In behavioral assays, insulin-treated mice show avoidance of NaCl at lower concentrations than controls. These differences were abolished when amiloride was added into NaCl solutions suggesting that insulin was regulating ENaC. To test for the ability of insulin to alter ENaC function, we performed patch clamp recording on isolated mouse TRCs. In TRCs, insulin caused a significant increase in Na+ influx (EC50 = 6.1 nM). Similarly, using the Na-sensitive dye SBFI, increasing extracellular Na+ from 0 to 140 mM elicited an increase in Na+ influx in a subset of TRCs and insulin treatment (20 nM) enhanced this Na+ influx. To investigate whether ENaC function is altered during the onset of diabetes, we performed Na+ imaging in isolated TRCs from a mouse model of Type 1 diabetes. TRCs from diabetic mice exhibit amiloride-sensitive Na+ responses similar to non–diabetic littermates. However, insulin enhancement of Na+ influx via ENaC was abolished in diabetic TRCs. TRCs from diabetic mice exhibit greater responses to NaCl than those from non-diabetic mice and behaviorally diabetic mice showed avoidance of NaCl at significantly lower concentrations. In contrast, diabetic animals showed no significant avoidance to these NaCl solutions when amiloride was added to NaCl solutions indicating a role for ENaC in this increased sensitivity. Our results are consistent with the hypothesis that ENaC alterations during diabetes may be another example of the ability of the gustatory system to respond to nutritional challenges. Supported by NIH DC02507 (TAG).

SYMPOSIUM - CILIA, SENSORY DYSFUNCTION AND DISEASE

18 Olfactory Cilia: Linking Sensory Cilia Function and Human Disease

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Cilia are intimately involved with the fundamental biological processes of photoreception, olfaction, and auditory function. The olfactory system is one of the multiple sensory systems that are critically dependent on the proper function of cilia. Non-motile sensory cilia on OSNs compartmentalize signaling molecules necessary for odor detection allowing for efficient and spatially confined responses to sensory stimuli. Since OSN cilia lack the necessary machinery for protein synthesis, nascent proteins must be transported from the cell body into the cilium. Movement along the ciliary axoneme is tightly regulated and involves evolutionarily-conserved intraflagellar transport (IFT) proteins. Joel Rosenbaum will discuss the basic mechanisms and regulation of IFT. Randall Reed and Jeffrey Martens will present work that elucidates specific mechanisms that are required for the translocation of the transductory machinery to the sensory cilia, as well as the human pathologies associated with defects in these processes. While it is clear that olfactory dysfunction is a clinical manifestation of a subset of ciliopathies, there appears to be a selective penetrance of phenotypes between different cilia in the body highlighting the necessity for comparison of multiple systems. Anand Swaroop will discuss mechanisms and clinical disorders associated with cilia dysfunction in the visual system while Kirk Mykytyn will talk about G-protein coupled receptor signaling in the CNS and its alteration in specific ciliopathies. Finally, Dominic Cosgrove will discuss the discovery of proteins that may link actin-based steriocilia of the inner ear to primary cilia. This session will highlight new cell biological approaches that can reveal not only mechanisms of fundamental olfactory processes. Acknowledgements: NIDCD (1R01DC009606-01)

19 Molecular Organization of Olfactory Transduction Components in Cilia

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Bardet-Biedl Syndrome (BBS) is a pleiotropic, heterogeneous disease associated with renal, mental, retinal, and early developmental anomalies. These phenotypes are consistent with defects in cilia formation or function. Identification of *BBS8*, one of 14 implicated in BBS, led to the hypothesis that BBS is caused by basal body and/or cilia defects. We previously showed that BBS patients and BBS mouse models exhibit impaired olfactory function consistent with the critical role of cilia in olfaction. We genetically ablated the mouse BBS8 gene since it is particularly abundant in OSNs and BBS8 antibodies reveal staining in dendritic knobs in a shell-like structure surrounding the basal bodies. BBS8 null mice have reduced olfactory responses by EOG recording. Immunofluorescence analyses of the OE reveal a dramatic truncation of OSN cilia, a disorganized dendritic microtubule network, and mislocalization of proteins normally enriched in cilia. Visualization of proteins in

OSNs by genetic reporters reveals altered localization patterns. Although OSN numbers are largely normal, targeting of OSN axons to the olfactory bulb is aberrant; axons expressing the same receptor display reduced fasciculation and project to multiple targets. Using reagents that reveal the characteristic neuronal activity of each OSN, we observed altered activity in BBS8-null OSNs. We hypothesize that alterations in cilia structure, the essential signaling platform for olfaction, alters the uniformity of responses in populations of OSNs expressing the same OR. In parallel, we have examined the requirements for specific olfactory transduction components to localize to cilia in cell-culture systems. This has led to valuable new tools to evaluate the mechanisms of cilia enrichment and dynamic properties of these sub-cellular structures. Acknowledgements: This work was supported by NIH grant DC04553

20 Intraflagellar Transport functions in cilia assembly and signalling processes, and also in exocytosis

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A major revival in research on cilia and flagella and their relationship to certain pathologies rested on the discovery of the IFT (Intraflagellar Transport) in all eukayrotic cilia and flagella, and the function of IFT in ciliary assembly and signalling processes. The cloning of the IFT genes led, first, to the ciliary hypothesis of PKD (Polycystic Kidney Disease) and later to the discovery of many other cilia-dependent diseases, now termed ciliopathies. For a decade, mutations in the IFT genes and their relationship to ciliary sensory defects (chemo, mechano, and phtotosensory) received most of the research attention. A new aspect of IFT function has recently been discovered with data showing the role of IFT polypeptides in the process of exocytosis. IFT gene mutations, therefore, may not only affect ciliary assembly and function, but other cellular functions dependent on exocytosis. Among these are the roles of exocytosis and vesicle fusion with the cell membrane during cytokinesis promoting the formation of the cytokinetic furrow, and in vesicle exocytosis at the neuronal synapses and in formaiton of the immune synapse. Exocytosis and IFT are also important in the vesicle fusion at the base of the cilium which is required for ciliary membrane formation. Finally, another new role for eukaryotic cilia has recently been described, and that is the formation of exosomes or small vesicles from the ciliary membrane at the ciliary tip. It is felt that, in addition to being an organelle of motility and sensory function, the cilium may also be an important secretory organelle, signalling adjacent cells by means of these vesicles.

21 Genetic interactions dictate photoreceptor cilia biogenesis, homeostasis and survival

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Vertebrate photoreceptors are polarized post-mitotic neurons with a modified primary cilium that includes membrane discs and is critical for phototransduction. Photoreceptor cilium is a highly metabolically active organelle as almost 10% of outer segment discs are renewed everyday. Genetic defects that affect cilia biogenesis or transport process can lead to dysfunction or death of photoreceptors and consequently vision loss. We are specifically interested in two cilia-associated proteins CEP290 and RPGR and exploring their roles using zebrafish and mouse models. We find that these proteins interact with a specific set of NPHP and BBS proteins, and such interactions are critical for photoreceptor function and survival.

22 Loss of Bardet-Biedl Syndrome Proteins Causes Aberrant Localization of Ciliary GPCRs in Central Neurons

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It has been known for more than forty years that neurons in the brain possess primary cilia, but the specific functions of these organelles remain unknown. Certain G protein-coupled receptors (GPCRs) specifically localize to neuronal cilia, suggesting they perform sensory and signaling functions on neurons. The functional importance of neuronal cilia is suggested by the fact that several human ciliopathies, including Bardet-Biedl syndrome (BBS), Joubert syndrome, and Meckel syndrome, have prominent functional and structural CNS phenotypes. BBS is characterized by obesity, retinal dystrophy, renal anomalies, hypogenitalism, polydactyly, and cognitive deficits. We have discovered that the BBS proteins are required for proper localization of GPCRs to primary cilia on neurons in the mouse brain. We hypothesize that some of the BBS phenotypes are the result of altered signaling due to ciliary GPCR mislocalization. Acknowledgements: National Institute of General Medical Sciences

23 Usher protein function in ciliated neuroepithelium of the cochlea and retina

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Usher syndrome is the leading genetic cause of deaf/blindness (retinitis pigmentosa) in the world. It is a genetically heterogeneous disorder for which there have been 10 genes identified associated with three clinical subtypes. The proteins encoded by the usher genes come from diverse classes including an unconventional myosin motor, scaffold proteins, transmembrane cell-cell adhesion molecules, a tetraspanin, and a G-protein coupled receptor. Usher proteins are expressed in ciliated neuroepithelial cells of the cochlea (hair cells) and the retina (photoreceptors). Mutant mice (spontaneous or engineered) for Usher genes all show splayed cochlear hair cell stereocilia, resulting in deafness. Most of these proteins are expressed developmentally in hair cells, and thought to be involved in adhesive interactions required for stereocilia development and mechanotransduction. Interestingly some usher proteins are not present in stereocilia, yet mutants still show the splayed phenotype. A synonymous function for usher protein complexes in photoreceptors has not surfaced. The proteins localize to the region of the inner segments near the connecting cilia. We recently uncovered a universal defect in usher mouse models. All mutants show a large threshold shift in light induced translocation of the G-protein α-transducin. These same animals are more sensitive to light induced oxidative damage and photoreceptors degenerate in response to exposure to bright light at a significantly faster rate than strain matched wild

type mice. Thus, usher proteins are implicated in the regulation of the visual cycle in photoreceptors. Acknowledgements: NIH R01 DC004844

24 PLATFORM PRESENTATIONS - POLAK YOUNG INVESTIGATOR AWARD WINNERS

Nasal SCCs respond to bacterial quorum sensing molecules

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The airways are continually exposed to harmful compounds carried on the incoming airstream. The trigeminal nerve responds to such compounds as irritants and evokes protective reflexes, including sneezing, apnea, and local inflammation of the mucosa. While free intra-epithelial nerve endings can detect some of these irritants (e.g., mints, capsaicin, acetic acid), the epithelium also houses a population of trigeminally innervated solitary chemosensory cells (SCCs) which detect substances that cannot penetrate the epithelium to activate trigeminal endings directly. These SCCs express T2R bitter taste receptors along with their downstream signaling components including Gα-gustducin and TrpM5. We have previously described that nasal SCCs are necessary to evoke trigeminal reflex reactions to denatonium benzoate. Here we show that these SCCs also detect acyl–homoserine lactones (AHL) utilized as quorum-sensing molecules by Gram-negative bacteria. Isolated SCCs respond to AHL signals by increasing intracellular Ca^{2+}. Furthermore, activation of the trigeminal nerve by these same compounds is observed in the form of changes in respiration (respiratory depression and apnea). Genetic deletion of either Gα-gustducin or TrpM5 eliminates the trigeminal nerve evoked reflex to AHLs. Thus functional SCCs are necessary for the AHL-evoked trigeminal response. AHLs are the first natural ligand described for the nasal SCCs and the detection of bacterial quorum-sensing molecules by SCCs is the first demonstration of a clear function for these cells. Since the SCCs are innervated by peptidergic polymodal nociceptor fibers, activation of this system by bacterial AHLs will trigger a local neurogenic inflammatory and immune response to fight the bacterial invasion. Acknowledgements: Supported by NIDCD

25 GABA: an inhibitory neurotransmitter in taste buds

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Taste buds secrete ATP upon taste stimulation, with Receptor (Type II) cells being the source of such secretion. Data from isolated taste buds, single taste cells, and most recently lingual tissue slices (Dando and Roper 2009) demonstrate that ATP is secreted from Receptor (Type II) cells via pannexin 1 hemichannels. The findings indicate that ATP mediates cell-cell communication between Receptor cells and Presynaptic cells as well as between Receptor cells and sensory axons. Besides ATP, other synaptic transmitters have only very recently been identified with confidence in mammalian taste buds. For instance, serotonin and norepinephrine are now known to be released during gustatory stimulation. Recent evidence (Herness, 2009) suggests that γ-aminobutyric acid (GABA) also plays a role in taste buds. We have used confocal calcium imaging on lingual slices to test GABAergic mechanisms in semi-intact taste buds. GABA and the GABAergic agonists muscimol (GABA-A receptor) and baclofen (GABA-B receptor), bath-applied at 10 μM, reduced taste-evoked-Ca^{2+} responses in taste buds in the lingual slice. These inhibitory effects were only observed in Presynaptic cells. GABA, muscimol, or baclofen did not alter taste-evoked responses in Receptor cells. GABAergic inhibition was partially reversed when lingual slices were incubated with the GABA receptor antagonists bicuculline (GABA-A), or CGP55845 (GABA-B), at 10 μM. Taken together, these results suggest that GABA inhibits gustatory signaling in taste buds, probably by interfering with cell-to cell communication pathways. Experiments are currently underway to identify if taste cells secrete GABA during gustatory stimulation and thereby identify the source of GABA. Acknowledgements: This work was funded by grants from NIH/NIDCD, 5R01DC007630 and 5R01DC000374 (SDR).

26 Birthdates of mitral cells regulate the soma location in mouse olfactory bulb

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Odor information acquired by olfactory sensory neurons (OSNs) is relayed to the dendrites of mitral and tufted cells in glomeruli of the olfactory bulb (OB). Each OSN expresses only 1 of ~1200 odorant receptors (ORs), and OSNs having the same OR converge into only 2-3 glomeruli/OB. Thus, the molecular specificity of the OSN is preserved in the target glomerulus. Glomeruli are therefore functional units for odor information processing and the spatial representation of glomeruli may be considered as an 'odor map'. What is the logic used by mitral/tufted cells to decode the odor map? Spatial information of the odor map should be reflected by the spatial distribution of mitral cell soma in the OB since they have single primary dendrite which arborizes and receives OSN axon within a single glomerulus. Dividing the mitral cells into spatially distinct subpopulations is an important step toward understanding the logic. However, in contrast to OSN axons, mitral cells exhibit no molecular specificity that may account for the glomerulus they innervate. Here, we classified mitral cells according to the time of birth during embryogenesis. By carefully analyzing the soma location in postnatal OB, we found that early-generated and late-generated mitral cells were differentially distributed in dorsomedial and ventrolateral regions of the OB, respectively. These regions correlate with dorsal and ventral domains of odor maps, which are defined by OCAM expression.

Thus, mitral cells may be divided based on birthdate into subpopulations which have different roles in odor map decoding. We will also present results suggesting that tangential migration of late-generated mitral cells along pre-existing axons of early-generated mitral cells is an underlying mechanism for birthdate-dependent map of mitral cells in the OB. Acknowledgements: This work is generously supported by NIH/NIDCD.

27 Faf1 as a Regulator of Olfactory Axon Guidance

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The mammalian olfactory system provides an excellent *in-vivo* model to study axon guidance through its precise connections linking the peripheral olfactory epithelium to the centrally located olfactory bulb (OB). The axons of olfactory sensory neurons (OSNs) project to specific targets on the OB, establishing a system where axon guidance can be evaluated against a predictable pattern. As apoptosis signaling has been implicated in the process of axon guidance we sought to use the olfactory system to study this connection. Here we report that the apoptotic signaling molecule, Fas-Associated Factor 1 (Faf1), which has been shown to regulate Fas-mediated cell death, can also regulate OSN axon guidance. Studies have shown that Faf1 is highly expressed in the mouse olfactory system during embryonic development. Therefore, we use a transgenic strategy that combines an early olfactory-specific promoter with the tetracycline-inducible system to modulate Faf1 expression in immature OSNs when their axons are actively seeking targets in the OB. Surprisingly, immature OSNs overexpressing Faf1 do not undergo apoptosis but rather mature OSNs are lost instead, suggesting Faf1 plays a more complex role than direct induction of cell death. In OSN axons, Faf1 overexpression produces significant targeting defects and even misroutes terminals into the deep layers of the OB. Using P2-LacZ mice we further demonstrate that Faf1 overexpression in immature OSNs disrupts convergence of P2 axons, producing multiple P2 glomerular-like structures in the OB. Perhaps most striking is that all of the described phenotypes are reversible such that shutdown of Faf1 overexpression can restore OSN axonal projections to their wildtype pattern. Thus, we conclude that Faf1 signaling can regulate OSN survival as well as axon guidance. Acknowledgements: This study was supported by the NIH/NINDS Intramural Program.

28 Food for Thought: Processing of Food and Non-Food Odors in the Human Brain

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The flavor percept is mediated primarily by the olfactory sense, thus rendering olfactory stimuli as the main carriers of flavor information we receive when tasting foods. In addition, behavioral research has previously shown that subjects are better in identifying food than non-food odors. Therefore, we hypothesized that these odors would be processed not only within the main olfactory system, but also within cortical areas known to process taste. We explored potential differences in neuronal processing between food and non-food odors in 30 healthy subjects (15 women, age 19-32 yr) by functional magnetic resonance imaging (fMRI). Pleasant and unpleasant odors representing food (banana, fish) and non-food (hydrogen sulfide, grass) stimuli were presented by an olfactometer, and were embedded in a constant flow of odorless, humidified and heated air. BOLD responses were acquired in a block-design using a 1.5T Siemens scanner, and subsequent analyses were performed using SPM5. Whereas both odor categories activated common olfactory areas, a direct contrast between the two odor categories demonstrated that food odors activated the entorhinal cortex, insula, precuneus and fusiform gyrus significantly more than non-food odors. There were no brain regions activated more for non-food odors than for food odors. Paired with our recent finding that food odors are processed faster than non-food odors, this indicates that separate neuronal networks exist for food versus non-food odors, and that primary taste areas in the brain respond to food odors, thus strengthening the behavioral link between food, flavor and olfaction. Whether this demonstrated difference is due to signals inherent in the odor source or to a learned association between food odors and its corresponding flavor (taste) remains to be elucidated. Acknowledgements: Funded by the Monell Chemical Senses Center. We would like to thank Anna-Lena Cordts for her help in the experiment.

SYMPOSIUM - SENSORY INTEGRATION AND COMPETITION

29 Smelling sounds: olfactory–auditory sensory convergence in the olfactory tubercle

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The olfactory code is influenced by numerous factors, including behavioral state, odor-sampling patterns and cross-modal sensory convergence. Growing evidence supports the view that primary olfactory cortical regions are not unimodal, but instead represent information from several sensory modalities – providing a substrate for sensory convergence early in olfactory processing. Adding to previous reports of both gustatory and visual influences on the cortical processing of odors, here we report novel findings revealing that the olfactory code is subject to auditory cross-modal influences. *In vivo* extracellular recordings from the olfactory tubercle, a trilaminar structure within the basal forebrain, of anesthetized mice revealed that olfactory tubercle single-units selectively respond to odors – with 65% of units showing significant odor–evoked activity. Remarkably, 19% of olfactory tubercle single-units also showed robust responses to an auditory tone. Furthermore, 29% of single-units tested displayed supra-additive or suppressive responses to the simultaneous presentation of odor and tone, suggesting cross-modal modulation. In contrast, olfactory bulb units did not show significant responses to tone presentation, nor modulation of odor-evoked activity by tone – suggesting a lack of olfactory-auditory convergence upstream from the olfactory tubercle. Thus,

the tubercle presents itself as a source for direct multimodal convergence within an early stage of odor processing, and may serve as a seat for psychophysical interactions between smells and sounds. Acknowledgements: NIDCD grant DC003906 to D.A.W.

30 Multisensory stimulation modulates perceptual ratings and neuronal activity

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Over the last few decades, sensory perception and its neurobiological substrates have been extensively studied. However, up until recently, most of this research has focused on only one modality in isolation, thus standing in sharp contrast to how we experience multimodal stimuli in our everyday lives. Multimodal sensory experiences, such as seeing, hearing, and smelling a coffee brewer or a popcorn machine, have the behavioral advantage of being more easily identifiable than unimodal stimuli alone. The aim of this series of studies was to investigate the perceptual and neural correlates of multisensory integration using behavioral testing and event-related functional MRI. We used odors, short videos, and sounds originating from six distinct stimulus objects, evenly split between positive and negative percepts. Congruent as well as incongruent stimulus combinations were administered to allow for comparison of the unimodal sensation with the multimodal combination of the stimuli. The behavioral results show that perceptual ratings of multisensory stimuli differed compared to their bimodal equivalent. Moreover, increased cerebral activation in response to multisensory integration occurred bilaterally in areas known to integrate cross-modal stimuli and bilaterally in a not previously demonstrated integration area residing within the superior frontal gyrus. The benefits of multisensory experiences for perceptual and neuronal processing and their implication in our everyday life will be discussed. Acknowledgements: Supported by start-up funds from the Monell Chemical Senses Center awarded to JNL and a DAAD postdoctoral fellowship D/08/40252 awarded to JA.

31 fMRI and TMS studies of multisensory integration

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In the first part of the talk, I will discuss human brain areas that fMRI suggests are important for multisensory integration. The first area, the intraparietal sulcus, is important for integration of vision and touch. The second area, the superior temporal sulcus, is important for the integration of the visual and auditory modalities. In the second part of the talk, I will discuss how the brain performs multisensory integration when it is presented with conflicting information from different modalities; and, more generally, how it handles situations in which different sensory modalities provide information about the environment that is more or less reliable. The concept of Bayes-optimal integration will be introduced, and imaging experiments that explore the neural mechanisms for optimal multisensory integration will be described. Finally, I will discuss what happens to perception when brain areas for performing multisensory integration are disrupted using transcranial magnetic stimulation (TMS). Acknowledgements: NSF and NIH

32 Odor information processing by the olfactory bulb analyzed in gene-targeted mice

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In mammals, olfactory sensory neurons (OSNs) expressing a specific odorant receptor (OR) gene project with precise stereotypy onto one or a few glomeruli in the main olfactory bulb (MOB). In recent years we examined the functional connectivity of bilateral olfactory bulbs and the representation of olfactory signals by mitral/tufted cells, the projection neurons of the bulb. By focal injection of tracer into genetically identified glomeruli, we have found that the anterior olfactory nucleus pars externa (AONpE) links isofunctional olfactory columns in the bilateral MOB and serves an important role in bilateral exchange of odorant-specific information. By recording from OSNs expressing mouse I7 receptor and their postsynaptic neurons in the bulb, we found that I7 OSNs and their corresponding M/T cells exhibit similarly selective tuning profiles at low concentrations. Increasing the concentration significantly reduces response selectivity for both OSNs and M/T cells, although the tuning curve of M/T cells remains comparatively narrow. By contrast, interneurons in the MOB are broadly tuned, and blocking GABAergic neurotransmission reduces selectivity of M/T cells at high odorant concentrations. Our results indicate that olfactory information carried by an OR is channeled to its corresponding M/T cells and support the role of lateral inhibition via interneurons in sharpening the tuning of M/T cells. Acknowledgements: China MOST, NNSFC, HFSP

33 Binaral rivalry and olfactory awareness

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When two pieces of conflicting information are presented at the same time to a pair of sensory organs, the brain tends to resolve the conflict by switching between the information. Also known as perceptual rivalry, this phenomenon has been documented in vision and audition. Recently, we have shown that this competition also operates in olfaction (Zhou & Chen, 2009), and we have dubbed it ''binary rivalry''. In this talk, I will introduce the context of our work, summarize the initial data demonstrating the effect, and present results from our more recent work which further characterize this phenomenon. Some general implications of our findings will be discussed.

34 Evidence of a central gustatory map in humans

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There is a clear need to localize both appetitive (food, mates) and aversive (predators) elements in our environment via chemosensory

cues. In humans, it appears that lateralizing odors without somatosensory or movement cues is difficult. Yet other species, for example scorpions, can identify what type and where prey are located by how the olfactory organ (the comb) is stimulated. In humans, the gustatory receptors are embedded within the oral epithelium and appear to convey sensations that share properties with other skin sensations such as the location of stimulation. For example, in the absence of discriminative tactile cues, humans can lateralize taste stimuli of varying taste quality. This means that the gustatory sensory signals allow the observer to know both what is in the mouth (something sweet) and where it is located in the mouth (anterior, left). By logical extension, if several identically shaped items are placed in the mouth and only one conveys taste sensations, humans can identify, locate, and remove the one that conveys taste even without the movement of the objects. More difficult still is the lateralization of a stimulus of a particular taste quality while a competing taste quality is present and while tactile and taste intensity cues have been rendered irrelevant. The ability to localize a taste stimulus based solely on its taste quality and location is likely derived from a central gustatory map that can inform what and where a stimulus is in the oral cavity. These cues enrich the information from a potential food and may be used to help recognize oral items either alone or when a heterogeneous bite is taken. Acknowledgements: This work was supported by NIH DC02995.

SYMPOSIUM - CHEMORECEPTION IN CONTEXT: INTERACTIONS WITH ENDOCRINE SYSTEMS AND METABOLIC STATE

35 Olfactory epithelium, a tissue under metabolic influences

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The nutritional status of individuals influences odour detection. Fasting results in an increased perception of food-related odors, whereas satiety is correlated with a reduction in their olfactory detection. This suggests that metabolic signals are able to modulate olfactory functions. Leptin and insulin are good candidates to be such metabolic signals as they modulate electrical activity of olfactory bulb (OB) neurons. We suggest that, beside effects at the OB level, these hormones act at the olfactory mucosa (OM) level . We established the expression and localization of insulin and leptin receptors in rat OM and demonstrated that 48h fasting, leading to decreased plasma leptin and insulin levels, increased their expression. Surprisingly, this increased number of insulin receptors, evaluated by radio receptor assay, is not observed in the OB, suggesting a differential regulation at these two levels of the olfactory system. We further showed that insulin application on OM, mimicking a postprandial insulin surge, decreased the amplitude of electro-olfactogram responses to odorant. These data provide evidence that OM is under hormonal driven metabolic influences and that insulin is able to modulate the olfactory message amplitude from its first step. Furthermore, we found faint local production of insulin and leptin, which are increased by fasting. This suggest paracrine/autocrine role for these hormones in OM. We showed that insulin is involved in the proliferation/apoptosis balance regulating the OM renewing since it is able to inhibit OM apoptosis induced by bulbectomy through the regulation of p53-dependent pathway. The evaluation of OM function in insulino- and leptino-resistant obese rats is under investigation. Acknowledgements: This work was supported by the French National Research Agency (ANR-05-PNRA-1.E7 AROMALIM).

36 Olfactory neurons activity and olfactory perception are modulated by anorectic peptides, insulin and leptin

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The mechanisms controlling food intake are based on internal (endocrine, metabolic) and external (the sensory characteristics of food) signals. Olfaction is one of the external clues that leads to consume regardless of nutritional needs. Conversely, as we shown, the olfactory acuity is modulated by the nutritional status. This interplay could be mediated by neuroendocrine signals like insulin and/or leptin as their receptors have been described at peripheral (olfactory epithelium) and central (olfactory bulb, OB) levels. To test this hypothesis, we studied the modulation of the activity of the olfactory sensory neurons (OSN) by hormonal peptides. Using patch-clamp recordings in an *in vitro* intact epithelium, we showed that insulin- or leptin-perfusion significantly raises the spontaneous firing frequency of the OSN. Moreover, these hormones decrease the OSN transduction current and receptor potential recorded during odorant stimulation. Therefore by increasing the spontaneous activity but reducing the odorant-induced activity of OSN, an elevated insulin and leptin level may result in a decreased global signal to-noise ratio in the olfactory epithelium. This input to the bulb is further modulated by the main output neurons of the OB (mitral cells, MC).The MC are one of the central targets of these hormones: MC express insulin receptors and their discharge is modulated by insulin or leptin. This effect would underlie our behavioral tests where an icv injection of either insulin or leptin decreases olfactory detection. Thus insulin and leptin act in the olfactory system both at peripheral and OB levels to modulate olfactory sensitivity. The role of olfaction in the control of food intake appears crucial and needs to be investigated further in order to better understand alimentary pathologies. Acknowledgements: This grant was supported by the Agence Nationale de la Recherche (ANR), the French national research agency (project ANR-05-PNRA-1.E7 AROMALIM)

37 The olfactory bulb as a metabolic sensor via insulin modulation

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Gene-targeted deletion of a predominant voltage-dependent potassium channel, Kv1.3, in mitral cells of the olfactory bulb results in a gamut of phenotypic changes including a ''super-smeller'' ability, supernumerary axonal projections to heterogenous glomeruli, and an alternation in action potential discharge frequency. Kv1.3 is a substrate for tyrosine phosphorylation and the molecular targets for modulation by the hormone insulin have been mapped along the N- and C-termini of the channel. Our laboratory has developed awake, intranasal insulin delivery approaches that demonstrate a robust phosphorylation of the channel that is linked to the behavioral but not anatomical modifications of the knockout model. Recently the laboratory has discovered that the channel is also responsible for regulating body weight and that both diet- and genetically-induced obesity can be abrogated via gene-targeted deletion of the channel, or uniquely by olfactory bulbectomy that manipulates total energy expenditure. We have now recorded on acute and chronic insulin treated mice as well as diet-induced obese mice and will present an electrophysiological analysis performed on brain slices of how metabolic state alters neuronal responses in mitral cells of the olfactory bulb. High fat diet has additionally demonstrated an anatomical loss of olfactory sensory neurons, presynaptic to the mitral cell input. Acknowledgements: This work was supported by NIH DC03387 and NIH DC00044 from the NIDCD and sabbatical award from FSU.

38 Roles of taste signaling molecules in endocrine cells in pancreas and tongue

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Gα-gustducin, T1r receptors and other taste signaling elements are expressed in duodenal enteroendocrine L cells that express insulinotropic glucagon-like peptide 1 (GLP-1). Gustducin and T1r3, well known for their roles in taste signaling, are essential for L cell release of GLP-1 in response to glucose. We examined taste cells to determine if they too expressed hormones typical of intestinal enteroendocrine cells. Taste cells were found to express GLP-1, glucagon, PYY and other gut hormones. Patterns of expression indicated that gustducin-expressing type II taste cells and other subtypes of taste cells express GLP-1. We also examined the function of hormones released from these ''endocrine taste cells''. In wild-type mice, with or without esophagealectomy/vagotomy, application of glucose to the tongue induced a rapid elevation of GLP-1 in the bloodstream. Stimulation of taste cell explants from wild-type mice with glucose led to release of GLP-1 into the medium. Stimulation of gustducin-null mice with glucose did not lead to significant release of GLP-1 from taste cells in vivo or in explants. At least a portion of the cephalic phase rise in circulating GLP-1 depends on direct release of GLP-1 from taste cells into the bloodstream and requires gustducin. Taste receptors, Gα-gustducin and downstream proteins were also found to be expressed in several types of pancreatic islet cells. Gα-gustducin is expressed in alpha cells, and T1R3 is expressed in alpha and beta cells. Functional assays showed that in wild type mice, but not in T1R3 null mice, T1R3 receptors mediate increased secretion of insulin in response to artificial sweeteners and sweet tasting amino-acids. Acknowledgements: Supported by NIH grants DC03055 and DC03155 to RFM

39 Mechanisms of alimentary chemosensation and modulation

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Chemosensory cells throughout the alimentary canal use a common molecular toolkit to detect and respond to nutrients and other chemostimuli. For example, ''taste'' transduction molecules (e.g., TAS1R and TAS2R receptors, gustducin) regulate hormonal secretion in the gut. We have reported that a loss-of-function variant of a human TAS2R is associated with glucose dysregulation and the presence of Type 2 diabetes mellitus in a human population. Furthermore, bitter-tasting compounds can elicit glucagon-like peptide-1 (GLP-1) secretion from enteroendocrine L cells in a receptor-dependent manner, thus suggesting a mechanism by which TAS2R function could influence glucose homeostasis. Interestingly, ''gut'' hormones (e.g., GLP-1) can modulate peripheral taste function as well. We found that both GLP-1 and glucagon modulate sweet taste sensitivity. GLP-1 is produced in two distinct subsets of mammalian taste cells, while the GLP-1 receptor is expressed on adjacent intragemmal afferent nerve fibers. In contrast, glucagon and its receptor are coexpressed in a distinct population of taste cells. However, both GLP-1 and glucagon signaling appear to enhance or maintain sweet taste sensitivity: genetic and/or pharmacological disruption GLP-1 or glucagon signaling results in dramatically reduced taste responses to sweeteners in behavioral assays. Together, our recent studies of the interplay between gustatory and endocrine systems support a role for canonical ''taste'' molecules in the maintenance of metabolic homeostasis and suggest that sensory function may be modulated in the context of an animal's metabolic status. Acknowledgements: Supported by: NIDCD (R01 DC005786, R01 DC010110, F31 DC010113, T32 DC000054, P30 DC010364), NIDDK (P30 DK072488), NIDCR (T32 DE007309) and the Ajinomoto Amino Acid Research Program.

SYMPOSIUM - WIRING THE OLFACTORY SYSTEMS

40 Olfactory Ensheathing Cell Plasticity can be regulated by DNER, a protein highly expressed on Olfactory Receptor Neuron axons

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Olfactory ensheathing cells (OECs) ensheathe, guide and promote growth of olfactory receptor neurons (ORNs) throughout life. Because OECs can promote axonal re-growth across a PNS:CNS boundary in the olfactory system, they have become a candidate

for cell-mediated repair following CNS lesion, with enormous variation in results. Given that different methods used to culture OECs may change their innate characteristics, we hypothesized that factors within the olfactory neuraxis maintain OECs in a plastic state that is lost during culture, and this affects the ability of OECs to promote CNS repair and regeneration. As OECs are passaged, they flatten and senesce, progressively lose the ability to promote axonal outgrowth, and down-regulate expression of several developmentally-regulated proteins, including Brain lipid binding protein (BLBP; FABP-7). BLBP is highly expressed by embryonic CNS radial glia, and is induced via canonical Notch signaling. OECs retain the highest levels of BLBP expression in the adult nervous system, but Notch ligands (Delta, Jagged) are not highly expressed in the adult olfactory system. Instead, we have found that Delta/Notch-like EGF-related receptor (DNER), a trans-membrane non-canonical Notch ligand which can stimulate deltex-mediated signaling, is highly expressed in ORN axons (and mitral cell dendrites) throughout development. OECs in close apposition with DNER-expressing ORN axons *in vivo* maintain high BLBP expression, which decreases when ORN axons are removed following bulbectomy, or in a DNER null. Cultured LP-OECS which have reduced BLBP expression after passaging can be induced to re-express BLBP by stimulation with recombinant DNER. We are now testing if restoring non-canonical DNER-mediated Notch signaling in OECs also promotes their functional plasticity. Acknowledgements: Canadian Institutes of Health Research

41 Axon - matrix interactions regulate olfactory wiring

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Olfactory sensory neuron (OSN) axons follow stereotypic spatiotemporal paths in the establishment of the olfactory pathway. The topography of olfactory projections from epithelium to olfactory bulb (OB) is an essential determinant of odor coding. The mechanisms subserving the sorting and targeting of axons are complex. Recent studies highlight the importance of guidance molecules including odor receptor proteins which have been shown to be necessary for correct targeting. We have identified extracellular matrix (ECM) molecules expressed early in the developing pathway which we have proposed to play a role in its initial establishment. During later embryonic development, when axons sort out and target specific glomeruli, we have hypothesized that ECM cues may also act to help establish the complex olfactory topography. In a screen of ECM molecules expression during the period of glomerulogenesis we identified tenascin-C (TNC) in a boundary-like expression pattern, which appears to prevent axons from prematurely innervating deeper layers of the OB and initiating glomerulogenesis. To investigate this hypothesis we developed an *in vitro* assay of OSN neurite outgrowth and demonstrate that TNC is inhibitory in a dose dependent manner, and axons avoid growing on TNC substrates in stripe assays. Analysis of glomerulogenesis in TNC null mice reveals that glomerular development is delayed in the absence of TNC. These data correlate with previously published behavioral reports of TNC null mice which display impaired olfactory function in the early postnatal period, but recover function over the first postnatal week (de Chevigny et al. 2006 Mol Cell Neurosci. 32:174-86). Together, these data demonstrate that TNC acts to restrict OSN axons to the nerve layer during a key period of olfactory pathway development. Acknowledgements: Supported by NIH DC005706 and DC007600 to HBT, HHMI and NIH-NHLBI Fellowships to LVD, Deutsche Forschungsgemeinschaft to MS, and NIH DC00210 to CAG .

42 Faf1 as a Regulator of Olfactory Axon Guidance

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The mammalian olfactory system provides an excellent *in-vivo* model to study axon guidance through its precise connections which link the peripheral olfactory epithelium to the centrally located olfactory bulb (OB). Since axons of olfactory sensory neurons (OSNs) project to specific targets in the OB, the system offers a predictable pattern from which to evaluate changes in axon guidance. As apoptosis signaling has been implicated in the process of axon guidance we used the olfactory system to explore this connection by focusing on the apoptotic signaling molecule, Fas-Associated Factor 1 (Faf1). Faf1 has been shown to play a regulatory role in Fas-mediated cell death and is expressed in the olfactory epithelium during development. Using a transgenic strategy, which combines an early olfactory-specific promoter with the tetracycline-inducible system, we overexpressed FAF1 in immature OSNs, when their axons are actively seeking targets in the OB. Our data show that while overexpression of Faf1 does not induce cell death in immature OSNs, it does result in severe mis-targeting of axons to the OB. Interestingly, these phenotypes are reversible as OB organization returns to normal when Faf1 overexpression is shut down. Thus, we propose that Faf1 may play a functional role in axon guidance possibly through the modulation of apoptosis signaling. Acknowledgements: This study was supported by the NINDS/NIH intramural program.

43 Reduced Avoidance Response to Predator Odorants Associated with Wiring Defects in the Olfactory System of Robo-2 Mutant Mice.

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The formation of complex stereotypic connections between olfactory sensory neurons (OSNs) and second order neurons in the olfactory bulb (OB) is believed to be important for accurate odorant information processing. Ablation of OSNs that innervate the dorsal (D) region of the OB leads to a loss of avoidance behavior in mice in response to aversive odorants such as trimethyl-thiazoline (TMT) and 2-methylbutyric (2MB) acid[1]. It remains to be determined whether more refined disruption of the glomerular map in these regions of the OB could have an effect on innate responses in mice. To examine this question, we have generated mice lacking expression of the axon guidance receptor Robo-2 specifically in OSNs and

analyzed the targeting accuracy of axons projecting from two subsets of OSNs innervating the dorsal region of the OB. While MOR1-3-expressing axons that project to the DI region of the OB target accurately in *robo-2* mutant mice, MOR174-9-expressing axons that innervate the DII region coalesce on glomeruli located more ventrally in *robo-2* mutant mice. To examine the functional consequences of these wiring defects, we evaluated the avoidance behavior of wild type and mutant mice in response to TMT and 2MB acid. *Robo-2* mutant mice showed a robust decrease in their avoidance behavior towards the predator odorant TMT but could be conditioned to avoid TMT using LiCl injections. Interestingly, *robo-2* mutant mice showed similar avoidance behavior as wild-type mice in response to 2-MB acid. Taken together, our results indicate that defects in the wiring of the olfactory system can lead to selective loss of some innate behavioral responses in mice. 1. Kobayakawa et al. (2007) Nature 450:503-508. Acknowledgements: Canadian Institutes of Health Research

CLINICAL LUNCHEON (Ticketed event) - NEW CLINICAL TRIAL FUNDING OPPORTUNITIES AT NIDCD

44 New clinical trial funding opportunities at NIDCD

Gordon Hughes

Program Officer, Clinical Trials, NIDCD

The National Institute on Deafness and Other Communication Disorders is committed to building and expanding its clinical trials program to promote the development of interventions to treat or prevent disorders in hearing, balance, taste, smell, voice, speech and language. Three new clinical trial initiatives and funding opportunities, issued in July, 2008, can be found at http://www.nidcd.nih.gov/research/clinicaltrials. Individual application information can be found at http://www.nidcd.nih.gov/funding/foa/ . The Phase I/II Preliminary Clinical Trial specifically targets studies that will provide preliminary data and optimize the design of the eventual phase III trial. The Phase III Clinical Trial Planning Grant is designed to permit early peer review of a proposed phase III clinical trial and is used to develop a detailed Manual of Procedures, train clinical sites and prepare case report forms. The Phase III Definitive Clinical Trial should have the potential to significantly impact on clinical practice or public health policy. NIDCD strongly encourages clinical trial applications in chemoreception sciences.

SYMPOSIUM - TRANSIENT DYNAMICS, METASTABLE STATES AND TEMPORAL CODING IN CHEMOSENSORY PROCESSING

45 Frequency Transitions in Odor-Evoked Neural Oscillations

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Odor-evoked neural oscillations are ubiquitous in olfactory systems. How do these oscillations originate, and what determines their properties? In insects, odor-elicited oscillations are produced in the antennal lobe by interactions of excitatory projection neurons (PNs) and inhibitory local interneurons. In moths, we found that lengthy odor presentations elicited oscillations that were initially fast (~40 Hz) but then rapidly shifted to a slower rate (~10-20 Hz). Because this shift in frequency closely paralleled the intensity of output from the antenna, we first thought the oscillation rate was driven by the strength of the stimulus. However, we also found that changing the intensity (concentration) of the odor had almost no effect on the initial oscillation frequency. Individual olfactory receptor neurons (ORNs) showed a surprisingly narrow dynamic range owing to sensory adaptation and saturation. But because ORNs became less selective as the odor concentration increased, the size of the population of ORNs that responded to an odor increased with the concentration. Thus, in the periphery, the great majority of the olfactory system's dynamic range derives from the size of the responsive population of ORNs, not the intensity of its response. Our recordings and a computational model showed that lengthy stimuli caused each ORN to adapt; this reduced the intensity of input to the oscillator circuit, reducing its output frequency. In contrast, changing the concentration of the odor changed the number of responsive ORNs, but not the intensity of the most responsive ORNs providing input to the oscillator circuit; thus the oscillation frequency remained stable. Acknowledgements: This work was supported by the Japan Society for the Promotion of Science (00169, 70510) to I.I., Joint NIH-NIST postdoctoral fellowship award by National Research Council to B.R., grants from NIH-NIDCD and NIH-NINDS to M.B. and an intramural grant from NIH-NICHD to M.S.

46 Multiple Roles for STDP in Shaping Olfactory Representations

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Odor representations in insects undergo progressive transformations from the receptor array in the antenna, via the antennal lobe (AL), to the presumed site of odor learning, the mushroom body (MB). Broad activation of the AL by an olfactory stimulus gives rise to oscillatory population activity and diverging trajectories of projection neuron (PN) activation. Different points along these trajectories can be thought of as representing different aspects of the odor stimulus, and Kenyon cells (KCs), which decode PN activity in the MB, respond sparsely at specific time-points along the PN trajectories. Previous work suggests that individual oscillation cycles are meaningful units for the encoding and decoding of olfactory information by PNs and KCs. This appears to be the case also for extrinsic neurons in the mushroom body beta-lobe (bLNs), which decode the KCs' sparse responses. I will describe the results of intracellular recordings made from KCs and bLNs to examine synaptic transmission, plasticity and odor representation in this circuit. We have found that KC-bLN synapses undergo Hebbian spike-timing dependent plasticity (STDP) on a timescale similar to the oscillatory population discharge, which is generated by the AL and propagated throughout the circuit. This plasticity has a homeostatic effect on the phase of bLN firing, facilitates

the synchronous flow of olfactory information, and maintains the segregation between oscillation cycles. We also address how the interaction of STDP with phase-locked inhibition and neuromodulation shapes the way odors are represented in the MB. Considered within the context of the circuit in which the KC-bLN network is embedded, these results suggest a mechanism for learning different aspects of an odor stimulus. Acknowledgements: Broad Fellows Program in Brain Circuitry. Multi University Research Initiative (ONR)

47 Timing in olfaction

Dmitry Rinberg, Roman Shusterman, Matt Smear, Thomas Bozza

Olfaction is traditionally considered a 'slow' sense. However, recent evidence demonstrates that rodents are capable of making difficult odor discriminations rapidly, in as little as a single sniff. Can olfaction operate at even faster time scales? Odors can vary on a time scale finer than the period of a sniff cycle (roughly 100-500 ms) - odors are temporally structured by air turbulence, the sniff waveform, and the complex geometry of the nasal turbinates. Does the olfactory system preserve this precise timing information? And can an animal use this faster variation to guide behavior? To understand the temporal aspects of olfactory information processing, we combine electrophysiological, optogentic, and behavioral approaches. We have analyzed the temporal structure of mitral cell activity in awake mice. We found very precise spiking patterns relative to the sniffing cycle, with jitter as small as 20 ms during odor presentation. This precise timing may carry information about the stimulus during odor presentation: different odors evoke different patterns in the same cell and different cells respond differently to the same odors. To better control the timing of our stimulus, we have generated transgenic mice that express Channelrhodopsin2 in all olfactory sensory neurons (OSNs), which allows us to decouple sniffing from stimulus delivery by stimulating sensory neurons with light. We trained animals to discriminate different temporal patterns of stimulation. We found that mice can discriminate temporal difference as small as 10 ms relatively to the sniffing-breathing cycle. Precise temporal neuronal responses to odors and the ability of mice to discriminate spatially-identical OSN stimuli on the basis at sub-sniff-cycle timing differences provide strong evidence that precise stimulus timing plays as crucial a role in olfactory neuronal coding and perception as it does in other sensory modalities. Olfaction may not be such a 'slow' sense.

48 Meta-stable states in taste processing

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Both mammalian gustatory cortical (GC) and amygdalar neurons respond to taste administration with time-varying progressions of firing rates, different ''epochs'' of which containing different types of information. When complex analyses are brought to bear on simultaneously-recorded GC ensembles, these temporal codes are revealed to be sequences of meta-stable population states; the progressions themselves are reliably taste-specific, but the timing of state-to-state transitions vary from trial to trial. In this talk, I show that such coherent sequences of meta-stable states are a common feature of decision-making networks under certain parameter settings, and that networks working in a ''state-sequence regime'' have many advantages over other networks with regard to correct decision-making. I go on to present data demonstrating that the trial-to-trial variability of the observed sequences is an intrinsic part of attentive processing, quite possibly reflecting the recruitment of more distributed inter-regional neural networks to the task of taste identification. This leads me to preliminary quantification of amygdala-cortical state-sequence coupling during attentive and inattentive taste responses. These data and analyses offer the beginning of a wholly new characterization of perceptual neural processing in the mammal. Acknowledgements: DC 006666, DC 007703, Swartz Foundation

49 Analyzing neuronal networks using discrete-time dynamics

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We present mathematical techniques for analyzing detailed Hodgkin-Huxley like models for excitatory-inhibitory neuronal networks. While these networks arise in many important applications, a primary focus is to better understand mechanisms that underlie temporally dynamic responses in early processing of olfactory sensory information. The models presented here exhibit several properties that have been described for olfactory codes in an insect's Antennal Lobe. These include transient patterns of synchronization and decorrelation of sensory inputs. Our strategy for studying a given network is to first reduce it to a discrete-time dynamical system. The discrete model is considerably easier to analyze, both mathematically and computationally, and parameters in the discrete model correspond directly to parameters in the original system of differential equations. By reducing the model to a discrete system, we are able to systematically study how properties of the dynamics, including the complex structure of the transients and attractors, depend on factors related to connectivity and the intrinsic and synaptic properties of cells within the network. Acknowledgements: NIH grant 1 RO1 DC007997-01

50 Lessons from olfactory processing for odor recognition using artificial sensor arrays

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We will investigate what aspects of olfactory processing are useful for practical implementations of artificial electronic noses. Olfactory receptor neurons have high selectivity to odorants while metaoxide sensor arrays display a broad spectrum of responses. Despite the limitations of the artificial sensors we can use the structural organization of olfactory processing to properly classify a set of analytes. Robustness is another prevalent feature of information processing in the brain. We will identify what aspects of the organization are resilient to damage and drift or changes in the stimulus. The role of time is definitely important due to the time-varying nature of the sensory responses. We will also discuss the role of transient sensor dynamics for the classification of odorants. Acknowledgements: ONR/MURI N00014-07-1-0741

PLATFORM PRESENTATIONS - THROUGH THE NOSE

51 CO_2 Receptor Response Modifying Odors; Novel Tools for Control of Mosquitoes

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Female mosquitoes use the sense of smell to identify human hosts and are responsible for transmission of many deadly diseases that effect hundreds of millions of people every year. CO_2 present in exhaled air is considered to be one of the most important olfactory cues for mosquitoes, causing activation of long-distance host-seeking flight behavior, as well as increased sensitivity to other skin odors. Here we show that volatile odorants from fruit can strongly inhibit the CO_2 receptor in *Drosophila melanogaster* and completely abolish CO_2-mediated behavior. Using the 'empty neuron' *in vivo* expression system we establish that the odorants act directly on the CO_2 receptor Gr21a/Gr63a. Following our work on *Drosophila*, we have used electrophysiology assays to perform a comprehensive analysis of structurally related odorants in multiple vector mosquito species that have conserved CO_2 receptor proteins. We identify three novel classes of odorants that dramatically alter the response of the CO_2-sensitive neuron. These three classes of odors include: odors which inhibit the CO_2-sensitive neuron and are candidates for use in disruption of host-seeking behavior, odors that activate the neuron and can be a substitute for CO_2 as a lure in trapping devices, and odors that cause strong and prolonged activation of the CO_2 neuron which blocks the ability to detect changes in CO_2 concentration and therefore offers a novel approach for disruption of host-seeking. Detailed behavioral analyses for some of these odors show a dramatic disruption in the ability of mosquitoes to be attracted to CO_2. The CO_2-response modifying odors offer a powerful approach to develop a new generation of economical, and environmentally friendly insect repellents and lures that can reduce the ability of mosquitoes to seek out humans. Acknowledgements: Supported by a Gates Foundation Explorations Grant.

52 RNAi-mediated dissection of olfactory behavioral response profiles of odorant binding proteins in Drosophila melanogaster.

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Chemosensation in Drosophila is mediated by large multigene families of chemoreceptors, including olfactory receptors, gustatory receptors, and odorant binding proteins (OBPs). The latter are highly divergent soluble proteins, which in the antennae and maxillary palps are secreted into the aqueous perilymph where they are thought to facilitate transport of hydrophobic odorants to olfactory receptors on the chemosensory membranes of olfactory sensory neurons. Although some OBPs have been functionally implicated in pheromone responses and host plant selection, their functions in general odorant recognition are thus far poorly characterized. To assess molecular response properties of OBPs, we have systematically suppressed the expression of 17 OBPs by crossing a tubulin-GAL4 driver line to lines from the Vienna Drosophila RNAi Center collection that express RNAi corresponding to individual Obp transcripts under UAS promoters inserted in the neutral PhiC31 integration site. RNAi expression was enhanced with a UAS-GAL4 enhancer and real time qRT-PCR showed effective suppression of target RNAs in the GAL4-UAS hybrid offspring. We measured behavioral responses to a spectrum of odorants, and different odorants revealed altered behavioral responses in subsets of lines in which Obp gene expression was suppressed. Moreover, the response profiles showed sexual dimorphism. Our results demonstrate that our approach can delineate odorant response profiles of OBPs and confirm that interactions between general odorants and OBPs are combinatorial. Furthermore, our results show that males and females experience the chemosensory environment through different expression patterns of chemoreceptors. Acknowledgements: NIH grant GM059469.

53 Calcium imaging of retronasal odor responses in the olfactory bulb (OB) in the anesthetized rat

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The perception of food flavor critically involves retronasal olfaction. Little is known about the detailed neural circuitry and mechanisms underlying retronasal stimulus coding, experience and sensory integration of flavor. To complement our optical imaging studies in awake animals, we perform controlled ortho- and retronasal odor stimulation in the anesthetized rat. We employ a novel positive-pressure odor delivery system and record flow-resistance of each route. By optical calcium imaging of retronasal olfactory bulb responses to gas-phase odorants in anesthetized rats, we ask whether rats could smell retronasally, and how these responses compare to orthonasal olfaction under tightly controlled conditions. Besides odor route, we further investigate the effect of odor concentration, flow rate and lipophilicity. We present preliminary evidence that both ortho- and retronasal stimuli evoke consistent and overlapping response patterns in the dorsal OB, suggesting that at physiologically relevant flow rates retronasal odorants can reach the ORNs. Further, we report reliable effects of route and flow rate on response patterns, which themselves depend on odor. These data provide the first evidence of spatio-temporal retronasal response patterns in the rodent OB and make retronasal smell feasible in the rat. Acknowledgements: R01 DC009994-01

54 Systems Level Decoding of Odor Receptor Chemical Space *In Silico*

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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

performed a systems level analysis of odor coding covering a large chemical space for the antennal repertoire of Odorant receptors (Or) in *Drosophila.* We apply chemical informatics on each receptor to determine structural features shared by activating odors and utilize this to screen a large library of compounds (>240,000) *in silico* for novel ligands, including an extensive collection of plant and animal volatiles. Functional validation through single unit electrophysiology demonstrates the strong predictive ability of our approach (>70%). We identify ~150 novel activators and predict hundreds more for each receptor. Additionally, We find that the degree of cross-activation is extremely low, as on average each Or was cross-activated by <4% of the non-predicted odors. We have also modified the cheminformatics platform to successfully analyze special cases; narrowly tuned pheromone receptors, and neurons whose receptors have not been decoded. Finally, we apply our *in silico* platform to decode this sizeable chemical space for a large repertoire of mammalian odor receptors which have been previously tested to a small set of odors. Since the activity of odor receptors is the driving force behind the computation in the neural network that leads to odor identification and subsequent behavior, the ability to generate such a systems level view of receptor activity will significantly aid our understanding of odor perception. Acknowledgements: This work was supported by the initial complement provided by the University of California, Riverside to A. Ray and an NSF IGERT fellowship to S. Boyle.

55 The Missense of Smell: Functional Variability in the Human Odorant Receptor Repertoire

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Humans have approximately 400 odorant receptors (ORs) with an intact open reading frame, but among this set there are a large number of variations between individuals. Previous work identified a number of segregating pseudogenes in the human population, but the functional consequences of segregating missense mutations are often impossible to predict from sequence alone. Using a heterologous expression system we examined the functional consequences of these segregating polymorphisms for ten odorant receptors with known ligands. We found 44 non-synonymous SNPs in our population of 20 subjects, representing 40 unique alleles. We cloned the 40 alleles into a mammalian expression vector and used a luciferase assay to measure the response to 60 odorants at three concentrations (1, 10 and 100 μM). We found that eight of the ten odorant receptors had at least one polymorphism in our population and 10% of the alleles had an abolished response to all tested ligands. This description of natural genetic variation and *in vitro* functional variation provides a platform for identifying the role of a single odorant receptor in human perception. Acknowledgements: This work was supported by grants from the NIH-NIDCD, HFSP and by an NRSA postdoctoral fellowship to J.D.M.

56 NCKX4, a calcium regulator, efficiently terminates the olfactory response and moderates the extent of adaptation

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In vertebrates, calcium plays a profound role in amplifying the primary olfactory response and in mediating olfactory adaptation. Thus, tight regulation of cilial Ca^{2+} in olfactory sensory neurons (OSNs) is critical for faithful coding of odor stimuli. Ca^{2+} enters OSN cilia through the CNG channel upon odor stimulation. Several proteins to remove Ca^{2+} from the cilia have been implicated, including Ca^{2+} ATPases and Na/Ca exchangers. We conducted a proteomic analysis of mouse olfactory cilial membrane preparations, and identified the Potassium-dependent Na/Ca Exchanger 4 (NCKX4) as a candidate exchanger for removing Ca^{2+}. Consistent with this identification, previous mRNA studies showed NCKX4 to be the most abundant Na/Ca exchanger (of 9 family members) in the olfactory epithelium (OE) and in OSNs. By in situ hybridization, we revealed that *NCKX4* mRNA localized exclusively to mature OSNs within the OE. We generated a *NCKX4* knockout mouse line, and conducted electrophysiological analyses. Mutant OSNs display a substantially prolonged response by electroolfactogram and single cell analysis, demonstrating that NCKX4 is necessary for rapid termination of the OSN response. Additionally, mutant OSNs display enhanced adaptation under paired-pulse stimulation, consistent with the idea that OSN adaptation arises from Ca^{2+}-dependent mechanisms. In single cell analysis, replacing extracellular Na^{+} with choline, which prevents Na/Ca exchange, significantly prolongs the response in wildtype OSNs, but only slightly in mutant OSNs, demonstrating that the majority of Na-dependent Ca exchange in OSNs is due to NCKX4. By rapidly terminating the OSN response and moderating the extent of adaptation, NCKX4 should have a profound influence on odor detection and perception. Acknowledgements: Supported by a Morley Kare Fellowship, the Human Frontiers Science Organization, Monell Chemical Senses Center and NIH R01 DC007395 and DC009946.

57 CAGE MATCH! Effect of Rodent Housing Conditions on Aggressive Behavior and P2 Glomerular Anatomy

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The effect of housing environment on the neuroanatomy and behavior of mice remains to be rigorously characterized. Here, we examined the effect that two distinct types of cage (high or low ventilation, HV or LV) had on aggressive behavior and neuroanatomy in a single strain of transgenic animal (P2iTLZ). In HV cages, the entire volume of air in the cage was exchanged with fresh air once every minute. In the LV cages, cage air exchanged passively through a filter in the cage lid with the ambient air. In all experiments, transgenic animals were kept in the HV cages from birth until 12 weeks. After 12 weeks, we randomly separated littermates into either the HV or LV cages for 4 weeks. After the 4-week trial period, we then tested the aggressive behavior of resident males toward intruder males. HV cage residents showed significantly more interaction and aggression than LV cage residents. In a parallel series of studies, we naris occluded a single naris in each animal at the 12-week time point before separation into the two distinct cage conditions. We then sacrificed the animals after the 4-week trial period and carefully characterized the P2 glomerulus. The P2iTLZ transgenic strain coexpresses lac-Z with the P2 odorant receptor, allowing us to visualize the P2 glomerulus in the main olfactory bulb of these mice using XGal dye. The P2 glomerulus has previously been demonstrated to be responsive to urine—a prominent odor in the cages that is used by mice for social and sexual

communications. We found dramatic changes in the number and average size of the P2 glomerulus after the animals were maintained in the separate housing conditions for the 4 week trial period. Such changes are all the more remarkable as they are occurring in an adult animal responding to a novel environmental condition. Acknowledgements: NIDCD

58 A Brain-Machine Interface Through the Nose: Wheelchair Driving

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Sniffs are precisely controlled sensory-motor acts that depend on enervation of the soft palate by three cranial nerves, the 5th, 9th, and 10th. In that this enervation is cranial and distributed, we hypothesized that it may be spared following injury. We developed a device that monitored nasal pressure with a nasal cannula connected to a MEMS pressure sensor followed by a USB data acquisition system. We first programmed the device to function as a sniff-controlled "trigger" in on-screen computer games. The device was as fast and accurate as a mouse and joystick (two games, n=36, reaction time game 1/2 sniff=270.51/612.62 ms, mouse=272.17/629.57 ms, joystick=249.74/579.88 ms, accuracy "shooting" a moving target distance in pixels: sniff=26.36, mouse=25.35, joystick=26.91, all tests=NS). Furthermore, whereas mouse and joystick reaction time was constant across a 5 minute game, sniff reaction time decreased ($p<.05$). We then interfaced the sniff-controller with an electric wheelchair using a sniff-based code that generated directional commands. We tested 10 healthy subjects at wheelchair control. All subjects were able to drive the wheelchair along a complex convoluted track (~35 m). Furthermore, all subjects significantly improved within two days. Mean distance from track at Day 1 was 29.5 cm (SD=7.4), and at Day 2 18.4 cm (SD=8) ($t(9)=3.2$, $p<.02$). Finally, we tested quadriplegic subject Q1. Q1 was able to drive the wheelchair along the track on his very first effort. His initial mean deviation from track was slightly but significantly greater than that of the healthy group (36 cm, $t(9)=2.7$, $p<.03$), yet his second day performance was in fact marginally better than that of the healthy group (16 cm, $t(9)=.9$, $p=.4$). We conclude that soft palate enervation allows precise device control through sniffing.

POSTER SESSION I: TASTE IMAGING & PSYCHOPHYSICS; CENTRAL TASTE; MULTIPLE MODALITIES; CENTRAL & PERIPHERAL OLFACTION

59 Bitter Taste can Induce Nausea

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In this study we show that acute oral exposure to bitter tasting solutions can directly elicit nausea. Teleologically nausea is the negative experience that punishes the ingestion of toxins. Since many toxins taste bitter, there is a rational link between tasting toxins in the mouth and the nausea that results from their ingestion and ensuing illness. Furthermore, bitter taste is strongly sensed by the glossopharyngeal and vagus nerves, which innervate the posterior oral cavity and the gastrointestinal tract respectively. The two projection fields of these sensory nerves are immediately adjacent within the nucleus of the solitary tract as well as in other brain relays, thus establishing a neuro-anatomical substrate for taste inputs to influence gastro-intestinal states. To demonstrate that oral exposures to bitter taste stimuli alone are capable of inducing nausea, we used both subjective and objective assessments. Healthy subjects were presented with strong bitter taste stimuli in an oral stimulation protocol without swallowing (0.8 uM sucrose octa-acetate hold in the mouth for 3 min). Nausea was then measured by self-assessment on a modified Muth Nausea Profile questionnaire and by the physiological measures of electrogastrography (EGG). Both the questionnaire and the EGG measurements established a nausea response to strong bitter taste, in about 50% of the subjects tested. Sucrose solution and water were used as controls. We therefore demonstrated for the first time that bitter taste alone can elicit nausea in many people. The establishment of this phenomenon is of human health relevance as many very bitter life-saving drugs elicit gagging and intense rejection, sometimes leading to treatment failure, especially among children. Acknowledgements: NIH DC06760

60 NIH Toolbox: Proposed Assessment of Taste Function and Phenotype

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The NIH Toolbox initiative aims to assemble brief, comprehensive tools for population studies. We proposed assessment of taste function and phenotype with the general Labeled Magnitude Scale (gLMS) to quantify regional (tongue tip chorda tympani nerve; CTN) and whole mouth intensity of water, quinine, NaCl and propylthiouracil (PROP; whole only). In a counter-balanced design with a sample of 100 healthy adults (18-30 yrs, 59 F), we compared the Toolbox to existing tests: lower QHCl level (0.3 vs 1mM) and PROP solutions vs taste strips. In the gLMS orientation, all Ss correctly ranked remembered sound and light sensations from weakest to strongest. Compared with a larger control and patient database, our sample had higher quinine ratings from CTN stimulation. Correlations across methods ranged from 0.50 to 0.57 (water to quinine); but the Toolbox test with the lower quinine level was more likely to classify Ss as low functioning. Whole mouth correlations were higher across methods (0.70 to 0.76, water to quinine). The sample was diverse in PROP tasting. The 3.2mM PROP solution and 600nmole PROP strip were correlated (0.59), but the solution corresponded better to non, medium and supertaster groups based on historical PROP to NaCl intensity ratio method. Quinine was modestly correlated with PROP (0.22 and 0.40 for 0.3 and 1mM), but some Ss were discordant, consistent with multiple bitter receptors. Ss reporting greater bitterness for weak PROP

concentrations also reported greater intensities for blank control strips. In summary, the Toolbox tests captured variability in taste function and phenotype. The higher QHCl level may avoid misclassifying taste dysfunction. The taste strips were more convenient but solutions may deliver cleaner phenotypes due to less oral sensations from the carrier media. Acknowledgements: NIH HHS-N-260-2006 00007-C, USDA Hatch Project CONS00827, NIH-NIA, NIDCD DC000283 and DC007291

61 Effects of BMI on fMRI Activation to a Pleasant Taste During Hedonic Evaluation in Older Adults

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Although obesity is currently recognized as a global health epidemic, little attention has been directed to the rising prevalence of obesity in the fastest growing segment of the population, older adults. The objective of the current analysis was to investigate associations between body mass index (BMI) and fMRI activation to a pleasant taste during a hedonic evaluation task. Twenty healthy older adults (ages 65+) were recruited from the community and were screened for exclusionary criteria including dementia, ageuesia, and anosmia. Participants fasted for 12 hours prior to being scanned, during which time they received 8 separate administrations of a .64M sucrose solution separated by water rinses. T2*-weighted echo planar images were acquired using an event-related paradigm on a 3T GE Signa EXCITE Short-Bore research scanner. Data were processed using both whole brain and region of interest analyses, and systematic associations between BMI and fMRI activation to sucrose were found for both analyses. Specifically, BMI was negatively correlated with activation of the insula, nucleus accumbens and caudate nucleus. Although little is known about how reward processing of food-related stimuli is altered during the aging process, these data indicate a strong association between decreased activation of reward regions and greater amounts of body fat in otherwise healthy older adults. Further research is warranted to investigate whether decreased activation of the reward system may precede weight gain, or if food-related stimuli become less rewarding after an unhealthy accumulation of abdominal fat. Exploring relationships between chemosensory processing and levels of body fat in older adults may aid in increasing understanding of age-related nutritional problems and changes in eating behavior. Acknowledgements: Supported by NIH Grant #1 R01 AG04085 to C.M.

62 Neuroanatomical correlates: psychophysical evaluation of different taste qualities during hunger and satiety

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The physiological states of hunger and satiety moderate brain activation in response to taste and flavor stimuli. We have shown that the psychophysical evaluation of taste stimuli influences the pattern of cortical activation. That is, different brain areas are involved in the evaluation of pleasantness (PL) relative to the evaluation of intensity (INT). fMRI was utilized to examine the patterns of cortical activation involved in psychological evaluation of PL and INT during hunger and satiety in response to 2 taste stimuli (sucrose and caffeine). During scanning, subjects were administered taste stimuli and were asked to evaluate the perceived PL or INT using the general Labeled Magnitude Scale. Image analysis was conducted using AFNI. A multiple linear regression was conducted to examine the potential relationships between perceived PL and INT of the taste stimuli and cortical activation. We have observed that during the PL evaluation, there are robust correlations between perceived PL and activation within the OFC. There was a positive correlation between PL and OFC activation for sucrose when hungry and negative correlation when sated; this effect is less robust for caffeine and relationships were positive across physiological conditions. In contrast, INT evaluation was associated with activation within the insula. Here we show that this relationship varies as a function of stimulus and physiological condition. There was a positive correlation between perceived INT and insula activation for sucrose when sated and for caffeine when hungry. These findings shed light on the impact of qualitative features on brain activation during psychophysical evaluation and may contribute to understanding the neural mechanisms of eating termination and over consumption. Supported by NIH grant AG04085 to C.M.

63 Validation of PROP Taste Strips for the NIH Toolbox Initiative.

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The goal of this study was to validate the use of edible taste strips for examining 6-*n*-propylthiouracil (PROP) bitter taste function in humans. This validation was carried out by presenting participants with taste strips that contained either 400 or 600 nmoles of PROP and asking them to report on the intensity and hedonics of the perceived taste. Taste intensity values for PROP strips were compared to PROP and NaCl solutions according to Tepper *et al.* (*Physiol. & Behav.*, 2001). These results were then compared to genotype analysis of the *TAS2R38* bitter taste receptor gene. This gene exhibits variations at three distinct nucleotide sites, which directly affect PROP taster status. Participants with PAV/PAV or PAV/AVI genotypes were readily able to discriminate PROP strips from control strips, and detected PROP strips as bitter tasting. For PROP tasters (PAV/PAV or PAV/AVI genotypes), 400 nmole PROP strips resulted in generalized Labeled Magnitude Scale (gLMS; 0-100 scale) average values of 35, whereas taste intensity values for 600 nmole PROP strips averaged 40 on the gLMS. Non-tasters (AVI/AVI) averaged 10 on the gLMS for the 400 and 14 for the 600 nmole PROP strips. Average intensity ratings for 3.20 mMolar PROP solution by tasters was slightly higher (54) than for PROP taste strips, while average gLMS rating for 0.32 mMolar PROP solution by tasters was comparable to

strips (33). Hedonics values were based on a three-face scale, and PROP tasters showed a dislike for both PROP strips and PROP solutions. PAV/PAV and PAV/AVI participants showed a larger difference between PROP strips and control strips, relative to AVI/AVI participants for the gLMS and hedonic data. These results indicate that edible strips are suitable for examining PROP taster status in humans. Acknowledgements: Supported by federal funds from the National Institute on Aging, National Institutes of Health, Contract HHS-N-260-2006 00007-C. (PI, R. Gershon), and by NIDCD R44 DC00729

64 Differences in endogenous bitterness of Rebaudioside A do not appear to impact psychophysical compression of the sweetness power function.

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Although natural high intensity sweeteners have long been a scientific curiosity, a stevia derived sweetener, Rebaudioside A (RebA), was recently granted GRAS status, resulting in renewed commercial interest and a new generation of natural sweeteners on the market. Because RebA is both bitter and sweet, variation in perceived bitterness may differentially impact RebA sweetness across individuals via mixture suppression. We speculated this would result in greater compression in the psychophysical function for RebA sweetness (eg sweetness would grow more slowly with concentration in those experiencing the most bitterness). Here, 21 subjects (5 men) tasted sucrose (1.8, 4.2, 5.6, 7.5, 18% w/v) and RebA (.013, .032, .042, .056, .13 % w/v) samples in triplicate, rating sweetness and bitterness using the generalized labeled magnitude scale (gLMS). Data were analyzed with a 'poor-man's multilevel model': a compound specific power function was estimated for each subject via regression, and then each subject's power exponent was used as a variable in a subsequent regression model. As anticipated, the power exponents for sucrose and RebA were correlated across subjects (eg greater growth in sweetness with concentration). Also, a subject's sucrose exponent was greater than their RebA sweetness exponent in all cases. Contrary to our hypothesis however, we did not find the expected negative relationship between maximal RebA bitterness and compression in a subjects' RebA sweetness function. Instead, we found a substantial trend in the other direction, such that RebA bitterness was positively correlated with an accelerating RebA sweetness function. Collectively, this suggests sweet specific or generalized supertasting may overwhelm mixture suppression in determining the sweetness of tastants that are also bitter.

65 Neural Correlates of Self-Initiated Tasting in Humans

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Taste sensations occur primarily during the act of eating and drinking. To date, neuroimaging investigations have focused exclusively on measuring brain response to taste during passive delivery, where the subject lies and waits for a taste stimulus to arrive on their tongue or into their mouth. Recent work in animals highlights the importance of the act of obtaining a taste stimulus upon its neural representation. Moreover, it is clear that many of the regions that respond to taste in humans also orchestrate oral movement and somatosensation. The goal of the current study was to use fMRI to compare brain response to taste vs. tasteless during self-initiated vs. passive receipt. A custom-built mouthpiece, equipped with a suction sensor that relayed signals to a computer to trigger immediate delivery, allowed subjects to self-initiate stimulus delivery. Ten subjects received tasteless and sucrose solutions during passive and self-initiated delivery. Delivery had no effect on perceptual ratings of intensity, familiarity, liking or sweetness. Imaging data were analyzed using a flexible factorial design based upon random effects models. Irrespective of stimulus, self-initiated vs. passive delivery was associated strong preferential response in bilateral insular and somatomotor mouth areas, striatum and cerebellum. Additionally, a stimulus by delivery interaction was observed in the frontal operculum, which responded maximally during sensation of a self-initiated taste. Trends towards a similar interaction were also observed in the insula and orbitofrontal cortex. These findings indicate that brain response to taste is significantly stronger when it is actively obtained and that the act of sucking is orchestrated by a network that overlaps with oral sensory areas.

66 Valid comparisons of food preferences

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Taste comparisons are commonly made with category scales (e.g., Natick 9-point), visual analogue scales and the Labeled Magnitude Scale, but labels on these scales generally fail to denote the same taste intensities to all, leading to invalid comparisons. To remedy this problem, we created a general Labeled Magnitude Scale (gLMS) encompassing all sensations (0=no sensation, 100=most intense sensation of any kind ever experienced). Because the most intense sensation ever perceived is rarely a taste, the top anchor of the gLMS acts as a standard for groups varying on a taste-related attribute (e.g., taste bud density), enabling valid taste comparisons. Similar logic for food affect led to the hedonic gLMS (-100=most intense disliking of any kind, 0=neutral, 100=most intense liking of any kind), but we need to confirm that the endpoints of this scale act as standards that are independent of food affect. One group (N=200) rated liking for 10 foods (5 sampled, 5 rated from memory) on a 9-point hedonic scale; another group (N=200) rated these foods on the hedonic gLMS. Subjects also named their most intense hedonic experiences. Food items were the most liked experience in only 8% of subjects; they were rarely the most disliked experience. Because hedonic gLMS labels do not correspond to food affect for most individuals, they allow valid comparisons of food affect. Finally, subjects in both groups were asked to rate their most and least favorite foods. Ratings fell near the top and bottom of the 9-point scale for all subjects; on the hedonic gLMS, supertasters rated their favorite foods higher and their least favorite foods lower than did others. The 9-point scale fails to show this difference because its labels are not independent of the variable of interest (in this case, food affect). Acknowledgements: DC000283

67 NIH Toolbox: Proposed Food Liking Survey

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We tested a liking survey for possible inclusion in the NIH Toolbox with the purpose of linking chemosensation, diet and health. Associations were tested between the liking survey items, anthropometric measures, and proposed Toolbox taste tests—regional (tongue tip chorda tympani nerve; CTN) and whole mouth intensity of quinine, NaCl and propylthiouracil (PROP; whole only). A convenience sample of 100 college-aged subjects (59 females) reported liking for 56 food and 19 non-food items on the hedonic general Labeled Magnitude Scale. Most Ss were normal adiposity (27 overweight/obese) and normotensive (21 prehypertensive or hypertensive), yet nearly half of males fell into the higher risk groups. In multiple regression analysis (MR) controlling for age, sex and non-food ratings, greater liking for vegetables, fruits, and bitter foods was associated with lower adiposity. Men showed positive association between adiposity and liking for sweet and fatty foods. In similar analyses and controlling for adiposity, greater salt liking was associated with higher blood pressures. We failed to see significant associations between adiposity and spicy food liking. Intensity of quinine (CTN alone and CTN to whole mouth ratio) best correlated with food liking. In MR, greater CTN quinine associated with more fruit and vegetable liking. Those with higher CTN ratio (normal taste function) reported greater liking for fat and sweet foods, rated their favorite food as more pleasurable than pleasant non-food items, and had lower blood pressures. PROP bitterness did not correlate significantly with food liking. However, adults discordant in PROP and quinine bitterness varied in liking for vegetables and fruits. These data continue to support the liking survey as a rapid tool to explain variance in taste and health outcomes. Acknowledgements: USDA Hatch Project CONS00827, NIDCD DC000283 and NIH HHS-N-260-2006 00007-C

68 Experience with Na-cyclamate induces increased human taste sensitivity for glucose, fructose and maltose, but not for sucrose.

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Experience (treatment) with Na-cyclamate, sugars or monosodium glutamate increases human taste sensitivity for glucose (Gonzalez et al., 2007, 2008). Human psychophysical, hamster chorda tympani, and Drosophila melanogaster receptor cell firing data indicate a peripheral mechanism for such changes (Faurion et al., 2002; Hassan et al., 2006; Gonzalez et al., 2009). We have suggested that binding of a treatment compound with the receptor molecule (T1R3 subunit in the case of humans) modulates, i.e. leads to changes in binding or other aspect of, the receptor response to the test compound (Gonzalez et al., 2007, 2008). If true, one would expect that experience with one sweetener would lead to sensitivity changes for various sweeteners. To test this prediction, subjects rinsed their mouths with Na-cyclamate 4 mM (binds to T1R3) or water for 10 sec once each day for 10 days. On day 11 or 12, they tasted isosweet concentration series of glucose, fructose, sucrose or maltose, each concentration paired with water, and indicated ''the sweetener'' of each pair. Subjects treated with Na-cyclamate had greater sensitivity to glucose, fructose or maltose, but not to sucrose, than those treated with water. Since fructose sensitivity was affected, the difference for sucrose is not because of different mechanisms for furanose and pyranose sugars. Since maltose sensitivity was affected, the difference also is not due to different mechanisms for monosaccharide and disaccharide sugars. Given the extensive use of sucrose in food, it may be that humans maintain sucrose sensitivity at a maximum level. The increased sensitivities for the other sugars support the prediction and are consistent with a receptor modulatory mechanism. Further tests with amino acid and high intensity sweeteners are in progress. Acknowledgements: Supported by NIH NIDCD grant # R15DC009042 to LMK

69 Sweet taste intensity is enhanced by temporal fluctuation of odor and taste, and depends on phase shift

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Pulsatile stimulation enhances taste intensity compared to continuous stimulation of the same net taste concentration. In the present work, we studied the effects of pulsatile stimulation on cross-modal aroma-taste interaction. We tested whether pulsatile stimulation amplifies aroma induced taste enhancement and whether the effect depended on timing of aroma and taste pulses. High-concentration sucrose pulses were alternated with water rinses every 2.5s by means of a Gustometer. Three different aroma (isoamyl acetate) categories were tested: (1) no aroma, (2) continuous aroma (3) pulsed aroma (in-phase or out-of-phase with taste pulses). Stimuli were evaluated for sweetness intensity by a 15-member trained panel using time-intensity analysis. Sweetness intensity was significantly ($p<0.01$) enhanced by pulsatile stimulation of sucrose or isoamyl acetate. The combined effect of pulsatile taste-aroma delivery behaved additively if taste and aroma pulses were given out-of-phase. In this case, sweetness intensity was enhanced by more than 35% compared to a continuous sucrose reference of the same net sucrose concentration. Aroma induced sweetness enhancement can be explained by cross-modal aroma-taste integration. Amplification of aroma-taste integration by pulsatile stimulation may be attributed to a higher afferent input of aroma and taste signals prior to integration. Other mechanisms including contrast effects and the importance of swallowing on pulse timing are discussed.

70 Responses to Different Temporal Patterns of Electrical Stimulation of the Chorda Tympani and Glossopharyngeal Nerves in the Nucleus of the Solitary Tract

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The chorda tympani (CT) and the glossopharyngeal (GP) nerves send input to the nucleus of the solitary tract (NTS) in the brain stem. Electrical stimulation of the CT and GP both evoked paired pulse suppression in NTS cells; however, paired pulse enhancement was only common following GP stimulation. To characterize convergent input from the GP and CT nerves onto NTS cells, we recorded evoked responses to taste stimuli and electrical nerve stimulation. CT and GP nerves were electrically stimulated with single pulses, pairs (20-1000 ms interpulse intervals) and triads of pulses. Triads consisted of 3 pulses: the first and third pulses were always 100 ms apart but the second pulse was presented at 25, 50 or 75 ms after the first, in separate blocks of trials. Twenty-seven NTS cells (7 taste-responsive) have been recorded thus far. Of these, 18 cells (5 taste-responsive) responded only to CT stimulation, 6 cells responded only to GP stimulation (1 taste-responsive) and 3 cells showed evidence of CT-GP convergence (1 taste responsive). Latencies of response were similar after CT or GP stimulation (range 2-35 ms). Of 17 cells tested with CT stimulation, 14 cells showed paired-pulse suppression. Of 5 cells tested with GP stimulation, 3 showed paired-pulse enhancement for at least one interpulse interval. Evoked responses to triads of pulses were well predicted from their responses to paired-pulse stimulation, resulting in different temporal patterns of response depending on the sequence of interpulse intervals within the triad. Collectively, these results suggest that 1) NTS cells receive recurrent inhibition from CT stimulation and recurrent excitation from GP nerve stimulation and 2) NTS cells both filter and transform the temporal pattern of input. Acknowledgements: Supported by NIDCD grant DC006914 to PMD.

71 Lick-evoked Taste Responses in the Nucleus of the Solitary Tract of Awake Rats.

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Rats can react to taste after only a single lick, suggesting that information encoded in the first ~150 ms of a response is sufficient for taste quality identification. The present study was designed to quantify the information contained in this early response interval in the nucleus of the solitary tract (NTS; the first central nucleus in the gustatory pathway) in awake, freely-licking rats. Thus far, activity of 23 taste-responsive cells was recorded from 8-microwire bundles chronically implanted in the NTS of 12 male, Sprague-Dawley rats. Rats had access to a lick spout that delivered 12 µl aliquots of 0.1 M NaCl, 0.01 M citric acid, 0.0001 M quinine HCl, 0.05 M sucrose, 0.1 M monosodium glutamate, or distilled water on a variable ratio schedule. Results showed that taste responses were apparent within 100 ms, and often began within 20-40 ms, after a lick. Most cells were broadly tuned across tastants. Water responses and inhibitory responses were found more frequently than in anesthetized rats. The contribution of temporal coding was assessed with a family of metrics that quantify the similarity of spike trains in terms of spike count and spike timing. These analyses showed that spike timing conveyed a significant amount of information about taste quality in a subset of cells; however total information contributed by spike count and spike timing was small (<1 bit) during the initial response interval. In two cells, recorded simultaneously, information doubled when they were treated as an ensemble, compared with when they were analyzed separately. When a tastant was delivered for five successive licks, the first lick provided more information than subsequent licks. Collectively, these data suggest that synergistic activity across groups of cells may underlie rapid taste identification. Acknowledgements: Supported by NIDCD grant 1-RO1-DC006914 to PMD and NIMH grant 1-R01-MH68012 to D. Gardner.

72 Somatostatin modulates GABAergic neuron activity in the rostral nucleus of solitary tract (rNST)

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Recently we characterized the properties of GABAergic interneuron in the rNST using a GAD67-GFP transgenic mouse (*GIN* mouse) that identifies a subset of inhibitory neurons that express somatostatin (Oliva, *J Neurosci*. 2000). In the rNST the GAD67 neurons were found to be surrounded by a network of somatostatin (SST) immunoreactive fibers. The source of these fibers is not known, but SST immunoreactive neurons have been demonstrated in the central nucleus of the amygdala (CeA) and injection of tracer into the CeA labels fibers throughout the NST (Saha, *Neuroscience* 2000). It is possible, therefore, that the SST fibers represent a descending modulatory pathway originating in CeA. Here we have used whole cell patch clamp recording in coronal slices of *GIN* mouse brainstems to study the role of SST on rNST GAD67-GFP interneuron. Neuron responses were recorded in current clamp mode at their resting membrane potential. The slice superfusate was changed to one containing either SST-14 or SST-28 for 1 – 2 min at concentrations of 0.1~1.0µM. 5 out of 8 neurons superfused with SST-14 responded with membrane hyperpolarization, 2 neurons did not respond to the SST-14 and 1 neuron responded with membrane depolarization. Of the neurons exposed to SST-28, 3 out of 5 responded with membrane depolarization, 1 neuron responded with membrane hyperpolarization and 1 neuron was unresponsive to the SST-28. The magnitude of the SST evoked change in membrane potential ranged from 2~14 mV. SST-14 and SST-28 also suppressed the spontaneously occurring spikes in the GAD67-GFP neurons. These results indicate that SST via a descending pathway has a modulatory action on GABAergic rNST neurons suggesting a significant role in brainstem chemosensory processing. Acknowledgements: NIH grant DC 000288 to RMB

73 Receptive Field Mapping of the Oral Cavity in the Rostral Nucleus of the Solitary Tract.

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The nucleus of the solitary tract (NST) receives input from three nerves that innervate discrete populations of taste buds in the mouth. While each nerve terminates in a unique pattern in the rat rostral NST, there is extensive overlap among these terminal fields. We seek to provide the functional counterpart to this anatomical organization. While seminal papers describe the receptive fields of single neurons,

with careful reference to the neurons' locations in the NST, the objective was not to comprehensively functionally map the oral cavity receptive fields. To accomplish this aim, we used a taste mixture that stimulates a wide range of transduction pathways to stimulate the whole mouth, anterior tongue, posterior tongue, nasoincisor duct, and soft palate in rats while systematically recording multiunit activity throughout the NST. We then quantified responses to each of these oral cavity regions and mapped the locations of these recordings onto a template of NTS afferent projection fields normalized to anatomical landmarks determined by cytoarchitectural markers. To date, we collected taste responses from 16 rats, representing 60 electrode penetrations, yielding over 600 recording sites. The receptive fields in the NTS are roughly segregated into anterior and posterior oral cavities in agreement with previously published single cell electrophysiology. However, individual recording sites rarely respond to stimulation of only a single region of the oral cavity. Thus, although there does appear to be a functional topography, each region of the NTS responds to a number of receptor populations in the oral cavity. These data will create a normative template to be used to examine developmental and/or nerve injury induced functional changes in NST organization. Acknowledgements: R01 DC00407, R01 006938 & R56-DC010183

74 Sucrose-best cells in the parabrachial nuclei preferentially project to the nucleus accumbens in the hamster

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The parabrachial nuclei (PbN) is the second central taste relay. From the PbN, gustatory information is further sent to the forebrain gustatory nuclei and insular cortex, including the lateral hypothalamus, the central nucleus of the amygdala, the bed nucleus of the stria termilalis. The nucleus accumbens (NAcc) is another forebrain target of the PbN projections. Anatomically, the PbN projections to the NAcc were demonstrated by tract tracing studies. It has also been reported that neurons in the NAcc respond to taste stimulation in free-behaving animals. We investigated whether gustatory information is sent to the NAcc via the PbN using electrophysiological techniques. Extracellular single-unit activity was recorded from the PbN and taste-responsiveness was confirmed by delivery of 32 mM sucrose, NaCl, quinine hydrochloride, and 3.2 mM citric acid to the anterior tongue. Following verification of a neuron's responsiveness to taste, the NAcc was stimulated (0.5 ms, £ 100 mA, 1/3 Hz) bilaterally. A total of 49 taste-responsive PbN neurons were tested successfully. NAcc stimulation activated 40 (82%) PbN cells. All orthodromic responses of the PbN cells to NAcc stimulation were inhibitory; the ipsilateral NAcc stimulation induced inhibition in 35 (72%) cells and the contralateral NAcc stimulation produced inhibition in 23 (47%) cells; seventeen cells (35%) were inhibited bilaterally. Among the 49 cells that were tested, 15 taste-responsive (31%) PbN cells were activated antidromically following the ipsilateral NAcc stimulation. Eight NAcc-projection cells were sucrose-best. These results demonstrated that nearly 1/3 of the taste-responsive neurons in the PbN project directly to the NAcc and that sucrose-best cells preferentially project to the NAcc. Acknowledgements: NIDCD006623

75 Parabrachial taste responses to sucrose, fructose and Polycose in the rat.

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Much attention has recently been given to the increase in sugars in the modern diet, and their contribution to the current incidence of obesity. Animals can distinguish between different types of carbohydrates based on their oral effects, but how this information is encoded within the central gustatory system remains unknown. Therefore, we used extracellular single-unit recording in the pontine parabrachial nucleus in anesthetized rats while stimulating the anterior tongue with various concentrations of sucrose (0.1-1.0 M), fructose (0.1-1.0 M) and Polycose® (0.02 and 0.2 M). Of the 56 taste responsive neurons recorded 46 (82%), 41 (73%), and 41 (73%) responded to sucrose, fructose, and Polycose, respectively. All sucrose (S)-best neurons also responded to fructose. In contrast, none of the S-best neurons responded to Polycose while twice as many citric acid (CA)-best neurons responded to only Polycose, compared to only sucrose (12% vs. 6%), 75% of CA-best neurons responded to both. Conversely, 3% vs. 11% of NaCl (N)-best units responded to only Polycose or sucrose, respectively, whereas 72% of neurons responded to both. Furthermore, sucrose was a more potent stimuli than fructose at 0.1 M (neuronal responses corrected to water baseline, spike/s mean ± S.E.: 4.75 ± 0.58 vs. 1.825 ± 0.75, $P < 0.05$), but not higher concentrations (1.0 M: 9.70 ± 1.05, vs. 8.23 ± 1.17, *NS*). Polycose, on the other hand, was an equipotent stimulus when compared to any sucrose concentration (0.02 M: 5.54 ± 0.78 and 0.2M: 8.39 ± 0.95). These data suggest that quality and concentration of carbohydrates are encoded differently in the rodent parabrachial taste relay and support the notion that neuronal activity elicited by oral Polycose may recruit alternative mechanisms. Acknowledgements: This research is supported by NIH grants DC00240, DK080899 and PA-TSF.

76 The Role of Amygdala-Cortical Cooperation in Taste Processing

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Previous work has demonstrated that temporal coding not only exists in gustatory cortex (GC), but is explainable in relation to taste processing. The time course of this processing can be defined as taking place over three epochs: detection (0-250ms), identification (250-1000ms), and palatability (1000-2500ms). Additional studies have shown that taste processing in the amygdala progresses through a similar sequence and may, in fact, influence processing in GC. However, these studies only provide indirect support for a role of amygdala in taste processing. We therefore have initiated a series of experiments to directly examine the impact of basolateral amygdala (BLA) on GC taste processing via *in vivo* recording from taste cortex while temporarily inactivating BLA (BLAx). Preliminary data shows that BLAx decimates activity in cortex in two specific ways: 1) perturbation of specific taste responses while leaving the baseline firing rate untouched and 2) dampening or outright elimination the baseline firing rate along with the taste responses. Across the population, while the spontaneous

activity in GC is diminished, taste-driven activity is reduced even more. Interestingly, BLAx affects all four basic tastants (NaCl, sucrose, citric acid, quinine) similarly. Further work already in progress will investigate if the impact of BLAx is particularly strong on specific epochs and aspects of taste processing. Acknowledgements: DC-006666

77 Roles of Gustatory Cortex and Central Amygdala in Processing Taste Concentration

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Single neurons in gustatory cortex and the amygdala respond to taste administration with time-varying firing rate patterns. Distinct epochs of these responses are sensitive to variations in particular properties of the taste (e. g., its quality or palatability), but little is known about whether any epochs are affected by changes in taste concentration. Here, I evaluate the taste responses of single-neuron ensembles in gustatory cortex and central amygdala to a concentration range of sodium solutions, and demonstrate that while the first 800ms of GC responses contain information about taste concentration and quality, the late-epoch of GC activity faithfully reflects the palatability of NaCl, with little apparent influence of taste concentration and taste quality. Further, I show that single neurons in the CeA respond to taste palatability and concentration in concert with GC, and suggest that a functional connection between GC and the CeA shapes single--neuron taste responses in both structures. Acknowledgements: Supported by R01DC007703 to DBK and F31DC009955 to BFS.

78 Influence of the *Soa* Genetic Locus on Responses to Bitter Stimuli in Mouse Central Gustatory Neurons.

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In mice, behavioral sensitivity to sucrose octaacetate (SOA) and other bitter-tasting stimuli is influenced by allelic variation at the genetic locus *Soa*. To explore how *Soa* genotype impacts gustatory neural processing we made electrophysiological recordings of taste responses from single neurons in nucleus tractus solitarius (NTS) in anesthetized mice whose genomes differ only at the *Soa* locus: C3.SW-*Soa*^{a} (C3.SW) congenic and C3HeB/FeJ (C3) inbred mice. C3.SW mice are sensitive to bitters and bred to carry the ''taster'' allele of *Soa* from SWR/J mice on the genomic background of C3 mice, which are less sensitive to bitters. Taste stimuli (33 total) included the bitters SOA (0.1, 0.3, 1 mM), cycloheximide (cyx; 0.3, 3, 30, 100, 300 μM), denatonium (den; 0.1, 0.3, 1, 3, 10 mM), nicotine (nic; 0.3, 1, 3, 10 mM), papaverine (pap; 0.1, 0.3, 1, 3 mM), quinine (qui; 0.3, 1, 3, 10 mM), and sweet, sodium salt and acidic stimuli. For each mouse, electrode tracts were marked and dye was used to verify sites of oral stimulation, which included posterior tongue papillae. Preliminary analyses of 6 C3.SW and 9 C3 cells indicate that *Soa* genotype and stimulus concentration interact to influence NTS unit responses (net spikes) to den ($F_{4,52}$ = 3.4, P = 0.01), pap ($F_{3,39}$ = 6.7, P 0.01) and qui ($F_{3,39}$ = 7.2, P 0.01), with responses at some concentrations larger in C3.SW cells (planned comparisons, P 0.05). Non-significant trends were noted for other bitters, with activity to SOA and nic tending to be larger in C3.SW cells. The collection of more data is underway. Our preliminary findings raise the possibility that allelic variation at *Soa* influences central taste responses to multiple types of bitter stimuli. How *Soa* genotype impacts the distribution of NTS cells and neural coding will be assessed with more data. Acknowledgements: NIH DC008194

79 Signal Detection Analysis of Oral Sensory Responses to Fat in Mouse Central Gustatory Neurons

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The gustatory properties of free fatty acids (FFAs) remain enigmatic. In rats, severing the chorda tympani taste nerve alters oral behaviors to FFAs, but orally-applied FFAs fail to evoke activity in integrated recordings from this nerve. A fat taste signal could be subtle, variable or contributed by additional afferents. We electrophysiologically recorded from single taste neurons in nucleus tractus solitarius (NTS) in anesthetized C57BL/6J mice while stimulating oral tissue with 0.5 (M) sucrose, 0.1 NaCl, 0.01 HCl, 0.01 quinine and 176 or 352 μM Na^+-linoleate (LA), a water-soluble form of linoleic acid used in behavioral studies. Stimuli were applied to the entire mouth, innervated by multiple oral sensory nerves terminating in NTS. Across 15 taste-sensitive cells the mean response (net spikes) to LA (6.6 ± 2.3 [SE] spikes) was low relative to that evoked by other tastants (least effective, quinine: 41.6 ± 8.7 spikes). In 7 cells held for a long time their ability to signal LA was assessed using signal detection methods. Cells were repeatedly tested with LA, an equimolar Na^+ control (176 or 352 μM NaCl) and water, making 414 trials (median trials/stimulus = 15) available for analysis. Receiver operating characteristic curves indexed the performance (P_D) by which each cell's firing rate could detect stimuli from water. Some cells discriminated (best: P_D = 1, perfect detection) LA and water whereas others did not (worst: P_D = 0.55, near-chance detection). Neurons (n = 4) that could detect LA from water (mean P_D = 0.94 ± 0.03) by 1 just noticeable difference (P_D > 0.75) showed some ability to also detect equimolar NaCl against water (mean P_D = 0.75 ± 0.04). Thus far, a subset of cells is sensitive to Na^+-linoleate but Na^+ may be involved in this sensitivity. Ongoing experiments will test other FFAs. Acknowledgements: NIH DC008194

80 Trigeminal input may compensate for taste loss during flavor perception.

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Loss of retronasal olfaction means loss of flavor; flavor loss diminishes the pleasure of eating and may contribute to lower quality of life in the elderly and neurodegenerative populations. To devise effective interventions against the loss of flavor perception, a better understanding of how taste damage affects retronasal olfaction is required. When the chorda tympani nerve, one of the three cranial nerves that mediates taste, is anesthetized to mimic common clinical damage, retronasal olfaction is reduced by half (Snyder, 2008). Real-life, localized taste damage leading to reduced retronasal olfaction was explored. Subjects (n=46: 28 female, 18 male, age 19-91, mean age = 36.78, 24 had localized taste loss) were trained to use the generalized Labeled Magnitude Scale (gLMS) to rate the intensity of various chemosensations. A spatial taste test was performed to detect taste loss localized to discrete areas of the tongue innervated by different cranial nerves. Whole-mouth taste and oral trigeminal sensations (perceived viscosity and oral burn) were also

assessed. Orthonasal and retronasal olfaction were evaluated using 20 food items that varied in smell, taste, and trigeminal intensities. Retronasal ratings were plotted against orthonasal ratings. ANOVAs compared individuals whose retronasal ratings fell below or above the regression line. For 6 of the foods (grape, butter, cinnamon, apple, mint, and strawberry), retronasal sensation was significantly ($p<.05$) reduced for those with lower whole mouth taste. Further analysis showed that the flavor perception of foods with the highest oral burn ratings (mustard, garlic, palak paneer, and curry) was not diminished in subjects with taste loss suggesting that increases in trigeminal input of oral burn may maintain retronasal olfaction in the face of taste loss. Given the loss of pleasure in eating that can be associated with age or neurodegenerative disease, the possibility that the addition of mild burn might compensate for loss of taste has important therapeutic implications for avoiding malnutrition. Acknowledgements: RO1DC000283, R33DC861, and TL1RR029889 from the National Center for Research Resources and by the NIH Roadmap for Medical Research

81 *The thermal grill illusion*: an investigation comparing responses on the hand and the tongue

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The *thermal grill illusion* (TGI) is a paradoxical 'heat' sensation induced on the skin when simultaneously applying adjacent innocuous warm and cool stimuli. This sensation can be painful and results from interactions in the spinothalamic tract between cold-sensitive neurons and nociceptive neurons. Although extensively demonstrated on the hand and forearm, limited information is known about the effect of skin composition on the TGI. The present study investigated the sensation induced by a thermal stimulation device (six 3mm bars) on the mucocutaneous surface of the anterior part of the tongue in comparison to a stimulation on the glabrous skin of the palm. Thermal stimuli were homogeneous cold (18/18, 20/20, 22/22 and 24/24), homogeneous warm (36/36, 38/38, 40/40 and 42/42) and mixed cold and warm bars (TG conditions: 24/36, 22/38, 20/40 and 18/42) temperature (°C±1°C) combinations. Forty subjects reported their sensation using scales of thermal (cold-warm) and pain intensities and qualitative descriptors (hot, cold, painful hot, painful cold, burning, prickling/tingling/stinging). Homogeneous temperature combinations induced similar sensations on the hand and on the tongue. In agreement with other studies, a 'hot', 'burning' sensation associated with higher pain score was reported for mixed combinations on the hand. The intensity of this TGI was positively related to the magnitude of temperature difference between the warm and the cold bars. Interestingly, under the same TG conditions, sensations induced on the tongue were perceived as cold and non-painful and described, in some instances, as 'tingling'. In conclusion, the TG conditions elicited a different sensation on the tongue and on the hand, most probably due to variation in thermal spatial sensitivity between these sites.

82 Flavor Integration of MSG and Citral: Response Time Measurement

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Previously, we investigated the integration of gustatory and retronasal olfactory components of flavorants by measuring simple response times (RTs) to three oral flavorants: sucrose (gustatory), citral (olfactory), and their mixture. Responses to the mixture were faster than values predicted by a model of probability summation of responses to the separate (independent) components, suggesting positive coactivation (integration) of gustatory and olfactory signals in flavor perception (Veldhuizen et al., Chemical Senses, 2010). In the present experiment, we tested for evidence of positive coactivation in speeded responses to a mixture, MSG-citral, that was less pleasant, less familiar, and less 'congruent' than the sucrose-citral mixture previously tested. Using our computer operated, automated flow system, on each trial we presented subjects a brief pulse (0.5 sec duration/5 ml volume) of one of three flavorants (MSG, citral, or their mixture) or distilled water. Subjects were instructed to press a button as quickly as possible when they detected any flavor but not respond to the water. Overall, RTs to the MSG-citral mixture were faster or equal to the value predicted by probability summation. The integration of MSG and citral does not appear to be as robust, however, as the integration of sucrose and citral in the earlier study. The present findings suggest that familiarity, congruence, and/or pleasantness with the mixture may influence the degree of integration of gustatory-olfactory signals during early stages of flavor processing. Acknowledgements: Supported by NIH grant R01 DC009021-03 to LEM.

83 Gustatory-Olfactory Interactions in Favor Perception?

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Three experiments examined possible interactions between gustatory and retronasal olfactory components of flavorants (sucrose and citral). The first experiment used a two alternative forced choice method in an unspeeded task to measure the detection of each flavorant presented in water and in a weak background of the other flavorant. Results showed the detection of both sucrose and citral to be largely unaffected by the presence of a background of the other flavorant. The second experiment used a single stimulus method in a speeded task to measure response times (RTs) to discriminate the concentration of sucrose. Subjects were instructed to identify the sucrose concentration as weak or strong when it was presented in water and in a background of citral. (Although we attempted to implement the complementary experiment, we could not produce discriminable concentrations of citral because of the small volumes of solute used in our automated delivery system.) RTs to sucrose were smaller in the presence of the background, indicating facilitation (negative masking). The third experiment used a two alternative forced choice method in an unspeeded task to measure the discrimination of weak versus strong sucrose in water and in a background of moderate citral, and the discrimination of weak versus strong citral in water and in a background of moderate sucrose. The results showed little effect of the background on discrimination, although there was a suggestion of masking. The presence of facilitation

(negative masking) in the speeded task but not in the unspeeded tasks suggests the possibility of distinct early and late processes in gustatory-olfactory flavor integration. Acknowledgements: Supported by NIH grant R01 DC009021-03 to LEM.

84 Taste-odor interactions: Enhancement of odor or taste?

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Previous studies have reported different types of odor-taste interactions: some have reported that odors can enhance the perceived intensity of tastes, and others have reported that tastes can enhance retronasal odors (or flavors). During informal testing we observed that the presence of a taste appeared to reduce adaption to a retronasal odor in a manner consistent with enhancement of odor by taste. We therefore designed a study to determine whether enhancement predominates in taste-odor interactions over trials. Three odors (0.56 mM furaneol, 0.00025% citral, and 1.8 mM vanillin) were presented alone, in binary odor-taste mixtures with 0.56 M sucrose, 10 mM CA, and 0.32 M NaCl, and in a ternary mixture with sucrose and citric acid. The stimuli were pipetted onto the tongue in 1-ml volumes, held in the mouth for 2 sec and expectorated. Ss then rated sweetness, saltiness, sourness, bitterness and "other" on the gLMS as they breathed normally through the nose. Stimuli were presented in blocks of 5 with a 1-min ISI. Data from 31 Ss confirmed the tendency for putatively congruent tastes (e.g. vanillin and sucrose) to reduce odor adaptation and to enhance odor intensity. Conversely, putatively incongruent mixtures (e.g. vanillin and CA) tended to show suppression of both taste and retronasal odor. No evidence was found for enhancement of tastes by odors. A follow-up study showed that the addition of sucrose significantly increased the perceived intensity of flavor in 2 food systems (vanilla pudding and a cherry flavored drink). However, the addition of vanilla flavor failed to increase taste intensity. These findings indicate that when Ss rate the intensity of both odors and tastes in congruent mixtures, the dominant effect of odor-taste interactions is enhancement of odors by tastes. Acknowledgements: Supported by NIH grant RO1 DC005002

85 The Crucial Role of Familiarity in Cross-modal Enhancement on Lotion Quality Perception

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Humans utilize cross-modal information to inform their perceptions of quality. In the present experiment the role of cross-modal information was investigated for its ability to influence perceptions of fragrance intensity, moisturization, and quality on lotions. Participants were divided into two groups, one received unfragranced lotions the other received a fragranced lotion. Within each group the participants received three samples with varying amounts of yellow colorant added. Data were examined for subject frequency usage in order to understand the influence of familiarity on perceptual evaluations. The data suggest that extrinsic factors (color and fragrance) alter the perceptions of lotions differently depending upon familiarity. Low amounts of color can positively influence quality evaluations ($F(2,150)=3.82$, $p=0.02$). Fragranced samples were evaluated as higher in quality for low usage users . Unfragranced samples were perceived as higher quality for high usage users. Finally color decreased the overall quality of unfragranced samples in high usage users . We suggest these findings demonstrate the powerful influence of multimodal sensory integration on our everyday quality judgments.

86 Functional and Anatomical Integration of the Chemical Senses: Is there a Flavor Sense?

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The field of neuroscience has gone from viewing the senses as isolated functions to considering them interlinked and functionally integrated entities. Unique with respect to functional integration is the supramodal sensation of flavor, a sensory phenomenon that merges information from all senses, though primarily from the three chemical sensations of smell, taste, and trigeminal irritation. Concordantly, side-by-side comparison of the three perceptions demonstrates considerable overlaps in neuronal activation; however, no systematic comparison has been made. Thus, similarities and differences in neuronal activation between these three perceptions were analyzed by means of meta-analyses allowing us to tap the combined power of independent studies. Whereas a common meta-analytical approach is to merely map the reported activations onto an anatomical template, we used the value-added meta-analysis technique Activation Likelihood Estimation (ALE) to obtain ALE maps of the separate sensations as well as comparative ALE maps. Common among the senses are high activation probability (AP) in the insula / frontal operculum (primary taste) and orbitofrontal cortex (secondary olfactory and taste cortex). Moreover, non-odorous trigeminal stimuli demonstrated high AP in the piriform cortex, an area commonly referred to as primary olfactory cortex. Thus, the three sensory modalities display a large functional and anatomical overlap. In light of recent data demonstrating reduction in function of one chemical sense disrupts the function of the others, these findings support the view that the chemical senses are connected not only via crossmodal linkage, but also via functional and anatomical co-location within the brain. Exploration of the chemical senses in unison rather than individually is ripe for future work. Acknowledgements: Supported by a start-up grant from the Monell Chemical Senses Center awarded to JNL and a DAAD postdoctoral fellowship D/08/40252 awarded to JA.

87 Additivity of Brain Activation to Odor and Taste during Judgments of Intensity and Pleasantness

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A question of fundamental importance in chemosensory processing is the additivity of neural response to stimulation of the gustatory and olfactory systems. We employed fMRI to investigate brain activation

in response to chemosensory stimuli representing sweet taste (sucrose), lemon odor (citral) and the combination, all presented in aqueous solution. Stimuli were delivered in the scanner intra-orally as .3 ml per 1 sec (See Haase et al., *J. Neuorscience Methods*, 2007 for stimulus delivery methods). Participants rated the chemosensory stimuli using the general Labeled Magnitude Scale to give psychophysical ratings of intensity and pleasantness. Rating scales were presented on a screen visible to the subject in the scanner. A joystick was used to position a cursor on the scale to indicate a rating and a MatLab program recorded the location. Functional imaging was conducted on a 3T GE Excite short bore scanner using a standard gradient echo EPI pulse sequence to acquire T2*-weighted functional images [(24 axial slices, FOV = 19 cm, matrix size = 64X64, spatial resolution 2.97x2.97x3 mm^3, flip angle = 90°, echo time (TE) = 30 ms, repetition time (TR) = 2 s)]. An event-related paradigm allowed for examination of brain activation in response to individual stimuli as the subject made psychophysical judgments of intensity or pleasantness. Image analysis was conducted using AFNI. Results indicate that brain activation to individual stimuli and additivity of brain activation to olfaction and taste significantly differed for intensity and pleasantness, and were determined by the psychophysical judgment made by the subject during the scan. We gratefully acknowledge the UCSD Center for Functional MRI. Acknowledgements: Supported by NIH grant number R01AG04085-23 to CM.

88 The nose smells what the eyes see: Modulation of olfactory perception by vision

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It is widely held that vision influences olfaction in humans and other primates but how this happens is little understood. Here we investigate visual modulation of olfaction in humans in the context of a unique olfactory phenomenon; called binaral rivalry, it refers to alternations in olfactory percepts when two different odorants are presented at the same time to the two nostrils. Subjects inhaled a pair of odorants while viewing a simultaneously presented image of rose or marker pen. The olfactory percepts were either in flux or stable. We showed that, when the olfactory percepts were in flux, subjects were significantly more likely to detect a smell that was congruent to the visual image. For olfactory percepts that were more stable, this enhancement effect was absent. The implications of our findings on olfactory-visual integration will be discussed.

89 Stinking Consciousness!

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Looking at the different theories of consciousness, one becomes aware that something does not smell right. Olfaction has been neglected. The olfactory system's anatomical structure, functional organization, and sensory states raise problems for the prevailing neuroscientific theories of consciousness, while providing a novel perspective for theorizing about consciousness. The anatomical structure of the olfactory system is problematic for the current neuroscientific theories of consciousness, which consider a thalamic relay or corticothalamic loops as a necessary condition for consciousness. A thalamic relay might be necessary for consciously analyzing odorants (Pially, *et. al.* 2007), but it is not required for consciously discriminating between odorants. Thus, providing reason to doubt Crick's (1984, 1994) theory (Smythies, 1997), Crick & Kock's (1998) theory (Shepherd, 2007), Koch's neurobiological theory (2004), and the Information Integration Theory of Consciousness (Tononi & Edelman, 1998; Tononi, 2004). The functional organization of the olfactory system further aggravates the problem for these theories, since the necessary thalamic connections cannot be replaced with a functional equivalence within the olfactory system. Using research on the mitral cell's functional encodings of odorants in the olfactory bulb (Friedrich & Lauent, 2001), I argue that the aforementioned theories cannot reply that the olfactory bulb plays an equivalent functional role to that of the thalamus for vision (Kay & Sherman, 2006). Furthermore, the necessity of cortical connections without thalamic relays for our conscious sense of smell suggests studying phenomenal consciousness as a necessary condition for access consciousness. using evidence from Blind Smell (Schwartz, 1994, 2000; Sobel *et. al.* 1999). Acknowledgements: CUNY Doctoral Research Grant

90 Model of dendrodendritic synaptic clustering along mitral cell lateral dendrites

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In the mammalian olfactory bulb, the lateral dendrites of mitral cells form dendrodendritic synapses with granule cell interneurons. Previous transsynaptic viral tracing results have suggested that dendrodendritic synapses cluster along the lateral dendrites and are not uniformly dispersed. Since recent evidence indicates that newly generated granule cells are required for perceptual learning, we test the hypothesis that dendrodendritic clusters arise through an activity-dependent synaptic mechanism. Using computational networks of mitral and granule cells stimulated with a spectrum of virtual odors, we describe how dendrodendritic clusters can form through activity-dependent synaptic learning. The results indicate that the activation of mitral cells to various odors and the resulting backpropagating spikes in their lateral dendrites are critical to this mechanism, yet the clustering phenomenon is robust to non-Hebbian, Hebbian, and spike-time dependent plasticity learning rules. We quantify and contrast cluster formation across time with these different learning regimes using various virtual odor concentrations and mixtures. Acknowledgements: NIH/NIDCD 1R01DC009977-01

91 The structure of human olfactory space

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We analyze the psychophysical responses of human observers to an ensemble of monomolecular odorants. Each odorant is characterized by a set of 146 perceptual descriptors obtained from a database of odor character profiles. Each odorant is therefore represented by a point in highly multidimensional sensory space. In this work we study the arrangement of odorants in this perceptual space. We argue that odorants densely sample a two-dimensional curved surface embedded in the multidimensional sensory space. This surface can account for more than half of the variance of the psychophysical data. We also

show that only 12% of experimental variance cannot be explained by curved surfaces of substantially small dimensionality (<10). We suggest that these curved manifolds represent the relevant spaces sampled by the human olfactory system, thereby providing surrogates for olfactory sensory space. For the case of 2D approximation, we relate the two parameters on the curved surface to the physicochemical parameters of odorant molecules. We show that one of the dimensions is related to eigenvalues of molecules' connectivity matrix, while the other is correlated with measures of molecules' polarity. We discuss the behavioral significance of these findings.

92 Spatio-temporal dynamics of olfactory processing based on event-related potential source imaging

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Functional neuroimaging studies provide evidence of olfactory neural networks but only in terms of spatial localization. In contrast, classical methods using conventional EEG and event-related potential (ERP) recordings show excellent temporal but poor spatial resolution. We intended to introduce high-density EEG recordings in order to unravel both spatial and temporal information processing in the olfactory pathway. Normosmic subjects (age 22-46 years; 6 w, 7 m) were studied. ERP were obtained in response to H2S from 64 electrodes (sampling frequency 1KHz). A k-means spatio-temporal cluster analysis was applied to grand mean ERPs to define the most dominant map segments within the first 1.2s post-stimulus onset. The sources of each time segment was estimated using a linear distributed inverse solution (LAURA). The source distributions were calculated for 2738 solution points in the MNI brain for each subject and the estimated current density was averaged within 26 regions of interest. For each region, the activity was averaged over subjects for each condition and the current density waveforms were analyzed. Source estimates indicated that activation started ipsilateral to the stimulated nostril in mesiotemporal/parahippocampal and lateral temporal structures (middle and superior temporal gyrus), crossed to contralateral mesio-temporal and lateral temporal structures but also was present ipsilaterally in the hippocampus and amygdalae; subsequently bilateral lateral frontal structures became active, and lastly large activation was seen in contralateral mesio and latero-temporal regions. In conclusion, deep frontal and mesio-temporal structures, known to be involved in odor perception, can be reliably detected by electric source imaging. Olfactory temporal activation sequences can be determined. Acknowledgements: Support by Neurosci. Center of the Univ. of Geneva to BNL, JSL, and CMM and SSMBS grant n° PASMA-119579/1BNL.

93 Odorant-Induced BOLD Signal in the Brains of Anosmic Subjects

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Congenital absence of the sense of smell, termed congenital anosmia, is typically incurable. Furthermore, whereas the underlying cause of congenital blindness and deafness is usually clearly identifiable, the cause of congenital anosmia, whether peripheral or central, often goes unidentified. We hypothesized that individuals with congenital anosmia due to central rather than peripheral mechanisms may nevertheless present with a brain-response to odors. To test this, we used an olfactometer to deliver 4 different odorants (2 trigeminal : isovaleric and eucalyptus and 2 non-trigeminal:PEA and ammonium solfide) in an event-related paradigm with 8 normosmic (4F) and 8 anosmic (3F) subjects inside a 3-Tesla Siemens scanner. Initial analysis suggested that nearly all anosmics had a BOLD response ($p < 0.001$) to odorants in several classical olfactory regions, and the anosmic response pattern appeared indistinguishable from that of the normosmic response pattern. This is a pilot result (n=8), however, if it remains robust it may have one of two implications: either our methods for fMRI of olfaction are invalid, or there is a new source of hope for congenital anosmics, as one may find a path to teach them what their brains "know" yet they do not.

94 Towards a Consensus Sensory Map of Perfumery Scents Based on Meaningful Psychological Dimensions of Odor Perception

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Two-dimensional sensory maps of odor character descriptors are valuable tools for understanding their similarities and, in the fragrance world, there is a high interest of achieving consensus on a universal odor descriptor map. However, this issue has long been elusive due to the high dimensionality of olfactory perception space. A recent work (Zarzo & Stanton 2009, Atten Percept Psychophys 71, 225-247) reports the multivariate statistical analysis of a numeric odor profile database containing 309 compounds. The loading plot corresponding to the first and second principal components was strikingly similar to the odor effects diagram proposed by P. Jellinek in the 1950s as well as to Edwards' Fragrance Wheel, which classifies 6500 commercial perfumes into 14 categories. A similar odor map was also obtained from the analysis of a second database, which contains the semantic description of 119 perfume materials. The dominant dimension discriminates fresh vs. warm scents, which might be reminiscent of low versus high temperature. I speculate that this dimension might discriminate odors typical from winter vs. summer. Consistent with this hypothesis, the second dimension basically discriminates floral vs. non-floral scents. The former are associated with spring, and odors most dissimilar to floral are typical from autumn. Thus, the resulting map of scents might reflect the role of olfaction in chronobiological annual rhythms. In conclusion, the findings suggest that there is certain 'universal' perceptual space of fragrances, and provide clues for better understanding the psychological aspects involved in the perception of fragrances which is of relevant industrial interest. Acknowledgements: Supported in part by a Fulbright grant sponsored by the Spanish Ministry of Education and Science.

95 ANDROSTENONE SUPPRESSES TESTOSTERONE RESPONSE TO SEX FEMALE PHEROMONES 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. We studied the role of androstenone (AND) in regulation social behavior in mice contrasting in sensitivity to AND and level of intermale aggression. CBA/J(CBA) mice are more than 2000-fold sensitive to AND than NZB/B1NJ(NZB) mice. NZB mice showed abnormal level of intermale aggression. 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. In standard odor preference test CBA males showed strong preference for receptive female odor relative to male odor ($p<0.001$, 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 (0.05%) suppressed plasma testosterone (T); 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). Addition of AND to urine from receptive females blocked testosterone response to female sex pheromones in CBA males ($p<0.001$, n=8). At the same time plasma corticosterone level stayed unchanged. Long lasting exposures to AND did not affect level of fecal glucocorticoid metabolites. Also we evaluated the influence of AND on aggressive and sexual behavior in a number of behavioral tests. The data obtained indicate that AND may show pheronomal activity in mice. Acknowledgements: RFBR 07-0401538

96 Androstadienone Modulates Attention-based Reactions in Men

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The androgen compound androstadienone (AND) has been suggested to act as a human pheromone. Documented results include modulation of human physiology, psychology, as well as altering women's subjective impressions of men towards a more positive evaluation. Based on this, we hypothesized that AND modulates social interactions and behavior in a subconscious manner. We investigated AND's impact on approach and avoidance tendencies in 39 subjects (26 women) with the approach-avoidance task (AAT) using a within subjects design. Subjects had to push away or to pull towards them a joystick, as fast as possible, in reaction to either an angry or a happy cartoon face, respectively, which was presented on a screen. This task was repeated twice, ones while exposed either to a low concentration of AND, masked with clove oil, or only the clove oil solution, both presented via a constant air flow olfactometer. The two odors were rated as iso-intense and with no difference in perceived pleasantness or familiarity. AND modulated men's general task performance (p <.001). In that AND reduced their overall reaction speed compared to the control condition, particularly so when reacting to happy faces (p = .031). This was not significant for women. The reduction in reaction speed when exposed to the putative human pheromone could be due to the competition of attentional resources by the combined presentation of emotional information in the emotional laden faces and the putative social/biological information in AND. This competition for attentional resources would result in less attention to the task at hand. Related data exploring attentional competition while exposed to AND using neuronal recordings will be presented. Acknowledgements: This study was funded by a Research Training Group RTG 1253/1 (EMOTIONS) from the DFG, awarded to MCMF, and by the NIDCD (R03DC009869) awarded to JNL.

97 Melatonin enhances olfactory bulb expression of gap junctions

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Synchronous activity of olfactory bulb neurons projecting to the same glomerulus is thought to be an important network component of odor processing by the olfactory bulb (OB). An important contributor to this synchronous activity is the direct electrical coupling provided by gap junctions formed by connexin proteins. The OB also exhibits circadian rhythms of both electrical activity and odor sensitivity, but the cellular mechanisms underlying these rhythms are unknown. Many circadian rhythms are under the influence of the daily rhythm of melatonin released by the pineal gland. Recent data suggest that melatonin regulates the expression of connexin 43 in myometrial cells and this contributes to synchronous rhythmic contractions of the uterus during childbirth. Collectively, these data led to the hypothesis that melatonin may contribute to the circadian activity of the OB by regulating gap junction expression and enhancing synchronous activity. To test this hypothesis, neonatal OB cells, grown in primary culture, were treated with 3 nM melatonin and connexin expression was examined by immunofluorescence. Connexin 43, in control tissue, was expressed primarily at the borders of contact between astrocytes. In contrast, melatonin-treated astrocytes displayed increased connexin 43 expression, which included a diffuse cellular expression pattern in addition to expression at cell-to-cell borders. These results suggest that melatonin may contribute to the circadian activity of the OB by enhancing electrical coupling via increased gap junction expression. Molecular analyses of these effects, and the effects of melatonin on connexins 36 and 45, are also being examined. Acknowledgements: Supported in part by the FSU Department of Biological Science and Program in Neuroscience

98 Odors eliciting Fear: a Conditioning Approach to Idiopathic Environmental Intolerance

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Individuals with Idiopathic Environmental Intolerance (IEI) seem to fear odorous chemicals. To determine whether IEI can be conceived of as an ''odor phobia'', we tested whether odors can elicit stress responses (1); and whether odors, after association with fear compared to before, are evaluated as less pleasant (2) and avoided more (3). **Method:** Using differential classical conditioning paradigm, an odor (either rotten egg as CS+ [CS: Conditioned Stimulus] and peach as CS-, or vice versa), were conditioned to an electrical shock as Unconditioned Stimulus (US). The acquisition phase consisted of 6 presentations of the CS+ followed by electrical shock and 6 presentations of the CS- alone. The extinction phase consisted of 6 CS+ and

6 CS- trials, with no US. Stress response was assessed via Skin Conductance Response (SCR); pleasantness via rating scales; avoidance via sniffing. **Results:** SCR increased significantly to both odors, but stress to rotten egg did not extinguish (1); a significant (p =.03) change in pleasantness during acquisition was encountered, with the CS+ being liked less after conditioning (2); sniffing volume associated with the CS+ decreased immediately after the first trial(s), but reduction in volume was not significant over 6 trials (3). **Discussion:** The finding that odors can be conditioned to stress responses, leading to reduced liking for these odors, support a fear conditioning conception of IEI. However, the absence of extinction and avoidance of the CS+ are problematic in the light of the theory and additional research is needed, especially since extinction would be the treatment of choice in view of the theory. Acknowledgements: NWO Vidi 452-03-334

99 Species specific regulation of the olfactory bulb dopaminergic phenotype

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Our previous studies indicated that the transcription factor ER81 regulates tyrosine hydroxylase (TH) expression in the mouse olfactory bulb (OB) by directly binding to the TH proximal promoter. A recent study suggested ER81 also regulates expression of other genes necessary for the dopaminergic phenotype by a molecular mechanism similar to TH, and this ER81-dependent differentiation of the dopaminergic neuronal phenotype is conserved from nematodes to mammals. However, a phylogenetic analysis of genomic DNA sequences corresponding to promoter regions for several dopaminergic genes in mammals revealed that the reported binding sites that mediate ER81 regulation of the dopaminergic phenotype are not conserved. Chromatin immunoprecipitation (ChIP) experiments with mouse OB tissue suggested that ER81 binds to several proximal promoter regions of many dopaminergic genes, but ChIP experiments with OB tissue from dog indicate that orthologous regions of these genes are not bound by ER81. Focusing on the expression of TH, which is the rate-limiting enzyme in dopamine biosynthesis, electromobility gel shift assays showed that recombinantly expressed ER81 can bind the TH promoter from rodents, but not humans. Transcription assays comparing human and rat TH promoters in a cultured murine OB cell line also suggested that there is species-specific regulation of TH. Together, our findings suggest that there are species-specific molecular mechanisms that regulate differentiation of OB dopaminergic phenotype. However, these findings do not exclude the possibility that there is also a conserved molecular differentiation pathway that functions in combination with the species-specific mechanisms. Acknowledgements: NIH DC008955

100 The olfactory capabilities of mice with long-term unilateral naris occlusion (UNO) and contralateral bulbectomy (bulb-x).

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UNO has been the most common method of effecting stimulus deprivation in studies of olfactory plasticity. However, despite the large corpus on the effects of this manipulation dating back to the 19th century, little is known about its behavioral sequela. Here we report the results of classical olfactory habituation and discrimination studies on adult mice that had undergone unilateral bulb-x and contralateral naris occlusion perinatally. The olfactory performance of UNO mice was compared to matched controls that had unilateral bulb-x but intact nares. Both experiments employed a masking protocol in which after successful dishabituation or discrimination to pure odors (0.1% isoamyl acetate or ethyl butyrate v/v in mineral oil), mice were challenged with test odors that were increasingly masked by dilution with the habituation odor. In the habituation experiment, UNOs (n = 9) and controls (n = 9) dishabituated to a 10% solution of test odor mixed with habituation odor however both groups generalized to a 2% mixture. However, UNO results for the 2% mixture approached significance ($p < 0.06$). Replication of this study (7 controls & 8 UNOs) confirmed that controls generalized to a 2% mixture of test odor but UNOs did not ($p < 0.05$). In the discrimination experiment, 4 UNOs and 4 controls were shaped to dig in one of two containers of sand that contained the S+ odor to obtain sugar pellet rewards. Controls and UNOs had an average mixture discrimination threshold of 1.6% (+ 0.4) and 0.22% (+ 0.102) respectively, a difference that was statistically significant ($p < 0.03$). These counterintuitive results suggest that UNO is neither an absolute method of deprivation nor does it appear to diminish olfactory capabilities and may in fact enhance them consistent with other recent evidence from our laboratory. Acknowledgements: National Science Foundation (# 0641433) to DMC

101 Olfactory Performance in Three Transgenic Alzheimer's Disease Mouse Model Strains

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Olfactory deficits develop in human Alzheimer's disease (AD) subjects prior to the onset of other behavioral symptoms. It is unclear whether the olfactory deficits lead other symptoms due to greater expression of AD causing factors in the olfactory system, or if properties of the olfactory network make it particularly susceptible to such factors. To determine if available mouse models are appropriate to address this question, we behaviorally tested three transgenic strains for olfactory and cognitive performance. Each model overexpresses proteins that are implicated in AD. One Tau mutant and two models with varying numbers of familial mutations in both amyloid beta and presenilin 1 (2XFAD and 5XFAD for number of familial Alzheimer's disease mutations) were assayed. No significant deficits were observed for the Tau or 2XFAD mutants until advanced age. The 5XFAD mice, however, showed significant deficits in detection thresholds and habituation by 8-10 months of age. These effects preceded spatial learning deficits. Further, preliminary data indicate that the amyloid beta plaque density in the 5XFAD mutants is reduced in the olfactory bulb and present in the olfactory cortex at a similar density to plaques in the rest of the brain. These results suggest that the 5XFAD mutants are a viable mouse model to study olfactory effects of amyloid beta overexpression, and may provide insight into the mechanism of AD related olfactory dysfunction. Acknowledgements: NIDCD R01 000086 NIDCD R03 008874

102 GC-D neurons respond to the semiochemical carbon disulfide and mediate the social transmission of food preference

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Social transmission of food preference (STFP) is a mechanism by which many animals, including mice, socially learn to make food choices. STFP depends on an observer animal's ability to detect both relevant semiochemicals and food odors in the breath of a conspecific demonstrator. However, the mechanism by which olfactory stimuli generate this behavior is unknown. We found that a specialized olfactory subsystem that includes olfactory sensory neurons (OSNs) expressing the receptor guanylyl cyclase GC-D, the cyclic nucleotide-gated channel subunit CNGA3 and the carbonic anhydrase isoform CAII is required for the acquisition of STFP in mice. Electroolfactograms (EOGs) recorded from the main olfactory epithelium show responses to physiological levels of carbon disulfide (CS_2), a component of rodent breath that is a known social signal mediating STFP acquisition. EOG responses to sub-micromolar concentrations of CS_2 are abolished in *Cnga3* $^{-/-}$ and *Car2* null mice (which lack CNGA3 or CAII, respectively) and are greatly reduced in *Gucy2d* $^{-/-}$ mice (which lack GC-D). Using cell-attached patch clamp recordings from the dendritic knobs of identified GC-D+ OSNs, we found that these cells show increased action potential firing in the presence of CS_2. As olfactory-mediated CS_2 detection is implicated in STFP acquisition, we next asked if *Cnga3* $^{-/-}$ mice show a deficit in this social learning paradigm. Indeed, *Cnga3* $^{-/-}$ observer mice, in contrast to wildtype and/or *Cnga3* $^{+/-}$ mice, fail to acquire STFPs from either live or surrogate demonstrators and do not exhibit neuronal activation of the ventral subiculum of the hippocampus, a brain region implicated in STFP formation. Our findings indicate that the GC-D+ OSNs are essential for the detection of chemosignals that facilitate social interactions related to food. Acknowledgements: Supported by the National Institute on Deafness and Other Communication Disorders (DC005633 to SDM, DC006603 to KRK) and the Deutsche Forschungsgemeinschaft (FZ, TL-Z, MB). TL-Z is a Lichtenberg Professor of the Volkswagen Foundation.

103 Gene Expression in the Olfactory Epithelium of β3GnT2 Mice.

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The glycosyltransferase β3GnT2 is highly expressed in olfactory neurons where it regulates poly-lactosamine glycan synthesis on several glycoproteins involved with olfactory axon guidance and signal transduction. Null mice for β3GnT2 have severe defects in olfactory epithelium development and glomerular targeting. Olfactory bulb innervation is delayed in early postnatal null mice, resulting in disorganized glomerular formation and a loss of specific odorant receptor-expressing neuronal subsets that persists in adults. To identify potential changes in gene expression associated with this phenotype we analyzed mRNA isolated from β3GnT2 mutant mice and wildtype littermates using Affymetrix mouse gene arrays. These chips contain over 34,000 unique target sequences including probes for more than 600 mouse odorant receptors. Preliminary studies suggest that many genes including those encoding for odorant receptors may be up- and down-regulated in the olfactory epithelium of β3GnT2 null mice. Regulation of individual gene expression is being confirmed by in situ hybridization and quantitative PCR studies. Importantly, these studies corroborate earlier work showing that adenylyl cyclase 3 (AC3) is significantly down-regulated in the OE of null mice. This suggests that some changes in gene expression may be due directly to the loss of β3GnT2-dependent glycosylation, while other differences may result in part from a decrease in AC3-dependent signaling in β3Gnt2 null olfactory neurons. Acknowledgements: Supported by NIH grants DC00953 and DC09034.

104 Genomic Effects of Unilateral Naris Occlusion (UNO) on the Olfactory Mucosa: A RNA Microarray Approach in Mouse

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UNO has been the method of choice for effecting stimulus deprivation in studies of olfactory plasticity. Early experiments focused on deleterious effects of UNO while more recent studies have pointed to 'compensatory' effects. However, the idea of deprivation-induced compensatory processes remains tenuous. High-throughput methods such as microarrays can help fill the deficits in understanding of UNO effects. Here we report an analysis of genomic effects on olfactory mucosa induced by UNO using the Mouse OneArray chip (Phalanx Biotech Group). RNA was extracted from mucosa of 25-postnatal day (PND) FVB strain mice using a Qiagen kit. Three mice received UNO on PND 1 and three untreated mice served as controls resulting in the treatments: UNO open side, UNO occluded side, and untreated mucosa. RNA samples were then hybridized to microarrays. Here we focus on the >700 olfactory receptor genes and 14 component genes of the olfactory transduction cascade that are represented on the Mouse OneArray chip. Twenty % of the olfactory receptor RNAs were significantly up-regulate in occluded side mucosa of UNO mice compared to the open side or control mice. RNAs from several genes of the transductory cascade were significantly modulated in the occluded mucosa in a direction that would amplify transduction. Twenty genes were selected for real-time PCR validation, 11 olfactory receptor genes, 8 transductory cascade genes, and a control gene (G6pdx). Taken together our analysis of olfactory mucosal RNA provides further support for the hypothesis that stimulus deprivation caused by UNO triggers a compensatory response in olfactory mucosa. This phenomenon is reminiscent of homeostatic

processes garnering considerable attention of late in the CNS but is among the first such demonstrations in primary sensory neurons. Acknowledgements: NSF Grant 0641433 to DMC

105 Unilateral smell loss- an early indicator for future global olfactory dysfunction

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Approximately 15% of subjectively normosmic individuals demonstrate clinical significant unilateral smell loss. That smell loss will not be noticed as long as olfactory function of the better nostril remains in the normal range. We were interested in the question whether individuals demonstrating clinically significant side differences of olfactory function are at risk to develop bilateral olfactory loss. Therefore those individuals ("difference- group", n=35) were re-tested on average 4.6 years after baseline investigations. Additionally, 58 subjects who did not demonstrate olfactory side differences ("control- group") were also re-investigated. All participants performed detailed olfactory testing using the "Sniffin' Sticks" involving tests for odor threshold, odor discrimination, and odor identification. The "difference-group" and the "control-group" were not significantly different regarding age (p=0.19), follow up period (p=0.41) and olfactory function of the better nostril at baseline (p=0.35). Olfactory testing at follow- up indicated lower olfactory function (p=0.005) in the "difference- group" than in the "control- group". The degree of side difference at baseline correlated negatively with the results from olfactory testing at follow-up (r=-0.29; p=0.01). These results suggest that individuals with side differences of olfactory function are at risk to develop bilateral olfactory loss within 4.5 years. Thus, the degree of unilateral smell loss is an indicator for the intensity of future bilateral olfactory loss. Acknowledgements: Deutscher Akademischer Austauschdienst

106 Gene expression and alternative splicing at the peak of proliferation during adult neurogenesis

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A complete view of the molecular events regulating adult neurogenesis in the olfactory epithelium requires more than our previous analysis of half the transcriptome (Shetty, et al. 2005 PMID16456926), so we identified differences in the abundance of exons in olfactory epithelia ipsi- and contra-lateral to unilateral bulbectomy of adult mice using the Affymetrix GeneChip Mouse 1.0 ST Array, which covers nearly all known exons. At 5 days after lesion, 4103 mRNAs were differentially abundant ($p<0.05$; FDR = 20%). Of these, 3,674 were not previously known to be altered by bulbectomy. Statistically overrepresented biological processes represented by the 1734 transcripts that increased after bulbectomy included neurogenesis, development, proliferation, differentiation, cell motility, transcriptional regulation, and apoptosis. In addition to these activated genes, exon level analysis predicted changes in alternative splicing for an additional 2996 genes (a total of 4365 exons). Gene Ontology categories overrepresented among these candidate alternatively spliced transcripts were largely related to cell signaling and metabolism: kinase activity and other types of post-translational modification, metabolic processes, and the cell cortex compartment. These data help complete our view of the mRNA changes that contribute to proliferation and differentiation during adult neurogenesis in the olfactory epithelium. Acknowledgements: Supported by award R01 DC007194

107 OR and V1R Genes share Common Promoter Elements

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Odorants and pheromones are detected by hundreds of odorant receptors (ORs) and pheromone receptors (V1Rs and V2Rs) expressed by sensory neurons that are respectively located in the main olfactory epithelium and in the vomeronasal organ. Even though these two olfactory systems are functionally and anatomically separate, their sensory neurons show a common mechanism of receptor gene regulation: each neuron expresses a single receptor gene from a single allele. The molecular mechanisms regulating OR and VR gene expression remain unclear. We investigated if OR and V1R genes share common *cis*-regulatory sequences in their promoter regions. We have conducted a comparative analysis of empirically determined promoter regions of 39 V1R genes, belonging to 11 different subfamilies. First, cDNAs containing the complete 5'-UTRs corresponding to the V1R genes were generated. Then, these cDNA sequences were aligned to the mouse genome so that the transcription start sites (TSSs) for each one of the genes could be precisely determined. The 39 promoter sequences were retrieved and browsed for common motifs. We then searched OR promoter regions for motifs that resemble the ones found in the V1R promoters. We identified motifs that are highly represented in the V1R and OR promoter regions. One of these motifs resembles binding sites for transcriptional repressors that belong to the BTB-ZF family, which are known to be associated with condensed chromatin in the nucleus. Our findings suggest that transcriptional repression may constitute an essential mechanism in the precise regulation of OR/V1R gene expression. Acknowledgements: Supported by FAPESP.

108 The Molecular Components of Anion-Based Signal Amplification in Olfactory Cilia

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The olfactory system detects a vast variety of odorants with a limited set of odorant receptors. Each receptor detects multiple odorants, but signal transduction operates with low efficiency. Sensory cilia of olfactory receptor neurons (ORN) solve this problem by amplifying the receptor potential through accumulation of chloride at rest and discharge of chloride upon stimulation. We studied this amplification process by examining the identity, the subcellular localization, and the regulation of its molecular components. PCR experiments revealed that the $Na^+/K^+/2Cl^-$ cotransporter *NKCC1* as well as the regulatory kinases *SPAK*, *OSR1*, *WNK1* and *WNK4* are expressed in FACS purified ORNs. Using immunochemistry we found that these proteins are localized in cilia of ORNs. Gene silencing experiments revealed that the activity of the NKCC1 mediated Cl^- accumulation depends on the kinases SPAK and OSR1 as well as on their own activating kinases WNK1 and

WNK4. PCR and *in situ* hybridization experiments demonstrate that *SLC4A1*, a DIDS-sensitive $Cl^-/HCO3^-$ exchanger, is specifically expressed in ORNs. Using immunochemistry we could detect SLC4A1 in cilia of ORNs where it may support Cl^- accumulation. In addition, we could show that the calcium-activated chloride channel TMEM16B is localized in the cilia and, thus, provides a pathway for the excitatory chloride current. These findings describe a molecular machinery that is necessary for anion-based signal amplification in olfactory sensory cilia. They provide a molecular concept for the unique strategy that allows olfactory receptor neurons to operate as efficient transducers of weak sensory stimuli. Acknowledgements: German research foundation to FM (MO1384 2-2) and SF (FR937 10-1)

109 Heterotrimeric G-protein βγ subunits in the Mouse Olfactory Epithelium

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G-protein coupled signaling cascades are involved in a variety of activities in olfactory sensory neurons (OSNs), such as odor transduction and axonal projection. While the roles of heterotrimeric G-protein α-subunits, such as $G_{\alpha olf}$ are well documented, relatively very little is known about $G_{\beta\gamma}$ subunits in OSNs. However, recent studies in other research fields have yielded a surge of data documenting the diverse functions of $G_{\beta\gamma}$ subunits. Here we investigate the expression of all known G-protein βγ subunits in the olfactory epithelium of mice using reverse transcriptase PCR (RT-PCR) analysis. Our data confirmed the expression of G-protein β1, γ8 and γ13 in the mouse olfactory epithelium found previously by other investigators. Additionally, we found the presence of four of the seven known γ subunits (γ2, γ3, γ5 and γ12) and three of the five known β subunits (β2, β4 and β5) in the olfactory epithelium. Further, we have used RNA *In situ* Hybridization (RISH) to study the expression of these $G_{\beta\gamma}$ subunits. We have so far confirmed in RISH analysis that mRNA transcripts of β4, γ2, γ5 and γ12 subunits are present in the olfactory epithelium. We have also observed positive labeling of these subunit mRNAs in the VNO as well as in the septal organ. Our results demonstrate that multiple $G_{\beta\gamma}$ subunits are expressed in olfactory sensory epithelia. Acknowledgements: Supported by NIH/NIDCD DC009269 to WL.

POSTER SESSION II: OLFACTORY PHYSIOLOGY & CELL BIOLOGY; TASTE MOLECULAR GENETICS; CHEMESTHESIS & TRIGEMINAL

110 Odor fear conditioning effects on piriform cortical odor processing in awake rats

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Odors that we encounter everyday are usually very complex. While the olfactory system is capable of discriminating complex yet similar odors (e.g. mocha and latte) with practice, the underlying mechanisms are not clear. As more data have been reported in anesthetized animals, data from awake animals are few. This experiment was therefore designed to investigate two related questions in awake rats: 1) odor coding of complex mixture in the anterior piriform cortex (aPCX) and 2) fear conditioning effects on odor coding in the aPCX. To record activity from awake animals, Long-Evans hooded rats were chronically implanted with movable bundles of microwires aimed at the aPCX. Up to 7 units were recorded simultaneously, and the electrode bundle was moved over time to sample additional cells. Odor-shock conditioning was performed to induce odor-related aversive experience on the rats, with a complex 10-odorant mixture as the conditioned stimulus (CS). The CS odor, along with overlapping odor mixtures and limonene were presented to the animals before the conditioning trials and for several days post-training. The results (n = 206 units) showed a slight decrease in percentage of units that showed excitation after conditioning, and a significant increase in suppression. A significant decrease in average spontaneous activity was observed after conditioning. Finally, an analysis of single-unit responsiveness revealed a late suppressive response after conditioning to all three mixtures overlapping with the CS but not limonene. Interestingly, while responsiveness to control odors decreased after conditioning, responses to the CS became temporally focused, with a more narrow range of onset and offset latencies. Together, odor fear conditioning should enhance signal:noise and CS coding acuity in aPCX. Acknowledgements: NIDCD to DAW

111 Physiological Roles of MOB CCKergic Neurons

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Axons from olfactory sensory neurons (OSN) expressing the same odorant receptor target a pair of glomeruli on the lateral and medial sides of each olfactory bulb (OB). These two ''mirror glomeruli'' are inter-connected via an intra-bulbar association (IA) system, which predominantly arises from superficial and middle tufted cells in the external plexiform layer (EPL). These IA cells: (1) exclusively contain CCK, indicating this peptide as their major or co-neurotransmitter; (2) have primary dendrites extending into glomerular layer where each glomerulus is surrounded by numerous juxtaglomerular (JG) cells. The vast majority of JG cells are GABAergic periglomerular (PG) and short axon (SA) cells; (3) have axons projecting to the contralateral internal plexiform layer (IPL) and synapsing onto the dendrites of local granule cells. Thus, these CCKergic IA cells are well suited to modulate both glomerular inhibitory circuits engaged by GABAergic PG cells and infraglomerular inhibitory circuits involving granule cells and mitral cells. We tested this hypothesis and found that: (1) CCK enhanced sIPSCs in ET and PG/SA cells and presynaptic inhibition of ON terminals by depolarizing and increasing GABA release from PG/SA cells via CCK-2 receptors. This suggests that CCK enhances intraglomerular inhibition of mitral/tufted cells and thus regulates sensory processing in glomeruli. (2) CCK enhanced sIPSCs in mitral cells. This could be due to the enhancement of the glomerular inhibition or the infraglomerular inhibition or both. Both possibilities are under investigation. In summary, our findings support the hypothesis that the CCKergic superficial/middle tufted cells shape the OB output to the olfactory cortex by modulating intraglomerular and possibly infraglomerular inhibitory circuits. Acknowledgements: Supported by NIH NIDCD DC005676

112 Mitral Cell Responses to Sensory Input Under Tonic Inhibition

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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 (MT) cells. MT cells are generally thought to be regulated mainly by inhibition at their lateral dendrites from GABAergic granule cells (GC). Less is known about inhibition occurring at their glomerular tuft by GABAergic periglomerular (PG) cells. We recently reported that the intrinsic bursting of ET cells results in strong spontaneous activation of most GABAergic PG cells to produce tonic presynaptic inhibition of ON terminals (Shao et al 2009). Since MT cells receive IPSCs from PG cells in response to ON input, we hypothesized that MT cells may also receive tonic *postsynaptic* inhibition. To test this hypothesis we measured spontaneous IPSC frequency in MT cells before and after restricted intraglomerular puff of gabazine (GBZ). GBZ significantly reduced the rate of sIPSCs and dramatically increased spontaneous spiking in MT cells. This indicates that tonic inhibition is of glomerular origin and potently regulates MT cell firing. To determine if tonic intraglomerular postsynaptic inhibition is due to ET cell drive of PG cells, we puffed L/T type calcium channel blockers into the glomerulus to block spontaneous ET cell bursting (Liu and Shipley 2008). As predicted, this significantly reduced spontaneous IPSCs in MT cells. These results, taken with our previous findings show that MT cell responses to ON sensory input are strongly regulated by tonic pre- *and* postsynaptic inhibition mediated by the ET-PG-MT cell circuit. Tonic intraglomerular pre- and postsynaptic inhibition may operate to set the gain and offset of the glomerular input-output function. Acknowledgements: Supported by NIDCD DC005676

113 Ethanol Reduces Olfactory Bulb Output by Reducing Excitatory Drive to Mitral/Tufted Cells

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In alcoholics, the smell of ethanol may be an important determinant of its acceptance because the drug's reinforcing properties could be associated with its chemosensory attributes. Moreover, it is possible that chronic alcohol abuse could make ethanol smell and taste better. While the effects of ethanol have been extensively investigated in many brain circuits, its effects on neuronal processing within the olfactory bulb are still unknown. In this study, we have used extracellular and whole-cell patch-clamp recordings in olfactory bulb slices to determine the acute effects of ethanol on output neurons of the olfactory bulb. Mitral and tufted cells appeared to be more responsive to ethanol application (50-100 mM) than external tufted cells. The most prominent effect of ethanol was a decrease in the amplitude and frequency of spontaneous EPSCs. Moreover, olfactory nerve-evoked EPSCs exhibited a decrease in amplitude and electric charge. These effects of ethanol persisted in the presence of the GABA-A receptor blocker, gabazine, but were attenuated in the presence of the NMDA receptor blocker, APV. Extracellular recordings revealed that ethanol decreased the firing frequency and the number of spikes per burst in most mitral and tufted cells. In the olfactory bulb, NMDA receptors have been implicated in synaptic plasticity, dendro-dendritic inhibition, self-excitation, and glutamate spillover. The attenuation of NMDA receptor activity by ethanol is therefore expected to reduce neuronal interactions and as a consequence attenuate olfactory bulb synchronous output activity in response to odor stimulation. This study provides insight into the mechanisms by which ethanol exposure could modulate olfactory bulb neuronal interactions, which may lead to an alteration in the sensory perception of ethanol odor. Acknowledgements: PHS grants: DC007123, DC007876, RR020146.

114 Lateral interactions in the in vivo olfactory bulb network of the rat show heterogeneous distance dependences and vary strongly with respect to respiratory phase

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The lateral connectivity of inhibitory granule cells in the mammalian olfactory bulb (OB) was previously shown to be sparse and distributed using viral tracers (Willhite et al. 2006). However, electrophysiological evidence of this distribution has not previously been shown in the in vivo network. To investigate this possible network organization we performed intracellular recordings of Mitral cells (MC) in vivo paired with focal electrical stimulations of the caudal olfactory nerve layer (ONL). This preparation allows for the activation of distant posterior (hypothetical "surround") glomeruli while avoiding presynaptic stimulation of the recorded cell (hypothetical "center"). Evoked inhibitory post-synaptic potentials (IPSPs) were recorded in MCs in response to posterior electrical ONL stimulations at varying distances from the recording location. IPSP amplitudes showed a heterogeneous distribution as a function of the distance between the stimulus and recording locations. This result suggests that the lateral network of the OB is not organized in a classical center-surround, distance dependent manner. However, there was a general tendency for more distant ONL stimuli to evoke smaller amplitude IPSPs than proximal stimuli. But, stimulus location alone could not predict the evoked IPSP amplitude – for example, the distance dependence nature could vary as a function of the stimulating current. The evoked IPSP amplitudes depended strongly on when the ONL stimulation was triggered with respect to the respiratory phase of the freely breathing animal. Taken together, these results imply that the OB network is highly distributed – within which there is a weak center-surround distance dependence as a function of stimulus strength relative to the respiratory phase.

115 Effects of Sniffing on the Temporal Structure of Mitral/Tufted Cell Output from the Olfactory Bulb

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We have previously characterized the temporal structure of olfactory receptor neuron (ORN) inputs to the olfactory bulb during natural sniffing (Carey et al. 2009; Verhagen et al. 2007). Here,

to investigate how sniffing affects olfactory bulb outputs, we recorded from mitral/tufted (M/T) cells in anesthetized rats using a custom ''sniff playback'' device to reproduce sniff patterns recorded from behaving rats. We found (as have others) that odorant stimulation elicited strong temporal patterning in M/T cells, with each sniff evoking a stereotyped response pattern: an action potential burst 100-200 ms in duration followed by a period of reduced activity. The latency from the start of inhalation to the excitatory burst was consistent within cells but varied among cells over the range of ORN input dynamics, suggesting that M/T activity is largely driven by ORN input. For some cells, we found that even small perturbations in the inter-sniff interval could cause qualitative changes in subsequent M/T responses. As a result, the odorant-evoked activity of M/T cell ensembles might change dramatically from sniff to sniff during natural (irregular) sniffing. At sustained higher sniff frequencies, the firing of some M/T cells became tonic and attenuated (similar to the effect observed among ORNs). Others retained strong patterning; these cells showed a decreased burst duration and slightly increased latency as sniff frequency increased, consistent with a predicted increase in glomerular inhibition at higher sniff frequencies (Wachowiak and Shipley 2006). Together, these initial results suggest that ORN input dynamics play an important role in shaping M/T cell response properties, and that rodents may qualitatively modulate M/T firing patterns by changing sniffing behavior. Acknowledgements: DC00416

116 NMDA Receptors modulate Spontaneous EPSC Bursts of Olfactory Bulb Superficial EPL Interneurons

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The superficial external plexiform layer (sEPL) of the main olfactory bulb (OB) contains GABAergic interneurons (INs), many of which exhibit spontaneous EPSCs (sEPSCs) that show periodic frequency increases (bursts). To examine burst modulation, we recorded sEPSCs from GFP^+ INs in the superficial EPL of OB slices from GAD65-GFP^+ transgenic mice. As described previously, IN sEPSC frequency was highly variable; a single autocorrelation lag time or burst detection inter-event interval could not be used to quantify bursts of all INs. We therefore developed a new method of detecting sEPSC bursts, which used a sliding scale that depended upon the average inter-event interval and sEPSC frequency. With this method, we found that AMPA receptor antagonists eliminated sEPSC bursts of most INs, as previously reported. However, we also found that the NMDA receptor antagonist APV reduced burst incidence (and sEPSC frequency), without affecting other sEPSC parameters. By contrast, the $GABA_A$ receptor antagonist gabazine (GZ) increased burst incidence. Adding APV eliminated the GZ effect. This suggests that NMDA receptor-mediated excitation could affect GABA release from presynaptic inhibitory INs. To localize the NMDA receptors, we examined APV effects on miniature EPSCs (mEPSCs) in TTX. As anticipated, mEPSC frequency, rise and decay slopes, and burst incidence were reduced relative to sEPSC values. However, adding APV further reduced the burst incidence, without affecting other parameters or the mEPSC amplitude distribution. Together, these results indicate that NMDA receptors modulate rhythmic excitation of sEPL INs primarily *via* effects on presynaptic inhibitory INs. Acknowledgements: Supported by NIH DC007876.

117 Mitral Cell Activity during Odor Discrimination in a Mouse Model of Schizophrenia

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Odor discrimination deficits are a common phenotype of schizophrenia, a psychiatric disease characterized by inaccurate perceptions of reality. In an animal model of schizophrenia, we have shown behavioral deficits in odor discrimination in mice with decreased alpha7-nicotinic acetylcholine receptor (alpha7) expression in the olfactory bulb (OB). With an aldehyde or acetate odor pair, wild-type mice had a significantly lower detection threshold compared to alpha7 heterozygous knockouts (HET) mice. However, it is not known whether a decrease in alpha7 expression corresponds to a decrease in the ability of the OB circuit to convey information necessary to discriminate among closely related odors. In adult HET mice, we implanted multielectrode microarrays in the ventral mitral cell layer of the main OB. Mice were then tested with a ''go-no go'' odor discrimination task (i.e., correct response = licking on a rewarded trial or not licking on an unrewarded trial). We characterized single unit and multiunit activity during the odor discrimination task. We will report on the differences in mitral cell odor responses between HETs and controls. Acknowledgements: NIDCD

118 Role of Group I and II Metabotropic Glutamate Receptors in Mouse Main Olfactory Bulb External Tufted Cell Responses to Olfactory Inputs

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External tufted (ET) cells in the glomeruli robustly express metabotropic glutamate receptors (mGluRs). Activation of Group I (mGluR1/5) or Group II mGluRs depolarize and enhance ET cell rhythmic bursting. Little is known about the role(s) of mGluRs in ET cell responses to olfactory nerve (ON) input. In the present study, we investigated the effects of mGluR antagonists on ON-evoked responses in ET cells in mouse main olfactory bulb slices. The mGluR1 antagonist LY367385 (100 μM) reduced the amplitude and integral of ON-evoked EPSCs by 20-30%. The mGluR5 antagonist MPEP (50 μM) significantly reduced ON responses by 40%. Combined application LY367385 and MPEP reduced ON responses by 50%. By contrast, the Group II mGluR antagonist LY341495 (1 μM) had no consistent effect on the amplitude of the ON-evoked EPSC, but produced a modest but significant 15% reduction of the integral. Taken together, these results indicate that both Group I mGluR subtypes on ET cells are engaged by ON input. The involvement of Group II mGluRs in ON-evoked responses in ET cell is modest. Acknowledgements: PHS Grant DC003195

119 Recognition and Coding of Social Cues by the Mammalian Grueneberg Ganglion

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We have begun to undertake a systematic effort to assess the mechanisms underlying recognition and coding of social cues by Grueneberg ganglion neurons (GGNs) of the mouse. A first step in this endeavor is the identification of sensory stimuli that are detected by individual GGNs. Here, we explore whether these cells could function as thermosensors by using live-cell imaging in acute tissue slices. By investigating the effect of acute temperature changes on the cytosolic Ca2+ concentration, we find that OMP-positive GGNs comprise a cluster a thermosensory neurons that are specialized to detect a temperature decline within a relatively narrow temperature window, and that GGNs might comprise a relatively homogeneous cell population with respect to temperature sensitivity. Since classical cold sensors such as TRPM8 and TRPA1 are unlikely to underlie GGN cold detection, we elucidate whether cGMP signaling could be necessary for cellular Ca2+ responses in GGNs. Although our results establish cGMP signaling via the CNGA3 channel as a vital mechanism for Ca2+ entry into GGNs, we find no evidence that this mechanism underlies cooling-induced Ca2+ responses in these cells. Several lines of evidence indicate that cold-evoked and cGMP-dependent Ca2+ signals in OMP-positive GGNs employ differential signaling mechanisms. Our results are consistent with the hypothesis that GGNs function in the detection of stress-related, thermosensory social cues. Acknowledgements: Supported by the Volkswagen Foundation and Deutsche Forschungsgemeinschaft (DFG).

120 Is the Olfactory Epithelium Tuned to Olfactory Perception?

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The principal axis of human olfactory perception, odorant pleasantness, has been linked to a physichochemical axis of odorant structure (Khan et al., 2007). We set out to ask whether electro-olfactograms (EOG) recorded directly from the human olfactory epithelium are tuned to this axis (PC1 of physichochemical space), or in turn better reflect known chemical families. We chose three pairs of odorants, pleasant (pl) and unpleasant (upl) from 3 distinct functional groups (Aldehydes: Butanal (upl) and Hydroxycitronellal (pl); Esters: Cresyl Acetate (upl) and Amyl Acetate (pl); Alcohols: Octanol (upl) and Geraniol (pl)). The odorants were diluted with PEG to a maximum vapor pressure of $0.01_{mm/Hg}$ at 25̈C outside the olfactometer, and further diluted with heated (36̈C) humidified (80%) air inside the olfactometer to obtain equated perceived intensities ($F(54,5)=1.26$, $P>0.29$). An overall flow of $6_{l/min}$ and maximum partial odorant flow of $3_{l/min}$ were kept. ISI=45 s, Stim. Dur.=0.5 s, 5 events per condition, Sampling rate=1kHz. Subjects held their breath 1.5s before stimulus onset and 3s thereafter. Following each trial subjects rated pleasantness and intensity. Preliminary findings (n=2) suggest that the evoked responses did not correlate with perceived pleasantness. Maximal response amplitude was obtained for Octanol (230μV, may involve a trigeminal component) and minimal for Hydroxycitronellal (24μV). A larger EOG area under the curve (AUC) was observed for the unpleasant odorant in the Aldehydes and Alcohols, and for the pleasant odorant in the Esters. Multi-descriptor chemical analysis revealed high correlations between carbon bond order descriptors ('nR=Ct' and 'nR=Cs') and AUC ($r>0.94$, $p<0.005$), suggesting specificity for aliphatic functional groups. Conclusive analysis depends on additional data. Acknowledgements: This work was supported by the FP7 ideas grant 200850 from ERC

121 Chemical determinants of rat olfactory epithelium response

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To further test the chemical determinants of rat olfactory epithelium response, EOG peaks in an exposed epithelium preparation (Scott et al., 2000) and in a preparation with intact air flow (Scott et al., 1996) were fit to the same equation using chemical parameters computed with Molecular Modeling Pro. The response distribution was characterized by the z-score of slope defined as the response size vs. epithelial position. That equation employed two parameters related to molecular solubility and charge distribution. Parameters were fit to the 21 odorants used in both studies with a correlation of 0.88 for the 42 total points. The same parameters and coefficients described the total set of 92 points ($r=0.77$). A new set of data has been collected with a series of intranasal flow rates for 41 odorants. This equation fit responses at high (500 ml/min $r=0.75$), medium (200 ml/min $r=0.67$), and low (100 ml/min $r=0.65$) flow rates. The success of one equation for three data sets argues for a consistent relationship between odor chemistry and spatial response pattern. The difference in response between high and medium flow rates was governed by different properties at different sites. For dorsomedial sites this difference correlated at $p<.01$ with the Hansen solubility parameter and solubility ($r=0.51$). For lateral responses this difference correlated at $p<.01$ with molecular length ($r=.45$) and compactness ($r=.45$). In spite of experimental variance, these data point to significant differences in the types of odorants that activate different epithelial regions and differences in the way they are affected by air flow in different regions. These results are consistent with the work of Mozell et al., (1991) on frog, and suggest that the anatomical arrangement in rat reinforces the air flow effects. Acknowledgements: Supported by NIH grants DC000113 and DC008648 to JWS

122 Diffusion limitation of cytoplasmic elements within the olfactory cilium

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Olfactory signal transduction starts at the cilia that display a nano-level tubular structure. Spatial distribution and temporal kinetics of cytoplasmic elements (cAMP and Ca^{2+}) are key factors to understand the transduction system, but experimental difficulties accompanying

such tiny structure have prevented experiments and analysis. In general, in water it is well known that elements can diffuse one-dimensionally over the length of cilium within 1 sec. This may cause non-linear amplification and/or adaptation in two different parts in the cilium. However, evidence suggests that there is a diffusion limitation of the factors in the cilium. In this work, we employed four techniques to overcome such technical difficulties. 1) Measurements of local current responses induced by laser-photolysis using whole-cell mode of patch-clamp, 2) Two point LMS-ROI local laser light stimulation to induce photolysis of caged cAMP, 3) $[Ca^{2+}]_i$–imaging after the photolysis of caged cAMP, 4) Digital modeling for one-dimensional diffusion of molecules within the cilia. As a result, current responses were shown to be independent at two points further apart than a few microns. With Fluo4 imaging, increased $[Ca^{2+}]_i$ by local cAMP jump were localized within the single cilium. It is theoretically suggested that the lateral spread of cAMP and Ca^{2+} is limited by the extrusion/degradation systems and the limited diffusion caused by the binding to the membrane cytoplasmic surface. We conclude that cytoplasmic diffusion of elements is limited within the cilia, so that the single cilium expresses a near-linear spatial summation of the odorant information.

123 Olfactory xenobiotic metabolizing enzymes have an impact on the stimulating properties of some odorants

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Xenobiotic metabolizing enzymes (XME) catalyze the biotransformation (oxidation, hydrolyse, conjugation, ···) of a large number of exogenous chemicals in tissues. Their high expression in the olfactory epithelium of various species, in the close environment of olfactory receptors, suggests that these enzymes could have a role in the regulation of the olfactory reception process. The degradation of odorants by XME has been shown in insects. However the role of these enzymes in mammal olfactory tissues remains unclear. Here, we studied the impact of olfactory XME on stimulating properties of odorants in the rat. We first showed that two odorant molecules, quinoline and isoamyl acetate, are respectively metabolized by cytochromes P450 and carboxylesterases *in vitro*. In a second study, using submerged electro-olfactogram (EOG) recordings, we observed that metabolites derived from these odorants elicited lower response amplitudes than the parent molecules. Further we analyzed the effects of specific cytochrome P450 and carboxylesterase inhibitors on EOG responses of quinoline and isoamyl acetate. Application of the cytochrome P450 inhibitor induced an increase of the quinoline response. Similarly, application of the carboxylesterase inhibitor increased the response to isoamyl acetate stimulations. This study demonstrates that metabolism of odorants by olfactory XME decreases their olfactory properties. This process can contribute to avoid the saturation of the peripheral olfactory system.

124 Integrating heterogeneous Odor Response Data into a common Response Model: A DoOR to the Complete Olfactome

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We have developed a new computational framework for merging odor response datasets from heterogeneous studies, creating a consensus meta-database, the Database of Odor Responses (DoOR). As a result we obtained a functional atlas of all available odor responses in *Drosophila melanogaster*. Both the program and the dataset are freely accessible and downloadable on the Internet (http://neuro.uni-konstanz.de/DoOR). The procedure can be adapted to other species, thus creating a family of ''olfactomes'' in the near future. *D. melanogaster* was chosen because of all species in this one we are closest to the complete olfactome, with the highest number of deorphanized receptors available. The database guarantees long-term stability (by offering time-stamped, downloadable versions), up-to-date accuracy (by including new datasets as soon as they are published), and portability (for other species). We hope that this comprehensive repository of odor response profiles will be useful to the olfactory community and to computational neuroscientists alike. Acknowledgements: BMBF grant 576/07 to MS and CGG, and DFG grant GA524/7-1 to DM. MS is an associated member of the DFG research training group GK-1042

125 The multiple PDZ domain protein 1 (MUPP1) – mediator of the olfactosome?

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The complex olfactory signal transduction pathway enables mammals to detect and discriminate between thousands of different odorants. But how the individual components of this complex signaling cascade get into close proximity to each other is an unsolved question. We recently showed that a PDZ protein called MUPP1 (multiple PDZ domain protein 1) is an interaction partner of olfactory receptors and thereby a putative mediator of a so called olfactosome (Baumgart *et al*, FEBS journal, Dec 2009). This scaffolding protein consists of 13 single PDZ domains and is known to be a mediator of diverse GPCR based signalling networks. We demonstrated that this scaffolding protein is highly expressed in the dendritic knobs and cilia of olfactory sensory neurons of mice. Further, we could show that different ORs are able to interact with MUPP1. In a new peptide microarray approach we investigate putative interactions of a great variety of ORs of all different known subfamilies in the mouse genome. In addition, we investigate other signaling components for their putative presence in the MUPP1 based complex by protein microarrays, as well as by direct protein-protein interaction assays.

126 Splice variants of the Ca^{2+}-activated Cl^- channel Anoctamin 2

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Olfactory receptor neurons (ORNs) in vertebrates use a calcium-activated chloride current to amplify the receptor potential in response to odor stimulation. Anoctamin 2 (ANO2, also called TMEM16B) has recently been proposed to be the long-sought calcium-activated chloride channel in the ORN (Stephan et al, 2009). Biophysical properties of heterologously-expressed ANO2 channels are similar to those of the native calcium-activated channel, but

with one noticeable difference: the native channel inactivates during prolonged opening at positive membrane potentials, whereas the heterologously expressed ANO2 channel does not. Different splice variants of ANO2 transcripts have been found in tissues including the olfactory epithelium and the retina. Using molecular techniques we found two alternative, hitherto not described ANO2 transcripts in ORNs, with alternative transcription initiation sites. Expression in a heterologous system and excised patch recordings yielded functional channels with biophysical properties largely similar to previously described ANO2 splice variants. To better understand the functional significance of various splice variants, we will further investigate biophysical properties of splice variants. Acknowledgements: This work is supported by a Morley Kare Fellowship, the Human Frontiers Science Organization and NIH R01 DC007395.

127 An electroolfactogram (EOG) study of odor response maps from the mouse olfactory mucosa?

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Olfactory sensory neuron (OSN) responses measured at the population level tend to be spatially heterogeneous in vertebrates and response ''maps'' vary with odor. One proximate explanation for this heterogeneity comes from evidence that olfactory receptor genes in rodents are expressed in OSN populations that are spatially restricted to one of four zones in the nasal cavity. An ultimate explanation for response anisotropy posits that it is the signature of a supplementary mechanism for quality coding, based on the sorptive properties of odor molecules. These theories are difficult to assess because most mapping studies have utilized few odors, have provided little replication, or have involved but a single species (rat). In fact, to our knowledge, a detailed olfactory response map has not been reported for mouse, the species in which most of the gene localization work has been done. Here we report the results of a study of the mouse olfactory mucosal response map using the EOG. We focused on the medial aspect of olfactory turbinates as viewed in midsagittal section. This limited approach still allowed us to test predictions based on the zonal distribution of OSN types and the previously mentioned sorption theory. In three separate experiments, 290 mice were used to record EOGs from a set of standard locations along each of four endoturbinates utilizing 11 different odors resulting in over 4400 separate recordings. Results confirm a significant spatial non-uniformity in odor responses that varies with odor. However, little support was found for the suggestions that odor specific response patterns across the mucosa are explained by OSN distribution restricted to zones or governed by the relative sorptiveness of odors. Alternative explanations for odor response maps will be explored. Acknowledgements: National Science Foundation (# 0641433) to DMC.

128 Neuropeptide Y modulates olfactory mucosa responses to odorant in fasted rat

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Neuropeptide Y (NPY) plays an important role in regulating appetite and hunger. In the hypothalamus, endogenous NPY stimulates food intake, under the control of the nutritional status. We have recently demonstrated that several nutritional and metabolic cues modulate the odor detection in the rat olfactory mucosa (OM). Previous studies have shown the presence of NPY and receptors in rodent olfactory system, and suggested a neuroproliferative role. NPY was also shown to directly modulate olfactory responses in hungry Axolotls. The aim of the present study was to investigate the potential effect of NPY on rat OM responses to odorants, in relation to the animal's nutritional state. Electro-olfactogramm (EOG) responses to odorants were recorded from rat endoturbinates, and their amplitudes were measured before and after local applications of NPY (*versus* vehicle), in both fed and fasted rats. NPY application significantly and reversibly increased the EOG amplitudes by ~30% in fasted rats, but not in fed rats. The effects of specific agonists of NPY receptor-subtypes were similarly quantified, showing that the NPY modulation operated mainly through Y1 and secondarily through Y5 receptors. We undertook the immunohistochemical localization of Y1 receptors in the OM, and preliminary results suggested an increased expression of these receptors in different OM cell types in fasted rats. These data provide the first evidence that NPY modulates the most peripheral step of odor detection in rat, at the OM level. This modulation strictly depends on the nutritional state of the animal, and provides another functional basis for the physiological link between olfaction and nutrition. Acknowledgements: This work was funded by INRA. Julia NEGRONI was financially supported by the INRA department ''Physiologie Animale et Systèmes d'Elevage''.

129 Investigation of Olfactory CO_2 Detection 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 small subset of olfactory receptor neurons allowing mice and rats to ''smell'' low concentration of CO_2. The second messenger cAMP is known to play a role in the detection of typical odorants while recent studies indicate that cGMP and the enzyme carbonic anhydrase (CA) are important for the detection of CO_2. The objective of this study was to investigate the transduction pathway for CO_2 detection by recording electro-olfactograms (EOGs) in response to CO_2 and odorants before and after topical application of L-cis-diltiazem, which inhibits cGMP activated Ca^{++} channels or niflumic acid, which inhibits Ca^{++} activated Cl^- channels. Wild-type (C47Bl6J) mice were euthanized and the olfactory epithelium was exposed. EOGs were recorded from the medial surface of the olfactory epithelium in caudal regions of endoturbinate II and II', areas known to contain high concentrations of CA. EOGs were recorded in response to CO_2 (0-50%) and odorants before and after topical application of the inhibitors. We found that application of L-cis-diltiazem attenuated the EOG response to CO_2 to a greater extent than the EOG response to odorants, indicating that cGMP activated Ca^{++} channels are important in the CO_2 transduction pathway but do not play a role in typical odorant transduction

pathways. The experiments using niflumic acid show that application of this inhibitor attenuated the EOG responses to both CO_2 and odorants, indicating that Ca^{++} activated Cl^- channels may play a role in sensing CO_2 as well as typical odorants. The results of these experiments provide further support for a unique olfactory transduction pathway for the detection of CO_2 in mice. Acknowledgements: This study was supported by funds from the Dr. and Mrs. Edward Shanbrom Foundation.

130 ATP Maintains Homeostasis in Olfactory Epithelium in Vivo and in Vitro

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Olfactory sensory neurons (OSNs) in olfactory epithelium (OE) undergo functional turnover throughout life. The balance of neuronal apoptotic death and neuroregeneration is tightly regulated to maintain homeostasis in the OE. Extracellular ATP exerts multiple neurotrophic actions in CNS, such as increasing cell proliferation and decreasing apoptosis. In the OE, we previously showed that ATP promotes neuroregeneration in vivo and in cultured OE slices. However, the effects of ATP on neuronal survival are not elucidated yet. The purpose of this study is to investigate mitogenic and protective effects of ATP in the OE in vivo and in OE primary cell culture. Adult Swiss Webster mice were intranasally instilled with saline or ATP (400 nmoles/kg). 48 hours later, the levels of proliferating cell nuclear antigen (PCNA)-labeled proliferation, and TUNEL- and activated caspase-3-labeled apoptosis in the OE were measured. We found that, compared to saline animals, intranasal instillation of ATP significantly increased PCNA+ cells in the OE. ATP also significantly decreased TUNEL+ but not activated caspase-3+ apoptotic cells even though the level of apoptosis in normal OE is relative low. Likewise, in OE primary cell culture, we found that ATP (100 μM) significantly increased BrdU and 5-ethynyl-2-deoxyuridine incorporation, suggesting an increase in proliferating cells. ATP also significantly decreased TUNEL+ and activated caspase-3+ apoptotic cells. Consistent with ATP-induced decrease of apoptosis, we observed a significant increase in the number of OMP+ mature OSNs, nestin+ neuronal progenitor cells, notch 2+ and NPY+ sustentacular cells following ATP incubation. Taken together, these data indicate that ATP plays a role in maintaining OE homeostasis via mitogenic and protective effects. Acknowledgements: NIDCD DC006897

131 Nickel Sulfate Induces Location-Dependent Atrophy of Mouse Olfactory Epithelium: Protective and Proliferative Role of Purinergic Receptor Activation

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Occupational exposure to nickel sulfate (NiSO4) leads to impaired olfaction and anosmia through an unknown mechanism. We investigated the mechanism of NiSO4-induced toxicity and the potential therapeutic role of ATP in olfactory epithelium. Male Swiss Webster mice were intranasally instilled with NiSO4 or saline followed by ATP, purinergic receptor antagonists or saline. We assessed the olfactory epithelium for NiSO4-induced changes 1-7 days post-instillation and compared results to olfactory bulb ablation-induced toxicity. Intranasal instillation of NiSO4 produced a dose- and time-dependent reduction in the thickness of turbinate OE. These reductions were due to sustentacular cell loss, measured by terminal dUTP nick end labeling staining at 1 day post-instillation and caspase-3-dependent apoptosis of olfactory sensory neurons at 3 days post-instillation. A significant increase in cell proliferation was observed at 5 and 7 days post-instillation of NiSO4 evidenced by BrdU incorporation. Treatment with purinergic receptor antagonists significantly reduced NiSO4-induced cell proliferation and post-treatment with ATP significantly increased cell proliferation. Post-treatment with ATP had no effect on sustentacular cell viability but significantly reduced caspase-3-dependent neuronal apoptosis. In a bulbectomy-induced model of apoptosis, exogenous ATP produced a significant increase in cell proliferation that was not affected by purinergic receptor antagonists, suggesting ATP is not released during bulbectomy-induced apoptosis. ATP is released following NiSO4-induced apoptosis and has neuroproliferative and neuroprotective functions. These data provide therapeutic strategies to alleviate the loss of olfactory function associated with occupational exposure to nickel compounds. Acknowledgements: CR contributed to this work as part of the Summer Research Opportunity Program of the Ronald McNair Program at MSU, and was supported by NINDS 1R25NS065777-01 and the RISE Program at the University of Puerto Rico-Cayey. This work was supported by NIDCD DC006897.

132 Using a 3-D Culture Model to Identify Factors that Regulate Olfactory Epitheliopoiesis

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Many facets of the molecular mechanisms that regulate the growth, maintenance, and regeneration of the olfactory epithelium (OE) remain to be discovered. The ability to efficiently test the regulatory role of candidate molecules or dissect complex molecular mixtures requires a tissue culture model that closely mimics the biology of the *in vivo* OE. When grown in air-interface cultures surmounting a layer of feeder cells, OE cells form "spheres" that closely resemble the OE *in vivo* and retain the capacity to engraft following transplantation (Jang *et al.*, 2008). We show here that an immortalized cell line derived from the lamina propria (LP_{imm}) stimulates sphere formation in 3-D cultures of mouse OE cells taken after MeBr lesion as 3T3 cells did in the original demonstration. Furthermore, conditioned media (CM) from both 3T3 and LP_{imm} cells greatly enhance the formation of spheres. In order to identify factors released by LP_{imm} to influence sphere formation, we took two approaches: 1) individual growth factors or a combination of them were added to base media; 2) proteins that are synthesized and secreted into CM by LP_{imm} were metabolically labeled using Click-iT™ chemistry and identified by proteomic methods. Some of the candidate molecules identified by the proteomic approach were tested by either adding them to base media or by using antibodies to block their function. Our culture system, by maintaining the capacity for progenitor cell engraftment and for easy manipulation *in vitro*, can provide a rapid "read" on factors that are worth the further effort required to define their role(s) in the process of epitheliopoiesis *in vivo*. In addition, this type of culture offers us the opportunity to expand neurocompetent stem cells for eventual therapeutic purposes. Acknowledgements: NIH R01 DC002167

133 Molecular markers of stem and progenitor cells are the same in human olfactory mucosa as in mice and rats.

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Our incomplete understanding of human olfactory pathophysiology has limited the range of therapeutic options for most common forms of olfactory dysfunction. We can identify different categories of progenitor cells as well as more differentiated cell types in the olfactory epithelium (OE) of mice and rats using a panel of antibodies to known proteins. Attempts to carry out a similar analysis of human OE have been limited by difficulties in obtaining tissue samples of adequate quality via biopsy, which has contributed to our lack of knowledge pertaining to olfactory epitheliopoiesis. Whole mount staining of septal epithelium obtained at autopsy (specimens harvested by the National Disease Research Interchange) enables us to identify regions ventral to the cribriform plate that are rich with olfactory neurons. As a consequence, we can obtain olfactory mucosa consistently and orient the tissue properly as required for in-depth analysis of the cell types of the human OE. Immunohistochemistry on sections of post-mortem human olfactory mucosa using a battery of antibodies directed against cytoskeletal proteins, surface antigens, and transcription factors expressed in stem and progenitor cells of rodents, generate staining patterns that closely reflect those observed in the OE of mice and rats. Our results reveal a high degree of similarity between the human and rodent olfactory systems, which was not previously appreciated. Further studies looking for alterations in the cellular composition of human OE obtained via biopsy from patients suffering smell loss and comparing them with patterns of staining in mouse models of injury-induced regeneration will help elucidate the pathophysiology of olfactory disorders in humans. Acknowledgements: R01 DC010242

134 Glomerular targets of olfactory sensory neurons in adult female mice heterozygous for mutated CNGA2 with TRPM5 knockout background.

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Activation of olfactory sensory neurons (OSNs) influences the proper axonal targeting to the olfactory bulb of adult mice. Odor activation in a majority of OSNs involves the cyclic nucleotide gated channel A2 subunit (CNGA2). We have previously shown that adult female mice heterozygous for the mutated CNGA2 (CNGA2KO-GFP +/-) have more GFP positive glomeruli than CNGA2KO-GFP +/- mice with a knockout of the transient receptor potential channel M5 (TRPM5) (Dunston et al AChemS abstract 2009). Here we further determined the location of the GFP positive glomeruli in these two mouse lines using an olfactory bulb mapping program. Consistent with our previous findings, the density of GFP positive glomeruli in mice that are CNGA2 KO-GFP +/- with TRPM5 knocked out was lower than that of the CNGA2 KO-GFP +/- mice. The overall distribution of these glomeruli was similar in the two lines. We also found that in both lines of mice many glomeruli are targeted by both GFP positive and negative axons in addition to glomeruli that homogenously receive either GFP positive or negative axons. The results imply that OSNs with null CNGA2 may exhibit different levels of activity. Further, we examined the immunolabel intensity for tyrosine hydroxylase (TH), which depends on sensory input. The intensity of the TH immunostaining in glomeruli receiving homogenous GFP positive axons in both lines of mice was decreased but not eliminated. Our data indicate that TRPM5 may contribute to different activity levels of OSNs. Acknowledgements: NIH/NIDCD grant 009269

135 G protein-dependent activation of PLC and PI3K in mammalian olfactory receptor neurons

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Odorants activate both PLC and PI3K in the cilia of olfactory receptor neurons (ORNs) (Klasen et al., 2009). To better understand the potential role of these enzymes in olfactory transduction we asked whether the activation of one or both enzymes was G protein dependent. Isoforms of both PI3K and PLC can be localized to the olfactory cilia by western blot and/or immunohistochemistry. Activation of both enzymes in ORNs can be independently blocked pharmacologically by G protein-specific inhibitors. The non-hydrolyzable GDP analog GDPβS, as well as suramin, a Gβγ inhibitor, independently blocked the activation of PLC and PI3K *in vitro* as detected by ELISA. The Gβγ inhibitor gallein and a closely related compound M119, which is reported to specifically disrupt *in vitro* G protein-dependent activation of PLCβ2, PLCβ3 and PI3Kγ, also suppressed odorant-dependent activation of rodent ORNs *in vivo*, as measured by calcium imaging. Taken together, these results provide evidence that odorants activate both PI3K and PLC signaling in a G protein-dependent manner in ORNs. Acknowledgements: Supported by grants from the Deutsche Forschungsgemeinschaft and the National Institute on Deafness and Other Communication Disorders.

136 Does Olfactory Marker Protein (OMP) function by interacting at calmodulin (CaM) binding sites?

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Behavioral, electrophysiological and Ca-imaging analyses have demonstrated the involvement of OMP in olfactory transduction, but the molecular mechanism remains elusive. Our recent studies of protein-protein and protein-peptide interactions have implicated CaM in this process. Bex protein, an OMP binding partner, also binds CaM, suggesting that these three proteins may interact to regulate the availability of CaM. We hypothesized that OMP might interact at CaM binding sites on various proteins in the olfactory transduction cascade. Last year we showed that OMP or CaM can each bind to the auto-inhibitory peptide (XIP) of Na/Ca exchanger 1 (NCX1), a protein involved in Ca2+ extrusion from OSNs. We are characterizing the interaction of OMP with putative CaM binding sites of other

proteins involved in olfaction. Peptides from PMCA2, CaM-KII, CNGB1b and PDE1c were analyzed by QCM-D (Quartz Crystal Microbalance with Dissipation Monitoring) for their interactions with CaM or OMP, in the presence or absence of Ca2+. Three of the four peptides (but not the PDE1c peptide) interacted with CaM only in the presence of Ca2+, with low uM Kd values. OMP also interacted with these three peptides, but independent of Ca2+, with 10x higher Kd (lower affinity) values than seen with CaM. These data provide additional evidence that OMP can selectively interact with several peptide sequences that bind Ca2+/CaM. NMR analyses of some of these interactions provide structural evidence for the nature of the complexes. 15N-NMR HSQC titrations of XIP/OMP, XIP/CaM, Bex (50-75)/OMP and Bex (50-75)/CaM demonstrate that OMP and CaM can compete for the same target sites. These data support our hypothesis that OMP can modulate the activities of some CaM binding proteins and thus influence the olfactory signal transduction cascade. Acknowledgements: Supported by Andrews FRG (HJK), NIH DC03112 (FLM) NIH GM58888 (DJW).

137 Isolation and characterization of immature olfactory sensory neurons

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To detect chemical stimuli, olfactory sensory neurons (OSNs) are necessarily exposed to damage, necessitating life-long neurogenesis to replace damaged OSNs. Mature OSNs (mOSNs) therefore always coexist with immature OSNs (iOSNs). To better understand the respective contributions of the two developmental stages of OSNs, we have simultaneously purified mOSNs and iOSNs from the same mice. We collected three cellular fractions: mOSNs, iOSNs and a residual population of all other cells in the olfactory epithelium. Affymetrix M430 v.2 GeneChip data for each fraction were compared in order to derive mRNA abundance ratios that were statistically evaluated against in situ hybridization data for 396 mRNAs, thereby allowing the assignment of validated probabilities of expression in mOSNs and iOSNs for each transcript. Of the ~10,000 genes that are expressed by OSNs (Sammeta et al., 2007, PMID 17444493) we identified 358 specific to mOSNs and 1,189 specific to iOSNs. The over-represented GeneOntology functions among the mOSN-specific transcripts were ion transport, neurotransmitter transport, synaptic transmission and cilia, consistent with the specializations that define the maturity of these neurons. Transcripts specific to iOSNs represented a more diverse set of biological processes, including axonogenesis, transcription, chromatin modification and nucleic acid metabolism, among others. The iOSN-specific list included 115 transcription factors (~10% of iOSN-specific transcripts) and numerous epigenetic regulators, consistent with the gene expression changes that must accompany neural differentiation. Acknowledgements: Supported by award R01 DC002736

138 Gene Expression Profiling of the Olfactory Neurogenic Lineage

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Olfactory sensory neurons (OSNs) are replaced throughout life during normal maintenance and after injury by the regulated proliferation and differentiation of stem and progenitor cells that persist in the olfactory epithelium (OE) throughout life. The stages in the progression from proliferating progenitor through cell cycle exit to the multistep differentiation of OSNs can be marked via the use of transgenic reporter mice and/or patterns of protein expression. To identify candidate pathways regulating this process we have examined a number of these stages by global transcriptional profiling. Using FACS–purified cells from ΔNgn1::eGFP (a BAC transgenic line) and ΔOMP::GFP (gene knock-in to the endogenous locus) mice that are otherwise unmanipulated and others after olfactory bulb ablation, we have obtained the transcriptome of cells at each of four distinct steps in the neuronal lineage: 1) immediate neuronal precursors, 2) activated immediate neuronal precursors, 3) newly differentiating OSNs, and 4) mature OSNs. Functional annotation clustering of highly regulated genes (adjusted p value <0.05 and fold change >2) from these data sets compared to one another and with normal olfactory mucosa has identified candidate pathways and genes for validation and functional studies. Annotation clusters relating to neurogenesis, regulation of transcription, establishment of chromatin architecture, mitotic cell cycle and axonogenesis had high enrichment scores in the globose basal cell and immature neuron–enriched samples, while sensory perception of smell and synaptic transmission were highly enriched terms in the differentiating and mature OSN datasets. These data provide a gateway to examining and understanding the genome–wide events that occur as neurons are born and mature within the OE. Acknowledgements: Supported by R21 DC008568 (J.E.S.) and F30 DC010276 (R.C.K)

139 Visualizing the Redistribution of Responses within the Rodent Olfactory Receptor Repertoire : Tracking Chemical, Conformational, and Concentration Changes.

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The combinatorial nature of odor encoding makes the study of the olfactory system fascinating, but at the same time it presents problems for data management. Although tables of probabilities, multidimensional vectors, and other descriptive statistics are the most thorough embodiment of complex data, such modes of presentation are not immediately intuitive to a broad audience. Here, we present a more visual representation that can be used to supplement other analyses. We show how it can provide a more accessible entry point to investigations of the relationships between odorant structure and physiological response across the suite of olfactory receptors. We apply this visualization method to three datasets, focusing on activation by octanal of rodent olfactory sensory neurons. This presentation helps underscore striking asymmetry in the response to odorants, highlights the presence of functional nodes / attractors in chemical space, and illustrates the dynamic nature of the population code in response to shifting concentration. Acknowledgements: supported via funding from the NIH NIDCD

140 Taste Preferences of the FHH-Chr n^{BN} Consomic Rat Strain Set

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Breakthroughs in the molecular genetics of the mouse have led to the discovery of several taste receptors and transduction mechanisms. However, findings in the mouse do not always generalize to other species, and the rat has long been the model of choice for studying taste and nutrition. To begin genetic studies of rat taste preferences, we exploited the FHH-Chr n^{BN} consomic strain set. Groups of 5–15 male rats from the FHH, BN, and all 22 consomic (Chr 1–20, X and Y) strains received 4-day two-bottle tests with a choice between water and each of the following: 10 mM NaCl, 237 mM NaCl, 32 mM $CaCl_2$, 1 mM saccharin, 100 mM NH_4Cl, 32 mM sucrose, 100 mM KCl, 4% ethanol, 1 mM HCl, 10 mM MSG, 1 mM citric acid, 32 μM QHCl, 1% corn oil, 32 μM denatonium, 1% Polycose, and 1 μM capsaicin. There were many significant differences in taste solution avidity between various consomic strains and the FHH strain, but few involving chromosomes harboring previously identified taste genes. As examples, (a) avidity for saccharin was influenced by loci on Chr 4, 10, 11, 16 and 17 but not Chr 5, which harbors *Tas1r2* and *Tas1r3,* the receptor subunits primarily responsible for variation in sweet taste preferences of the mouse; (b) avidity for sour tastes was influenced by loci on Chr 2, 4, 7, 10, 11 and 13 but not Chr 1 or 19, which harbor *Pkd2l1* and *Pkd1l3*, genes implicated in sour taste variation in mice; (c) avidity for fat (corn oil) was influenced by loci on Chr 2, 3, 5, 6, 7 and 8 but not Chr 4, which harbors *Cd36*, a gene implicated in fat taste in mice. Genes involved in taste transduction in the mouse do not account for the variation in taste preferences between these rat strains; instead, the results point to novel genetic contributions to taste preferences. Acknowledgements: Supported by NIH RO1 DK-46791. The rats were provided by a Seed Grant Program sponsored by Physiogenix Inc. (Wawatosa, WI).

141 Association Between Common Genetic Variation in the G-alpha Gustducin Gene and Human Sucrose Perception

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Variation in taste perception of different chemical substances is a well known phenomenon common in both humans and animals. We evaluated the hypothesis that sequence variations occurring in genes encoding taste signaling molecules can influence sweet taste perception in humans. Our population consisted of unrelated individuals (n = 160) of Caucasian, African and Asian descent. Threshold and suprathreshold sensitivities of participants for sucrose were estimated using a sorting test and signal detection analysis that produced cumulative R-index area under the curve (AUC) scores. Genetic association analysis revealed significant correlation of sucrose AUC scores with genetic variation occurring in the GNAT3 gene (single point $p = 10^{-3}$-10^{-4}), which encodes the taste-specific G_α protein subunit gustducin. Subsequent sequencing identified additional GNAT3 variations having significant association with sucrose AUC scores. Collectively, GNAT3 polymorphisms explain 13% of the variation in sucrose perception. Our findings underscore the importance of common genetic variants influencing human taste perception. In addition, because G_α gustducin also mediates bitter taste transduction, we propose variation at this locus may also underlie variation in human bitter taste perception. Acknowledgements: NIH intramural grant Z01-000046-10, CRADA DC-08-001, Givaudan Flavors Corp.

142 Do TAS1R3 promoter region SNP rs35744813 A allele carriers show a reduced response to concentrated sucrose?

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Recently, two polymorphisms in the promoter region of the TAS1R3 sweet taste receptor gene were shown to have functional consequences: in a signal detection based ranking task, A allele carriers (AA homozygotes and AG heterozygotes) for rs307355 and rs35744813 were found to have reduced sucrose sensitivity (Fushan et al 2009) at low sucrose concentrations (.12M and below). We attempted to extend this finding to higher sucrose concentrations using a different psychophysical approach (direct scaling) for one of the putatively functional SNPs. Here, 60+ participants tasted five sucrose (.08, .16, .32, .64 and .96M) stimuli (10mL) in triplicate, and rated the intensity of sweetness and other taste qualities using the generalized Visual Analog Scale (gVAS), an unstructured scale derived from the generalized labeled magnitude scale (gLMS). Similar to the gLMS, the gVAS has a top anchor of 'strongest sensation of any kind' and a broad usage context is encouraged through a brief scale orientation where participants rate the intensity of 16 remembered sensations. The gVAS differs from the gLMS in that all internal lines and adjective labels between no sensation and the top anchor have been removed. DNA was obtained via buccal swab and the TAS1R3 promoter genotype for rs35744813 was determined via custom TaqMan probe on an ABI Prism 7900HT Sequencer. The minor allele frequency for rs35744813 in our sample was similar to prior work. However, our data were not consistent with reduced sucrose response in A allele carriers. Presently, it is unclear whether conflicting results are due to the sample, the range of concentrations tested or the psychophysical approach (direct scaling vs. r-index). Also, more work is needed to determine whether the rs307355 polymorphism may be functional at high sucrose concentrations. Acknowledgements: Supported by funds from NIH T32AA07459, R44AA014118, and a VA Career Development Award.

143 Polymorphism in Bitter Taste Receptors of Primates.

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In mammals, bitter taste is mediated by *T2R* gene family members. Since T2Rs are directly involved in the interaction between mammals and their dietary sources, these genes likely evolved to reflect regionally specific diets during mammalian evolution. Human *T2R* genes (h*T2Rs*) have been observed to be polymorphic, however, polymorphisms in other wild animals has not been investigated so far. In order to elucidate the evolutionary process of bitter taste recognition, we started genotyping bitter taste receptors of individual primates living in the Primate Research Institute, Kyoto University. As a result, it

has been revealed that there are many Single Nucleotide Polymorphisms (SNPs) in T2Rs. Behavioral tests showed the presence of specific bitter-taste insensitive monkeys whose specific T2R is disrupted. These data demonstrate the presence of monkey models useful for bitter taste sensitivity, receptor expression, and neuronal processes. In addition to furthering analysis of molecular properties, in cooperation with the global COE program, we are constructing a genetic database of the individual captive primates in the institute. Acknowledgements: This work was financially supported by global COE program A06 and by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (2137009) and grants from the Takeda Foundation for Science and the Suzuken Memorial Foundation to H. I.

144 Community-Based Participatory Research in a Museum Setting

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As a museum, our vision is to "create a community of critical thinkers". Our *Genetics of Taste: A Flavor for Health* study accomplishes this by allowing the community to actively drive a real research project. The study has scientific and educational objectives: determine the relationship of the genotype and phenotype of *Tas2r38* to genetic ancestry and overall body composition and also to increase public understanding of how genetic research translates from the laboratory setting into meaningful information for society as a whole. These objectives are executed within a community-based participatory research laboratory within our health exhibit, *Expedition Health*. From inception to execution, this program is a thriving example of community-based participatory research at its best. The research question was chosen by the public and the study is conducted by volunteer citizen-scientists. Finally, museum visitors are enrolled as subjects using a series of simple, but highly specific tests: DNA collection for genotyping and ancestry analysis, a propylthiouracil taste test, body mass index measurement, and a fungiform papillae density count. The scientific benefit of this community-based approach is obvious: enrollment within the museum allows us to obtain an unprecedented sample size of greater than 1000 subjects, providing data subsets from a wide range of ages and diverse backgrounds. We anticipate this advantage will help build a broader picture of *Tas2r38* than would otherwise be possible. In conclusion, through community participation in authentic research, the *Genetics of Taste* study has and will continue to increase public understanding of genetic research, while also making strides to better understand the genetic ancestry of *Tas2r38* and its affect on the health of modern day humans. Acknowledgements: R25 RR025066-02 NIH NCRR SEPA

145 Morphological, physiological, and gene expression evidence for a supertasting phenotype in Gust-BDNF mice

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Brain-derived neurotrophic factor (BDNF) is the most potent neurotrophic factor in the taste system during development, which is also expressed in taste cells of adult mice. To study the roles that BDNF plays in the adult taste system, BDNF transgene expression was driven by the alpha-gustducin promoter in C57 taster background mice. BDNF overexpression in taste papillae was verified by in situ hybridization and quantitative real time PCR on laser capture microdissected taste buds. We performed immunohistochemistry on taste papillae using Troma-1, a taste bud specific antibody. Circumvallate taste buds in high BDNF expressing transgenic mice were significantly wider and fungiform taste buds were significantly larger ($p<0.001$) as compared to wild type controls. To examine whether innervation was affected in Gust-BDNF mice, we used antibodies to P2X3 and neural cell adhesion molecule (NCAM). The total density of innervation and specifically gustatory innervation was markedly increased in high BDNF expressing transgenic mice compared to low BDNF expressing transgenic mice and wild type controls. Moreover, using the two-bottle preference test we found that Gust-BDNF mice were able to detect 100 fold lower concentrations of sweet and bitter tastants. Microarray analysis showed an altered pattern of gene expression profile in all Gust-BDNF mice compared to wild type control mice. Interestingly, NCAM gene expression was significantly up-regulated in high BDNF expressing mice. We propose that Gust-BDNF transgenic mouse models can be employed to dissect the specific roles of BDNF in the adult taste system. Acknowledgements: R01-RDC007628 from NIH-NIDCD

146 Segregated populations of fish taste bud cells express T2R bitter taste receptor genes in a genomic cluster-dependent manner

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Bitter taste is thought to be a signal for toxic compounds that should be avoided. It remains controversial whether vertebrates can discriminate among various bitter compounds. Previous studies have indicated that most T2Rs, bitter taste receptors, are coexpressed with each other in mammals. In this report, we identified the entire repertories of T2R genes in teleost fish, and found that their expression relationships were different from those in mammals. A comprehensive genome search for T2R genes in four teleost species revealed the presence of 7 in zebrafish (*Danio rerio*), 6 in puffer fish (*Tetraodon nigroviridis*), 4 in fugu fish (*Takifugu rubripes*), and 1 in medaka fish (*Oryzias latipes*); 3 of the 7 T2R genes in zebrafish and the T2R gene in medaka fish were newly identified. Some teleost T2Rs were organized as clusters in the genome as in the case of mammals. Teleost T2R genes were actually expressed in the gustatory tissues. Double-labeling *in situ* hybridization demonstrated that T2R-expressing taste bud cells were distinctly divided into several populations expressing different sets of T2R genes in zebrafish and puffer fish unlike in mammals. The T2R genes in

the same cluster were coexpressed, and those in different clusters were expressed in different populations. These results strongly suggest that teleost fish can discriminate the taste modalities of various bitter compounds and/or T2R agonists. Acknowledgements: The Nissin Seifun Foundation

147 Changes in the Expression of Taste Receptor Genes in the Rat Circumvallate Papillae Caused by Zinc Deficiency

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Objectives: Zinc deficiency has been shown to cause dysgeusia, although the mechanism for developing dysgeusia due to zinc deficiency is unknown. In this study, we created zinc-deficient rats, and investigated changes in expression of taste receptor genes of the circumvallate papillae of the tongue. Subjects & Methods: SD rats were used in this study. As a control group (n=8), rats were raised on a normal diet. As a zinc-deficient group (n=15), rats were raised on a zinc-deficient diet (zinc content less than 0.06 mg / 100 g body weight). They were divided into 4 groups: 28 days of zinc-deficient diet (n=4), 56 days (n=4), 61 days (n=4), and 70 days (n=3). The animals were sacrificed to obtain tissue samples. The area of the circumvallate papillae was detached and subjected to RT-PCR and electrophoresis to examine the taste receptor gene expression. In this study, we examined changes in the expression of the rat taste receptor genes ; T2R4, 6, 40, 105, 118, 121, 136, and 140. Results and Conclusions: In the normal control rats, the expression of T2R4, 105, 118, 121, and 136 was observed in 100% of the rats, the expression of T2R40 and 140 was observed in more than half of the rats, and the expression of T2R6 was not observed in any of the rats. In the zinc-deficient rats, expression was significantly decreased for T2R4, 40, and 136, and the decrease of T2R4 was most remarkable. These results suggest that the expression of these taste receptor genes are affected by the zinc deficiency. When polaprezinc was administered to the zinc-deficient rats, an increase of expression (improvement) was observed in T2R4, 40, and 136, which had previously shown a decreased expression. These results seem to be suggesting that they are taste receptor genes linked to zinc.

148 Expression of vesicular glutamate transporters 1 in chemically defined cell populations in the rat lingual fungiform papillae

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In the central nervous system, glutamate is the major excitatory neurotransmitter involved in many neuronal processes such as neuronal plasticity or pain. In the rodent lingual gustatory papillae, vesicular glutamate transporter 1 (VGlut 1) (Braud et al. 2010), baso-lateral glutamate non-NMDA receptors on taste bud cells (Caicedo et al. 2000) and glial glutamate-aspartate transporters (GLAST) (Lawton et al. 2000) have been observed. However little is known about glutamate involvement in signaling pathways within taste buds. In order to identify glutamatergic cells within gustatory papillae, we examined immunohistochemically the expression of VGlut 1 in chemically defined cell population in rat fungiform papillae. Using chorda tympani (CT) and lingual nerve (LN) neuronal tracings, neuronal (PGP9.5), epithelial (keratin 19, *Ulex Europaeus* lectin) and glial (S100 protein) markers coupled to VGlut 1 labelling, we demonstrated that a subset of nerve endings, CT and LN, release glutamate whereas neither epithelial nor glial intragemmal cells seem to be glutamatergic. Taken together, these data suggest that glutamate might be involved in efferent signaling pathways within taste buds Refs: Braud a, Boucher y, Zerari-Mailly f. vesicular glutamate transporters localization in the rat lingual papillae. neuroreport. 2010, 6;21(1):64-7. Caicedo A, Jafri MS, Roper SD in situ ca2+ imaging reveals neurotransmitter receptors for glutamate in taste receptor cells. j neurosci. 2000 nov 1;20(21):7978-85. Lawton DM, Furness DN, Lindemann B, Hackney CM. Localization of the glutamate-aspartate transporter, GLAST, in rat taste buds. Eur J Neurosci. 2000 12(9):3163-71

149 Identifying trigeminal stimulants of TRPA1

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At high concentrations most volatile compounds activate the trigeminal nerve; however, the mechanism for activation is not completely understood for the majority of stimuli. Irritants often stimulate the nerve through TRP channels – particularly TRPA1. Over 90 compounds activate TRPA1 making it an excellent candidate receptor for stimuli working through unknown mechanisms. This project determined whether 4 compounds (amyl acetate: AA, α-terpineol: TE, benzaldehyde: BZ, toluene: TO) activate TRPA1. Intracellular calcium assays previously identified AA, BZ, TO and possibly TE as TRPA1 agonists. In the current experiments, behavioral and physiological assays were conducted using transgenic mice to determine if the whole animal requires TRPA1 for detecting these stimuli. The responses of TRPA1 KO, TRPV1 KO, and wild-type mice to the 4 trigeminal stimuli plus a TRPA1 positive control, allyl isothiocyante (AITC) were evaluated in a cotton-swab behavioral aversion assay. TRPA1 KOs, but not TRPV1 KOs, showed decreased aversion to AITC, TE, AA, and BZ compared to wild-type mice. There was no difference in responses of TRPA1 or TRPV1 KOs to TO compared to wild-type mice - possibly due to an olfactory aversion. To determine whether the identified TRPA1 agonists might activate the trigeminal nerve through receptors other than TRPA1, the respiration of wild-type and TRPA1 KO mice was monitored when stimuli were introduced through an intranasal drip. Exposure to TE, AA, and BZ stopped breathing in both wild-type and TRPA1 KOs. Exposure to AITC and TO stopped breathing in wild-type mice but not TRPA1 KOs. These experiments suggest TE, AA, and BZ activate the trigeminal nerve through multiple receptors including TRPA1. TO appears to activate the trigeminal nerve primarily through TRPA1.

150 TRPM5-Expressing Solitary Chemosensory Cells of Mouse Vomeronasal Organ: Regulation of Chemical Access.

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The mouse vomeronasal organ (VNO) primarily detects pheromones and other semiochemicals. Complex stimuli are drawn into the lumen

of the VNO, some of which may be contaminated by irritating and harmful environmental substances. In the VNO, the majority of the transient receptor potential channel M5 (TRPM5) expressing solitary chemosensory cells (SCCs) 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) and our recent study indicated the importance of the phospholipase C (PLC) pathway in SCC-mediated chemical responses (Ogura et al AChemS abstract 2010). Here we used a recently developed dye assay (Krosnowski et al AChemS abstract 2009) to determine the role of the TRPM5 ion channel and the PLC pathway in monitoring and regulating chemical access to the VNOs. TRPM5KO resulted in a significant increase in the amount of several bitter substances drawn into the VNOs. Local application of TRPM5 inhibitor triphenylphosphine oxide to the VNOs reduced the ability of wild type mice to limit bitter chemical access to the VNOs, mimicking the effect of TRPM5 knockout. Pharmacological inhibition of TRPM5 had no significant effect on the access of non-bitter chemicals in WT mice and bitter substances in TRPM5KO mice. Local application of PLC inhibitor U73122 to the VNOs also increased the access of bitter and odorous chemicals to the VNOs. These results indicate that the PLC pathway and TRPM5 expressed in SCCs in the VNOs are involved in monitoring and regulating chemical access to the VNOs. Acknowledgements: Supported by NIH/NIDCD DC009269 and UMBC startup fund to WL

151 TRPM5 and ChAT-expressing solitary chemosensory cells of mouse vomeronasal organ: anatomical and functional imaging studies.

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Sensory detection of semiochemicals in the vomeronasal organ (VNO) requires drawing in external stimulus fluids. It is not known whether the access of stimulus fluids to the organ is monitored to prevent potential chemical insults by irritating and harmful chemicals in the environment. We previously reported that TRPM5-expressing solitary chemosensory cells (SCCs) are densely populated in the vomeronasal entrance duct. These SCCs are innervated by trigeminal fibers and respond to irritants (Ogura et al. ISOT 2008; Lin et al. SFN meeting, 2008). Here we determined the percentage of trigeminal fibers innervating SCCs, the response profile, and signal transduction mechanisms in SCCs using immunocytochemical and Ca^{2+} imaging methods. The SCCs of VNOs express both TRPM5 and choline acetyltransferase (ChAT). We utilized GFP in TRPM5-GFP and ChAT(BAC)-eGFP mice to identify SCCs. Surprisingly, we found that at the entrance duct, almost all the trigeminal substance-P positive nerve fibers closely appose SCCs. A variety of stimuli including odorous chemicals at high concentrations and bitter substances induced increases in intracellular Ca^{2+} levels. Further, the Ca^{2+} responses were suppressed significantly by phospholipase C (PLC) inhibitor U73122, but not by the inactive analogue U73343, indicating involvement of the PLC pathway and Ca^{2+} release from the internal stores. The data suggest that the SCCs detect irritating chemicals in the stimulus fluids destined to VNOs. Acknowledgements: Supported by NIH/NIDCD DC009269 and UMBC Startup fund to WL.

152 Growth and Differentiation of Solitary Chemosensory Cells in Tracheal Epithelial Culture

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Solitary chemosensory cells (SCCs) are a population of specialized epithelial cells which were first identified in the epidermis of fish, but have since been demonstrated in both the upper and lower respiratory tract of mammals. SCCs express elements of the canonical taste transduction pathway including TRPM5, gustducin and "bitter" (T2R) receptors. SCCs respond to a variety of irritating compounds, including bitter substances and bacterial metabolites. Nasal and tracheal SCCs may represent two distinct cells types which share several important molecular and morphological features. Nasal SCCs are renewed, like the surrounding epithelium and taste receptor cells, but unlike taste receptor cells their differentiation is not dependent on neural innervation. In the present study, mouse tracheal epithelial cells (MTEC) were cultured to test whether tracheal SCCs can develop and differentiate *in vitro*. MTECs were recovered from either wild type (C57Bl/6J) or TRPM5-GFP mice and allowed to proliferate on Transwell membranes until confluent. Differentiation was initiated by removal of growth factors and establishment of an air-liquid interface (ALI day 0). Scattered TRPM5-GFP and Gustducin-immunoreactive cells were present in culture as early as ALI day 1 and increased in number over time. The majority of SCCs present in culture coexpressed TRPM5 and Gustducin and were triangular or bipolar, i.e. morphologically similar to SCCs *in vivo*. These results demonstrate that for tracheal SCCs differentiation is independent of innervation and provide an *in vitro* system for studying SCC determination. Acknowledgements: Supported by NIDCD & NHLBI

153 Subpopulations of trigeminal ganglion neurons are depolarized by GABA

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Sensory neurons of the peripheral nervous system, namely the dorsal root ganglia (DRG) and trigeminal ganglia (TG) are capable of temperature sensation, nociception and mechanosensation, as well as the detection of chemical compounds. Within the DRG sensory system, primary afferent depolarization (PAD) has been shown to be a mechanism critical for contrast enhancement and the modulation of sensory thresholds. PAD acts on presynaptic terminals of the afferent neurons which are high in intracellular chloride and thus depolarized following $GABA_A$ receptor activation. Despite considerable effort undertaken to promote our understanding of PAD and its function in the DRG, it is still unclear, if trigeminal sensory neurons also underly this mechanism. Here, we examine whether GABA-induced depolarization occurs in isolated TG neurons of mice at different developmental stages and with respect to their sensitivity to chemicals. In calcium-imaging experiments 49%, 71% and 88% of embryonal, early postnatal and adult TG neurons display calcium transients upon GABA stimulation indicating their depolarization. This GABA-induced depolarization

of TG neurons requires functional sodium-potassium-2-chloride cotransporters (NKCC1) and is independent of culturing conditions. Of each TG neuron subpopulation sensitive to either capsaicin, menthol or mustard oil a fraction of about 40% is depolarized by GABA at adult age. Based on these findings, we propose that PAD does occur in the trigeminal sensory system. As the proportion of neurons depolarized by GABA decreases with animal age, PAD appears to undergo fine-tuning during postnatal development. Furthermore, only a proportion of each sensory subpopulation seems to be submised to PAD suggesting two parallel paths of information processing on the brainstem level.

154 First and second-order trigeminal sensory neurons respond to two novel cooling componds that modulate lingual thermosensitivity.

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Oral somatosensory stimuli are transmitted primarily via the trigeminal nerve. Single trigeminal afferents can respond to agonists of cold-sensitive thermosensitive TRP channels TRPM8 (menthol) and/or TRPA1 (cinnamaldehyde=CA). We presently investigated if two novel cooling compounds (GIV 1 and GIV 2) excite menthol- and/or CA-sensitive trigeminal primary sensory neurons, using ratiometric calcium imaging of cultured trigeminal ganglion (TG) and dorsal root ganglion (DRG) cells, and if they modulate thermally-evoked responses of cold-sensitive second-order neurons in subnucleus caudalis (Vc) using in vivo single-unit recordings in rats. Effects of cooling agents on TRPM8 or TRPA1 were also investigated in cultured mammalian cells using modified baculoviruses. Compound GIV1 robustly activated TRPM8 (EC50 of ~400 nM) with some activation of TRPA1 at much higher concentrations. GIV1 (100 μM) directly activated 7% of TG and 11% of DRG cells. Approximately 80% of cells activated by GIV 1 were also activated by menthol compared to only 23% for CA and 29% for capsaicin. Lingual application of GIV 1 did not directly excite Vc neurons but significantly enhanced their responses to cooling 20-min post-application and briefly attenuated responses to noxious heat. GIV 2 appeared selective for TRPM8 and excited 62% of menthol-sensitive Vc neurons. Finally, in human sensory trials using toothpaste, GIV1 (0.16 mM) evoked a cooling sensation perceived as less intense but of longer duration (90 min) than that evoked by GIV 2 (0.17 mM, 45 min). Moreover, GIV1 elicited a pungent sensation that was perceived as stronger than that elicited by GIV 2. These novel cooling compounds provide additional molecular tools to investigate the neural processes of cold sensation. Acknowledgements: NIH and Givaudan Flavors Corp.

155 Tingle sensation by a sanshool derivative and its effects on primary sensory neurons

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Szechuan peppers are a preferred spice in some cuisines because of their tingling-numbing and cooling sensations. The tingle agent OH-α-sanshool activates a subset of sensory DRG neurons by inhibiting 2-pore potassium channels. We presently investigated the ability of a sanshool analog, isobutylalkenyl amide (IBA), to elicit tingle sensation in humans and to excite primary sensory neurons from rats. Using calcium imaging, IBA excited ~40% of cultured rat dorsal root ganglion (DRG) neurons of different sizes. Of IBA-sensitive cells, 30% also responded to menthol and/or cinnamaldehyde (CA) and 66% to capsaicin (CAP), with many responding to multiple TRP agonists. There was significant self-desensitization to repeated application of IBA. CAP did not cross-desensitize responses to IBA in CAP-insensitive DRG cells. A modified time intensity procedure was used in human studies to assess if lingual IBA (0.5%) evokes temporally distinct tingling, pungent or cold sensations. IBA elicited a sensation initially described as tingling and pungent, but after approximately 15 min, as evoking a cooling sensation. Similarly, using a half-tongue, 2-AFC methodology, pre-treatment with CAP (10 ppm) or mustard oil (MO; 0.125%) did not cross-desensitize the tingle sensation evoked by subsequent IBA application. The cellular responses elicited by IBA are remarkably similar to the sensations observed in human psychophysical trials. The ability of IBA to excite menthol- and CAP-sensitive DRG cells is consistent with sensory qualities (tingle, pungency, cool) elicited by IBA. The lack of cross-desensitization by CAP or MO suggests that separate populations of IBA-sensitive cells are likely involved in conveying sensations of pungency and tingle. Acknowledgements: NIH DE-013685, AR-057194

156 Ni^{2+}-Ions directly activate transient receptor potential V1

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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 ganglia, 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 potentially harmful 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 whole-cell voltage-clamp recordings, we observed that low millimolar doses of $NiSO_4$ induced non-specific cation-currents in cultured capsaicin-sensitive trigeminal neurons of mice. Furthermore $NiSO_4$ led to an activation of recombinant rat and human TRPV1 heterologously expressed in HEK293 and CHO-cells. Usage of a voltage step protocol revealed a strong outward rectification of these currents. Application of $NiSO_4$ to the cytoplasmic face of the membrane failed to induce any currents. However, when delivered to the extracellular face of the membrane $NiSO_4$ induced an increase in the channels open

probability paralleled by a decrease in the channels conductance. Both resulted in an increased net activity of TRPV1. In this context we identified three amino acids localized in the channels pore region, which are involved in the channels interaction with Ni^{2+}. When combined with other TRPV1 agonists, $NiSO_4$ produces a bimodal effect on TRPV1 activity which depends on the strength and concentration of the second stimulus. Outward-currents induced by low doses of capsaicin, higher temperatures (30°C – 40°C) and nearly neutral pH values (~pH = 7.0 – 6.5) were augmented by low doses of $NiSO_4$. In contrast, responses to stronger stimuli were reduced by $NiSO_4$.

157 Chloride HOMEOSTASIS IN TRIGEMINAL SENSORY NEURONS

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The trigeminal system is known to play an important role in chemo- and thermosensation as well as the perception of pain. Recent studies have shown a connection between intracellular chloride accumulation and pain perception by neurons of the dorsal root ganglia (DRG), but little is known about the chloride homeostasis in TG neurons. The intracellular chloride concentration is mainly controlled by cation-coupled chloride cotransporters. The $Na^+K^+2Cl^-$ cotransporter NKCC1 accumulates Cl^- intracellularly and is highly expressed in embryonal neurons of the central nervous system. During late embryonal to early postnatal development NKCC1 is downregulated accompanied by an upregulation of Cl^--extruding cotransporters. Due to this "chloride switch" opening of Cl^- conductances leads to hyperpolarization of adult central neurons. However, some peripheral neurons like olfactory sensory neurons and neurons of the DRG maintain high intracellular Cl^- levels even in adulthood resulting in cellular depolarization after opening of Cl^- conductances. Here, we show that isolated neurons of wild type (WT) mice display robust Ca^{2+} transients upon GABA stimulation in Ca^{2+}-imaging experiments. In neurons of $NKCC1^{-/-}$ mice, however, these responses are dramatically diminished with respect to the number of responsive cells and signal amplitude. Furthermore, we use the chloride imaging technique to investigate changes of intracellular Cl^- levels upon GABA stimulation of TG neurons of WT and $NKCC1^{-/-}$ mice. Additionally, we determine the intracellular Cl^- concentration of neurons of both WT and $NKCC1^{-/-}$ mice using the double-ionophor technique. We conclude that NKCC1 is the main Cl^- accumulating transporter in TG neurons. Further investigations aim at clarifying the role of NKCC1 in connection with trigeminal pain perception.

158 Pain Processing Networks Revealed Using Fully Exploratory Analysis: An FMRI Study Using Trigeminal Stimulation

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Fully Exploratory Network Independent Component Analysis (FENICA) on functional MRI data is based on the assumption that group networks develop on the basis of spatially consistent single-subject components. The analyzing method introduced in this study includes two different processing stages. First Independent Component Analysis (ICA) on a single-subject level is conducted and the second step includes analysis based cross-correlating the derived single-subject components. The most important aspect of this exploratory method without the need for a priori definition of the applied stimulus time course or the specification of a template including expected regions of interest is the single-subject character the method is based on. To test this new method a trigeminal stimulation experiment was performed. It is known that the processing of intranasal CO2 stimuli evokes specific activation involving a part of the general pain processing network. Functional images were obtained from 22 healthy volunteers using a 3T MRI scanner. We used an intranasal CO2 event-related birhinal stimulation paradigm. Image preprocessing was performed using SPM5. For further artefact corrections two regions of interests (white matter and ventricles) were defined and time courses were extracted and regressed out for each single-subject. Single-subject ICA was performed using probabilistic ICA as implemented in FSL. Group analysis with FENICA revealed areas known to be specifically activated for the processing of intranasal trigeminal stimulation and was able to clearly obtain the network involved in the processing of olfactory trigeminal stimulus processing. We can conclude that FENICA provides a truly exploratory, data-driven, operator independent and therefore unbiased way of assessing trigeminal networks.

159 Real-time PCR of trigeminal receptor mRNAs in human nasal biopsies

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Background: Previous research suggests that chemosensory stimuli mediated by branches of the trigeminal nerve are perceived differentially dependent on the location within the nasal cavity. Aim: The aim of this study was to acquire data on the occurrence of various trigeminal receptor mRNAs in different locations of the nasal mucosa using real-time PCR. Subjects & Methods: Biopsies of 12 healthy individuals (mean age: 37.8 years) were taken from the insertions of the middle and inferior turbinates, as well as anterior ventral and posterior dorsal septum. Real time-PCR was performed using primers for TRPA1 (ankyrin-like receptor with transmembrane domain I), ACCN3 (acid-sensing ion

channel), TRPV1 (transient receptor potential vanilloid receptor 1), TRPM8 (transient receptor potential receptor M8), and CALCB1 (calcitonin gene-related product). As housekeeping gene, HMBS was used. All primers were positively tested in a human trigeminal ganglion. Results: Strongest expression of all receptor genes was shown for TRPV1 and ACCN3, especially in septal regions of male individuals, TRPM8 was not detected. TRPA1 mRNA was more strongly expressed in anterior septal areas of female subjects in comparison to males. Younger subjects presented stronger receptor mRNA expressions than older ones. Conclusions: Present data of this small group tend to show a stronger expression of TRPV1 and ACCN3 in anterior septal areas of the nasal cavity compared to posterior and lateral locations. Supporting previous electrophysiological data, the results fit to guarding functions of the trigeminal system at anterior entry sites of the respiratory tract. Acknowledgements: Supported by Philip Morris USA Inc. to TH and MW

POSTER SESSION III: OLFACTORY PERCEPTION, HUMAN PSYCHOPHYSICS & ANIMAL BEHAVIOR; PERIPHERAL TASTE DEVELOPMENT & SIGNALING

160 Determinants of Measured Olfactory Sensitivity: Reprise

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An extensive literature outside chemoreception has long indicated that human subjects increase their measured acuity with repeated testing or training. Although slow to develop such information, chemosensory psychophysics has directed some attention to understanding sequential effects in testing. The scattered studies, not always focused on the issue but sometimes revealing it incidentally, show a consistent pattern: More testing yields lower thresholds. This holds for smell or taste or flavor. Thresholds measured over time decline exponentially over days and can reach a gain of an order of magnitude. One study implied gain within a day. That study, like the other olfactory studies, used a solvent to dilute the odorant and it seemed possible that its odor interfered with detection of the signal, though less effectively over trials. Those studies also used squeeze bottles that added another possible parameter for inadvertent increase in facility. In the present experiments we worked without a solvent and afforded the subjects a very friendly interface that required only sniffs from glass cones. In the course of a day of testing (> 6 hr) subjects (n=55) made 240 judgments in a 3 AFC task. Testing involved four odorants on different occasions. Even in these easier circumstances, performance increased within a day of testing, with subjects about 20-25% more sensitive at the end than at the beginning. Preliminary evidence suggests that the effect appears to repeat itself beyond the first day. The perceptual learning evident in all of the relevant chemosensory results occurs without training, but with mere experience. The addition of training may amplify it, which would indicate further that the ''typically'' measured threshold underestimates human physiological sensitivity. Acknowledgements: Supported by NIH grants DC05602 and DC002741.

161 The relationship between nasal cycle and cognitive processing.

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INTRODUCTION Many people experience a periodic fluctuation of relative nasal resistance between the two nostrils – the so-called nasal cycle. Some, but not all, studies have suggested that this alternation, which need not be totally reciprocal and reflects relative sympathetic:parasympathic tone, is related to cognitive function. In this study we tested this hypothesis using a simple laterality task. METHODS In large groups, 72 college-aged students (26 male) determined the side of the nose that was most open by alternately closing each naris with their thumb, and rated their confidence of this judgment. Participants then completed two paper-and-pencil tests (Klein & Armitage, 1979) in which two visual items were judged as ''same'' or ''different'': a ''language'' (left hemisphere) task comprised of pairs of letters (one upper and one lower case letter) and a ''spatial'' (right hemisphere) task made up of pairs of dot patterns. Participants were asked to judge as many pairs as possible, avoiding errors, in a 3 minutes (per task) interval. RESULTS A two-way ANOVA indicated a main effect of task (performance was significantly higher on the language than the spatial task) but not of nasal resistance. The interaction of task and nasal resistance was significant. Participants performed significantly better on the language than the spatial task when the left side of the nose was more open. No gender differences were observed. CONCLUSION These results accord well with reports that lateralization of function in the human brain is related to the relative left:right airflow patterns observed in the nose. Additional research is needed to determine whether associations observed in this study generalize to more complex cognitive processes. Acknowledgements: None.

162 Characterizing the Relationship between Naming and Recognition Memory for Odors and Sounds

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Over the past few decades, the issue of whether olfactory memory represents a separate memory system compared to other sensory modalities has been a topic of much research. As part of our efforts to better understand the nature of odor memory, we have developed a dual odor naming/recognition memory task, and in a recent series of experiments, we have demonstrated a very close relationship between semantic and episodic odor memory. The basic finding in these studies is that consistently and/or correctly named odors are associated with excellent recognition memory while memory is poor or non-existent for odors named inconsistently and/or incorrectly. The aim of the current study was to determine if a similar relationship between naming and memory would be observed with auditory stimuli. The auditory stimuli were computer-generated musical notes associated with a variety of musical instruments. Participants named the stimuli during the first phase of the test, and then, following a 10 minute retention interval, named the stimuli

again and were asked whether they had experienced each stimulus during the initial phase of the test. During the second phase of testing, half the stimuli were from the first phase, and half were new. Some participants were provided with four possible names for each stimulus as they named them, while others were required to generate their own names. The results were remarkably similar to those found previously for odor and flavor stimuli, that is, consistent and correct naming was highly predictive of recognition memory performance. We conclude that similar processes are involved in naming and remembering of the olfactory and auditory stimuli, and we speculate that object identification processes played a critical role in the naming-memory relationship.

163 The Effect of Odor Naming Feedback on Odor Naming And Recognition Memory

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The current study examined the effect of corrective odor naming feedback on subsequent odor naming and recognition memory performance. It has been established that feedback can improve odor naming performance. The current study examined the relationship between odor naming and recognition memory with and without naming feedback to determine if the feedback-mediated improvement in naming was accompanied by an improvement in memory. Participants were separated into two groups; a feedback group and a control group. The performance of these two groups on an odor naming and odor recognition memory task was then compared. Results showed that odor naming and recognition memory performance improved as a result of corrective odor naming feedback. As expected, those who received feedback scored higher in naming and memory compared to those without feedback. More importantly, when corrective feedback was effective (changing incorrect naming to correct naming), memory performance approached 100%. When naming feedback was ineffective, no evidence for memory was observed. The results provide compelling evidence for a strong relationship between odor naming and recognition memory. We propose that when it was effective, the feedback allowed participants to access odor knowledge that was then used to support both odor naming and episodic odor memory.

164 Smell and Prejudice: Affect influences on olfactory threshold

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Objectives of the study: To delineate the effect mood has on olfactory threshold. **Methods :** Five male and five female subjectively normosmic non-smokers, between ages 20 to 55,without known psychiatric disorders, odor sensitivities, or allergies, on no psychotropic medications, were recruited for this IRB approved study. Each subject was shown three randomly presented mood altering 10 minute audiovisual clips in a standardized method as per Chen and McClintock. After each tape the impact on mood was delineated by using the Ottawa-Georgia Mood Scale and olfactory thresholds were mapped as per the recommendations of the phenylethyl alcohol(PEA) smell threshold test of Doty. **Results:** All subjects underwent expected mood changes in response to the video segments. Average threshold in the positive, negative and neutral states were -5.4,-6.1 and -5.3 respectively with all values falling between-2 to -9. Of all participants, 30%, 20% and 60% showed greatest threshold in the positive, negative and neutral states respectively. Results were analyzed comparing positive affect vs. all conditions, negative affect vs. all conditions and both positive and negative affects vs. neutral conditions. The statistical significance was determined for all subjects, both sexes and orders of presentation, by using the paired T-test and Signed –Rank test for non parametric conditions. Analysis revealed no significant results ($p < 0.5$) under any of the derived conditions.

Conclusions: The null hypothesis was verified and no effect of mood on olfactory threshold was observed.

Sources of funding: none

165 Effects of odor on time perception

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OBJECTIVE/BACKGROUND: To extend T. Lorig's (1990) work and determine if odors of different hedonic valences would impact upon estimation of time. **METHODS:** Twenty nonsmokers (19 males, 1 female), average age – 34 years (range 28-43), without asthma, unusual smell sensitivity, or pregnant, were recruited into this IRB approved study. All perceived they had normal sense of smell. All scored 3/3 on Quick Smell Identification Test. In a single blind fashion, all individuals underwent three one minute epochs of time estimation while wearing 3M Aseptex molded surgical masks impregnated with Custom Essence Columbian Coffee aroma, International Favors and Fragrances Baby Powder aroma, or no aroma. Subjects were randomly assigned as to the order of masks presentations. After placement of mask, a 10 sec interval elapsed before timing began. Subjects were left alone in a room, without time keeping devices, and indicated when they perceived 60 sec had elapsed. Repeated measures analysis of variance was used to test for differences in time perception using significance criteria $p<.05$. **RESULTS:** Perception of 60 sec was significantly different across the three exposure groups ($p<.0001$). Columbian coffee aroma reduced estimated duration of 60 Sec by 8.6 sec ($p = .01$). Alternatively baby powder increased estimated duration of 60 sec by 34.3 sec ($p <.0001$). Eighteen (90%) of subjects reported positive hedonics toward coffee, and 100% described familiarity. No one had positive hedonics toward baby powder although eighteen (90%) found it to be familiar. Subanalyses excluding those who were unfamiliar with baby powder or those who disliked coffee was also performed (n=16). In this subgroup, coffee decreased perceived perception of time by 9.4 sec ($p <.02$) and baby powder increased time perception by 32.9 sec ($p<.0001$). **CONCLUSION:** These results have potential implication in situation associated with desire for shorter perceived duration of time, as in conditions of acute pain, or in situations where perceived prolongation of time would be desired i.e. when enjoying hedonically positive sensory experiences. **SOURCE OF FUNDING:** None.

166 Odor-related Affective Feelings: Structure and Inter-individual Variability.

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The Geneva Emotion and Odor Scale (GEOS, Chrea et al. Chem. Senses 34: 49–62, 2009) has been developed to provide a comprehensive approach of the wide range of affective feelings elicited by everyday odors. The final scale was obtained by a 3-step reduction of 480 affective terms taken from the literature, rated by 529 participants for their relevance to describe affective states triggered by 68 contrasted odorants. The same procedure has been used to develop a similar scale in England (Liverpool, N = 540 participants) and in Singapore (N = 354). Familiarity, intensity, pleasantness and identification answers were also collected. A model with 36 to 37 affective terms structured in 6 to 7 dimensions explained the major part of the relevant affective feelings in response to odors in the three countries. A comparison between the three cultures showed that, on the one hand, some dimensions were invariant: Sensuality/Desire, Disgust, Energy, Happiness/Well-being and (only in Europe) Soothing/Peacefulness. On the other hand, culture-specific dimensions emerged, such as a Spirituality dimension in Singapore, that was associated to the odors of incense and its ingredients and that certainly reflects the widespread religious use of incense in the Chinese community of Singapore (79% of our sample). Sex and age were investigated as factors of inter-individual variations: Women scored higher than men on the Disgust dimension and on the odor identification task, and affective ratings decreased with age, especially on the Disgust dimension. Finally, familiarity and pleasantness of the odors were highly correlated and associated to higher positive affective responses, whereas identification was positively linked to only some affective dimensions (including culture-specific dimensions). Acknowledgements: Firmenich SA

167 THE EFFECT OF TWO AMBIENT AROMAS ON HUMAN PHYSIOLOGY AND FOOD CHOICE

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Ambient aromas are supposed to affect human functioning but scientific evidence is relatively scarce. The present study verified whether 1-hr exposures to barely detectable concentrations of vanillin or a citrus aroma affected food choice, mood and/or physiological functioning of 22 human participants. A single-blind randomised controlled trial with within-subject design was conducted. Once a week, participants performed tests in one of three similar rooms filled with either the test aroma or with an odourless control, during which they had free access to snacks that matched one of the aromas (cookies, cheese, and fruit). Participants were equipped with physiological sensors that measured Galvanic Skin Response (GSR), heat flux, activity (Sensewear system, BodyMedia Inc.) and heart rate (Polar). Only the physiological and food choice results will be reported here. The results showed significant selective effects of ambient aroma on food choice with highest cheese consumption in the odourless condition and highest mandarin consumption in the citrus condition. Average heart rate was lowest during exposure to vanillin and highest during exposure to the citrus aroma. Exposure to citrus aroma also increased energy expenditure and heat flux compared to exposure to vanillin. In summary, the results demonstrated measurable effects of ambient aromas on human physiology and food choice. Follow-up studies will include studies with ambient aromas in the real-life setting of an instrument lunch restaurant. Acknowledgements: Inside Consumer Experience, an innovation project funded by Food & Nutrition Delta.

168 Perfume Masculinity/Femininity Affects Face Gender Judgments

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The human face provides a great deal of information that is valuable for social situations. When information from a face is ambiguous, however, people look to the immediate environmental context for cues, including odors, that may help to clarify the situation. Biologically relevant odors can influence decisions about gender (Kovacs et al, 2004; Zhou & Chen, 2009) and odor valence can influence social preferences (Li et al, 2007). The present experiment asked whether the presence of commercially available perfumes (both marketed and evaluated for masculinity/femininity) would provide information that might influence the classification of gender in faces that were either gender distinct (male or female), or ambiguous. Male participants were divided into two groups: one of which sniffed a masculine perfume (*Caesars Man*, Caesars) while classifying two 8-trial blocks of faces as "male" or "female" as quickly and accurately as possible, and the other of which sniffed a feminine perfume (*Shania*, Stetson) while performing the same activity. Results showed that although the frequency of classification of ambiguous faces was not influenced by the presence of perfume, men were slower to classify an ambiguous face in a way that was inconsistent with the gender denoted by the perfume, $t(16)=2.55$, $p=0.03$. Although a ceiling effect was present for the classification of the gender distinct faces, errors tended to be made with faces that were inconsistent with the perfume that participants were given, $t(17)=2.05$, $p=0.03$, one-tailed. These results suggest that the masculinity/femininity of a fine fragrance influences decisions regarding gender, and that perfume acts as a relevant cue in guiding social interactions.

169 Olfactory Brown

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In audition, a sound that contains every frequency within the range of human hearing, each at equal amplitude, is perceived as a hum

termed ''white noise''. In vision, a mixture that combines all wavelengths in the visible spectrum at equal energy is perceived as ''white'', and a mixture of primary paint colors is perceived as ''brown''. In fact, a careful mixture of just three primary paints, red; blue; and yellow, is sufficient to generate a brown. Here we set out to ask whether we could similarly generate an ''olfactory brown''. We took advantage of the recent development of an olfactory perceptual metric space from which we could select odorants that span perception. Critically, we first diluted those odorants to equal intensity. We then found that discrimination accuracy between one 28-component mixture and another 28-component mixture was equal to discrimination accuracy of a 14-component mixture from the 28-component mixture. More critically, discrimination difficulty, and hence similarity, between the 14-component and 28-component mixture was not different from that of one 28-component mixture and another. These results suggest that mixtures containing large numbers of different odors that span perceptual olfactory space may generate a similar percept: olfactory brown. These results reflect an initial exploration of olfactory perceptual space. Due to the mammoth effort of equating intensity and the endless combinatorial possibilities in exploring this question, the current results should be considered a pilot effort only. That said, this pilot suggests that mixtures containing large numbers of different odorants that span perceptual olfactory space, and that critically were equated for intensity, may generate a similar percept: olfactory brown.

170 Influence of Odor Pleasantness on Perceived Intensity in Binary Mixtures

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It has been reported that human subjects tend to estimate the intensity of unpleasant odor stronger than that of pleasant odor. This result suggests odor intensity could vary according to perceived pleasantness as well as stimulus concentration, which is obviously related to intensity. On the basis of these studies, we hypothesized that if odor pleasantness is improved, this will cause the reduction of perceived intensity. To address this issue, we evaluated odor intensity and pleasantness of binary mixtures, of which pleasant odor was gradually added under the presence of constant concentration of unpleasant odor. Firstly, we asked 10 human subjects to evaluate odor intensity and pleasantness as a function of concentration, and we chose 2 pleasant (dl-limonene and cycloten) and 2 unpleasant (isovaleric acid and ethanethiol) odors from these results. Then, they consecutively rated odor intensity and pleasantness of the mixture of which dl-limonene was gradually added to the constant concentration of isovaleric acid. Although odor pleasantness was improved as concentration of dl-limonene was increased, the intensity of mixture did not decrease significantly at any concentrations of added dl-limonene. However, there was the particular concentration range of dl-limonene in which the pleasantness of mixture was improved without increasing the intensity. The mixture of cycloten and ethanethiol was also made and the same result was obtained. On the other hand, we could not observe such a particular concentration range in the mixture of 2 unpleasant odors. These results indicate that odor intensity of mixtures would be affected by pleasantness of components, and that unpleasantness of odor can be improved without increasing perceived intensity by adding pleasant odor in a carefully adjusted concentration.

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

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Adaptation refers to a reduced sensitivity to a stimulus as a result of continuous exposure. It is standard to classify olfactory adaptation as short-term versus long-term, with the assumption that brief exposures (seconds to minutes) lead to fast recoveries from the odorant (within minutes) while prolonged exposures (hours to days and weeks) yield slow recoveries (hours to weeks or longer). In the current study, we assessed the olfactory threshold of phenylethyl alcohol (PEA) in five female subjects and that of n-butanol in another five female subjects. Each subject was tested once every four days over a course of two months. Each time, the subjects were exposed to peri-threshold concentrations of an odorant intermittently for approximately half a minute, with at least 30s in between the exposures. We showed a significant monotonic reduction of sensitivity in the course of repetitive testing of both PEA and n-butanol. Our findings demonstrate 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. Acknowledgements: This work was supported by NIH R03DC4956.

172 The relationship between positive odor-evoked memories and product evaluation

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Odors evoke more emotional and evocative autobiographical memories than other sensory modalities. We speculated that if positive emotional memories were evoked by an aromatic product, it would elicit a positive mood and the product would be evaluated more favorably. Thus, the purpose of our study was to explore odors that evoke positive emotional memories and to investigate whether those odors would enhance various product evaluations. In Experiment 1, 54 young (22- 31 years) and 68 older (42- 51 years) US women smelled 17 odors and described the emotions evoked and rated their emotional quality. Next they were asked to describe any autobiographical memories evoked by the odors, and to evaluate its pleasantness, specificity, emotional intensity and evocativeness. Then, they rated the odor itself for: pleasantness, relaxation-stimulation, and likability. Results showed that the pleasantness of autobiographical memories was highly correlated with the quality of emotions evoked and how relaxing and pleasant

the odor was perceived to be. It was further found that of the 17 odors, three most consistently evoked positive memories and one was consistently responded to neutrally. These odors were then selected for further study in Experiment 2. In Experiment 2, 271 US women aged 22- 31 years evaluated and used a lotion scented with 1 of the 4 odors from Experiment 1. Participants first evaluated the odor and reported emotions and memories as in Experiment 1, then they used the lotion for one week in their normal routine. Following the usage period, participants assessed how much they liked the lotion and evaluated various features of lotion quality. Results showed if the odor had evoked an emotional memory the lotion was rated higher on more measures than if the odor was non-evocative.

173 A Compact Multi-functional Olfactometer for fMRI Examinations

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The study of olfaction with fMRI is limited partially due to magnetic susceptibility artifacts in the inferior frontal-temporal olfactory territory and inadequate knowledge of neural activity and subsequent local hemodynamic function (BOLD) in the central olfactory structures. To address the latter issue requires a device that is capable of delivering odorants with a precise synchronization to the subject's respiration pattern. Several MRI compatible olfactometers have been developed for odorant delivery for fMRI. However, few of those devices are capable of monitoring and recording the subject's respiration pattern and subsequently applying that data to the experimental paradigm. The subject's respiratory pattern can modulate the experimental paradigm in terms of timing and odor perception (intensity and threshold), which presents a critical issue for fMRI post-processing and data interpretation. In this design, we present a highly integrated multi-functional olfactometer, developed specifically to overcome the aforementioned problems that are common to olfactory fMRI examination. It delivers up to six different odorants with accurate timing and without any optical, acoustic, thermal, or tactile cues to the subject. The multi-functional device also monitors and records the subject's respiration pattern, which can be synchronized with the subject's task response in real-time. The olfactory fMRI data quality and acquisition reliability are significantly improved with this olfactometer design. Acknowledgements: The Leader Family Foundation Laboratory for Alzheimer's Disease Research and NIH R01 AG 027771.

174 Behavioral characteristics when smelling odors and making selections

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When people smell several odors, how do they decide which one they prefer? For vision study, participants were shown pairs of human faces and required to decide which face was more attractive. Their gaze gradually shifted toward the face that they eventually chose a few seconds before making the choice. Are there any behavioral characteristics of making selection in olfaction as well? In our study, participants freely smelled seven odors (e.g., grapefruit or wash soap) in squeeze bottles and chose one odor which they liked the most among them. When the odor names were not shown, participants could smell the same odor repeatedly and there was no time limit for the selection. This situation was recorded and the duration time smelling an odor measured. As results, three behavior patterns on the choice of the most favorite odor were observed; 'Swift decision type' that made a decision immediately after smelling every odor just once, 'Stable position type' that chose an odor in fixed sequences without changing the bottles position, and 'Grouping type' that grouped odors according to preference before making a decision. Most of the participants in the later two types occasionally smelled the same odor again. Smelling duration time was converted into the standardization score for each participant. The average score for the odor finally chosen was higher than the odors not chosen (using a mean score for six odors), but the difference was not significant ($t(27)=0.91$, $p=0.37$). Especially, participants in 'Grouping type' smelled longer the preferable odor than in the other two behavior patterns. As a consequence, the odor which they liked most was smelled for a slightly longer time. Conversely, it might be possible that people make selection of the odor when it was smelled longer.

175 Odor Interactions among Ternary Mixtures by Human

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Mixture-interactions are a first-order concern in olfaction. At peri-threshold levels, people can often detect a mixture even if they cannot detect any of the individual mixture components when presented alone. The exact rules of this summation remain unclear. We have measured detection of both single chemicals and binary (two-component) mixtures over a range of concentrations. Significant deviations from additivity occur, and depend on both stimulus concentration and molecular properties. However, a simple response addition model describes peri-threshold mixture interactions reasonably well. Here, we extend this work to ternary (three-component) mixtures. We measured detection functions for four homologous carboxylic acids (acetic, butyric, hexanoic, octanoic), and for maple lactone, which is different from the acids in both structure and supra-threshold quality. We also measured detection functions for three ternary mixtures: 1) acetic + butyric + hexanoic (greatest overall similarity), 2) acetic + butyric + octanoic, and 3) acetic + butyric + maple lactone (least overall similarity). Analysis of variance showed that mixtures 1 and 3 (the most and least similar) showed approximately additive interactions across the full range of measured concentrations, whereas mixture 2 showed substantial sub-additivity across concentrations. These data suggest that a tendency toward peri-threshold additivity may continue as mixtures become more complex, but that the degree of additivity will depend on the molecules that comprise the mixture. Structure-activity models will require further research.

176 The Monell Odor Identification Task for the NIH Toolbox: Comparing Response Alternatives for 3 and 4 Year Olds

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The National Institutes of Health (NIH) Toolbox aims to develop a brief, inexpensive test to assess olfactory function in individuals between the ages of 3 and 85 years old. Here we provide an update on the evaluation of the youngest cohort of subjects (50, 3-4 year olds) with whom we explored the effects of the number of response choices on odor identification performance. Children and a parent were tested individually at the Monell Center. After familiarization with the study procedures, they were presented with 8 odorants in a scratch-and-sniff format in 3 versions. After smelling each odorant, the subject was asked to identify the odor by pointing to the matching image from either 2, 3 or 4 choices. All subjects were administered the 3 versions of the task in a single session and each version was completed in less than 5 minutes. While we saw an inverse relation between the response options and probability correct, this relationship is driven by the results of individual odor items within each task such that some odors are better for inclusion on a child-specific task than others. It was further noticed that some images used to represent the target odors were difficult to identify for three-year-olds. When performance above chance was considered, the difference between performance on the 3 and 4 alternative forced choice (AFC) tasks diminished and certain variables, like pre-school attendance, increased accuracy, particularly on the 4 AFC task. In conclusion, three- and four-year-old children can quickly and reliably perform all versions of this task, with each task having its own advantages and disadvantages. The removal of less effective odors, adjustment of odor images and further consideration of variables like pre-school attendance will yield a best-fit of task to population. Acknowledgements: NIH Blueprint for Neuroscience Research, NIH contract No.: HHS-N-260-2006-00007-C.

177 Process differences between physical and physiological odor mixtures

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We investigated whether a mixture will be perceived differently if it has been presented as a mixture in one nostril, in comparison to if two odorants are presented simultaneously in separate nostrils. In parallel, we investigated whether the different types of presentation gave rise to differences in event related potentials (ERP). Twenty-four healthy persons, 12 men and12 women, between the ages of 18 to 35 years participated in the study. Using the Sniffin' Sticks threshold test, all participants were screened for threshold differences between both nostrils separately. We excluded those with threshold differences over 2 points between the two nostrils (Gudziol et al, 2006). We compared single odorants A or B monorhinally, mixtures of A and B monorhinally, and simultaneous presentation of A and B birhinally. The odorants used were eugenol (A) and l-carvone (B). The stimuli were presented, using a computer-controlled air-dilution olfactometer (OM6B; Burghart instruments, Wedel, Germany), in a constant flow of odorless and humidified air of controlled temperature (250 ms stimulus duration; 80% relative humidity; total flow of 7 L/min; 36 degrees C). Participants were asked to rate the composition of the stimuli on a visual analogue scale. Hence, they rated to which extent they perceived a single odorant, A or B, or a mixture. The participants also rated the overall intensity of the stimuli. Additionally, ERPs for each type of stimuli were recorded. Analyses will focus on the differences between monorhinal and birhinal mixtures. Hence, both the differences in the ERPs and in the psychophysical ratings will be analyzed. The implications of potential difference between these physical and physiological mixtures for the understanding of mixture processing will be discussed.

178 Rapid yet short-lived olfactory plasticity in wake and in sleep

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Several studies have demonstrated that long-term repetitions of relatively brief olfactory exposures can lead to functional plasticity reflected in reduced (improved) olfactory detection thresholds for the exposed odorant. Here we set out to ask whether we could accelerate this process using a more aggressive exposure paradigm. We measured detection thresholds for the odorant citral (CAS #5392-40-5) using 20 log dilutions (highest concentration set at 3.125 % v/v citral in mineral oil) delivered within the maximum-likelihood adaptive staircase method. An exposure group of 20 female subjects smelled the odorant for 3 continuous minutes every 15 minutes for 20 times summing at one full hour of exposure. In addition, we measured threshold on 3 occasions: in the morning, 7 hours later, and one day later. A control group of 18 female subjects participated in the 3 threshold tests only. We found a significant decrease in thresholds in the exposure group ($M = 11.55 \pm 0.4$ in the first test, $M = 10.8 \pm 0.4$ in the second test, $p > 0.002$) but not in the control group ($M = 10.75 \pm 0.3$ in the first test, $M = 10.02 \pm 0.5$ in the second test, $p = 0.27$). This plasticity, however, was short lived, as thresholds returned to baseline the next day (Exposure group: $M = 11.42 \pm 0.5$ in the third test, $p = 0.66$; Control group: 10.3 ± 0.4 in the third test, $p = 0.3$). That functional plasticity occurs at such short time-frames may help pinpoint the neural substrates of this behavioral change. Finally, we are currently replicating this study during sleep in order to assess the role of conscious perception in the plasticity process.

179 Effect Of Eye Closure On Olfactory Detection Threshold.

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OBJECTIVE: To determine the effect of eye closure on olfactory detection threshold. INTRODUCTION: Part of the evaluation of cranial nerve I is the assessment of olfactory threshold. Smell Threshold Test (STT) of Doty measures olfactory detection threshold (ODT) to phenyl-ethyl alcohol (PEA). Currently, the effect of visual stimuli on olfaction is unclear. METHODS: Ten healthy, subjectively normasmic volunteers (7 women, 3 men), mean age 29 years (range 18-57 year) underwent STT based upon

single staircase-forced choice paradigm. Subjects kept their eyes open and then closed, or vice-versa, as ODT was determined by presenting them with varying concentration of PEA (-10.0 to -2.0 log units) in half log steps. Order of testing alternated after each subject, so that one half had odor testing with eyes closed first, while the other half had testing with eyes open first. A 20 minute washout period existed between the tests in each individual. Each subject served as their own control. RESULT: Mean ODT with eyes open was -3.7 (range -4.6 to -2.2), and with eyes closed was -4.0 (range -6.5 to -2.2). No significant difference was noted ($p>0.05$) in threshold values for eyes open versus eyes closed. CONCLUSION: We had anticipated that the absence of visual stimuli would lower the olfactory detection threshold, possibly due to reduction of competing sensory stimuli, allowing focusing of attention. However, no discernible effect was found. SOURCES OF FUNDING: NONE

180 Newly Discovered Specific Anosmias

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Specific anosmia is an inability to perceive an odor when others can readily detect such although the general sense of smell is intact. To date, specific anosmias for only a handful of odorants have been described. We explored human responses to 10 compounds. Herein, we note that some human subjects could not smell some of the odorants, viz., 3-hydroxy-3-methylhexenoic acid, 2-nonenal, skatole and geosmin, malodorants commonly encountered by people. We determined olfactory detection thresholds for these compounds by using triangle tests (2 blanks versus the target) beginning at the lowest concentration of each (n of human subjects = 99, 177, 174 and 134, respectively). The highest concentrations were 0.1%, 0.01%, 0.01% and 0.067% and 9 subsequent dilutions were 3-, 5-, 3- and 3-fold, respectively. For each triangle test the subject had to choose the odorant-containing plastic squeeze bottle (270 ml capacity containing 10 ml of diluted odorant or diluent [mineral oil]) and provide a confidence rating for the choice (3=certain; 2=possible; 1=guess). Each series was subjected to an ascending method of limits, which terminated when four correct steps were satisfied with a confidence rating summed across the last four trials of at least 7). We defined specific anosmia as reaching the highest concentration without meeting the criteria or at least 3 standard deviations above the mean. The percentage of people with specific anosmias was 14.1%, 6.2%, 2.3% and 6.0% respectively. These data suggest that there may be far more specific anosmias than previously reported: In the 10 selected for in-depth study, 40% could not be detected by a subset of those tested. Perhaps these result from altered or absent gene expression. Acknowledgements: Supported by NIH RO1 DC00298 to CJW and support from TAKASAGO.

181 Early Odor Learning in Tree Swallows (*Tachycineta bicolor*)

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Perching birds (Passerines) have exceedingly small olfactory bulbs and their chemosensory abilities are only minimally studied. We investigated the Tree Swallow (*Tachycineta bicolor*), a common migratory passerine that breeds in North America. We tested: (1) whether swallow chicks could detect odors, and (2) whether exposure to an experimental odor early in life would alter a chick's response. Our field-study involved chicks from two nest types. In experimental nests (n = 15) all chicks were exposed to the scent of peppermint oil during incubation and chick rearing, whereas those in control nests (n = 31) were not. At nine days of age we tested chicks for their reaction to peppermint oil, fox urine (a putative predator scent), and an odorless control using the Porter method (Porter *et al.* 1999). Chicks from both treatments detected test odors relative to the odorless control ($2.28 \leq t \leq 3.39$, $0.004 \leq P \leq 0.031$). Next, we compared the degree with which birds from each treatment discriminated between our two test scents by subtracting a chick's response to mint from its response to fox. Chicks reared in mint odor exhibited values that were nearly twice that of controls ($t = 2.05$, $df = 44$, $P = 0.046$), suggesting that early exposure to mint altered a bird's ability to differentiate between mint and fox odors. Birds in experimental boxes appeared more familiar with mint in that they reacted less strongly to mint than to the unfamiliar odor of fox. By contrast, birds from control boxes reacted similarly to mint and fox, both of which were unfamiliar odors. Our results suggest that chemosensory learning can occur early on in passerine development and we suspect this may be true of other birds. Studies of odor imprinting are a promising future step for avian research. Acknowledgements: An HHMI Undergraduate Summer Research stipend was granted to AAM. MPD was supported by a summer research grant from the Natural Sciences Division of Swarthmore College.

182 Exploring the Olfactory Ability of the Kea (*Nestor notabilis*), an Endangered Parrot from New Zealand

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The Kakapo (*Strigops habroptilis*) is a critically endangered, endemic parrot of New Zealand known for its nocturnal habit and olfactory ability (Hagelin 2004). Most parrots, however, are diurnal and their chemosensory abilities are practically unstudied. The Kea (*Nestor notabilis*) offers a particularly interesting comparison, because this endangered species is a diurnal relative of the Kakapo. We studied two captive Keas at the Philadelphia Zoo to determine whether birds could detect amyl acetate (banana odor). As part of an enrichment regimen birds were given either toy balls of perforated plastic that had been internally treated with scent or identical balls that were unscented. Pilot testing led us to focus on an unusual behavior, ''face rubbing,'' in which a bird wipes its cheeks, nostrils or beak against the outside of balls. Over 60-minute trials the behavior of each bird was scored by an observer blind to the toy treatment. Face-rubbing was more likely to occur when birds played with scented balls (N = 21 trials, Fisher's exact $P = 0.03$). Our result indicates that Keas can detect and react differently to toys treated with banana odor. Scented toys are a practical means of providing base-line information about the chemosenses

for many avian species. We also suggest that this method may be a potentially useful but overlooked means of enrichment for captive birds.

183 Songbird Chemosignaling: Differentiation and Detection of Volatile Compounds by Dark-eyed Juncos

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Although chemical communication is known to play an important role in reproduction in many animal species, little is known about this mode of communication in birds. In previous work (Soini et al 2007, J. Chem. Ecol.) we found that concentrations of volatile compounds in preen gland secretions of Dark-eyed Juncos (*Junco hyemalis*) increased markedly when individuals were moved from short to long days, suggesting a role for these compounds in reproductive behavior. Chemosignals involved in mate recognition and mate assessment are hypothesized to differ 1) among species, 2) among individuals within a species, 3) between sexes and 4) among reproductively isolated populations. We tested these hypotheses in passerines, focusing on captive dark-eyed juncos from two populations: 1) a recently isolated population at the University of California, San Diego campus that has shown rapid evolution of behavioral, morphological, and physiological traits and 2) the assumed ancestral range population at Laguna Mountain. Using gas chromatography - mass spectrometry, we measured volatile organic compound profiles in preen oil and found high individual repeatability, as well as significant differences among individuals, sexes, and populations. We also sampled preen oil from an additional 30 species in 10 families that breed in southern Indiana and found divergence in volatile composition among species. Finally, we conducted behavioral tests and found preliminary evidence suggesting that juncos may be able to discriminate among preen oil odors from different species, sexes, and individuals. Together our data suggest that volatile compounds in avian preen oil may fulfill criteria for mate recognition and mate assessment chemosignals. Acknowledgements: This work was funded by the Indiana University Faculty Research Support Program, National Science Foundation, and the Indiana Academy of Science. This work was also partly sponsored by Lilly Chemistry Alumni Chair (Indiana University), and by the Indiana METACyt initiative of Indiana University, funded in part through a major grant from the Lilly Endowment, Inc.

184 Impact of Complexity on the Processing of Odour Mixture in Newborn Rabbits

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Humans have limited capabilities to elementally process odour mixtures. In a mixture including more than 16 odorants, we do not recognize any individual component, and no more than 4 in a less than 16 odorants mixture (Jinks et Laing, 1999). Are those characteristics conserved within the Mammalia class? Here, we evaluated whether European newborn rabbits process elementally (perception of each component's odour) or configurally (perception of a typical odour different from components' odours) a 6 odorants mixture inducing the configural perception of "red cordial" in Human (Le Berre et al., 2008). We started to examine i) how many odorants rabbit pups discriminate in the mixture and ii) whether the perceptual effect evidenced in human is also observed in rabbit. To that goal, we followed a validated method of conditioning and behavioural testing (Coureaud et al., 2008, 2009). In Experiment 1, 70 pups have been conditioned to the mixture by association with the Mammary Pheromone and dispatched 24h later in 3 groups, each one tested in response to the mixture and 2 of its components. Pups highly responded to the mixture (>73.7%) and its components (>81.2 %). This suggests that newborn mammals efficiently extract elements from complex odour mixtures. In Experiment 2, 4 groups of pups (n = 17-20/group) were each one conditioned to a different component of the mixture and tested to this odorant, one not learned component, and the mixture. The pups strongly responded to the learned odorant (>68.42%) but only weakly to the unfamiliar component and to the mixture (<5.3%). This lack of response to the mixture may be due to its high level of complexity or to its partially configural processing. Overshadowing can be rejected since pups respond to all the components after being conditioned to the mixture. Acknowledgements: Supported by grants from Burgundy Region and UE FEDER, by IFR 92 and by French MESR.

185 Developmental and Odor-induced Changes in Odorant Receptor mRNA Expression During Olfactory Imprinting and Homing in Pacific Salmon, *Oncorhynchus spp.*

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Pacific salmon are well known for their extraordinary homing migrations from oceanic feeding grounds back to their river of origin to spawn. These migrations are governed by olfactory discrimination of homestream odors that juvenile salmon learn (imprint to) prior to their seaward migrations. Our previous studies have suggested that one component of imprinting may involve long-term sensitization of the peripheral olfactory system to specific odorants. In this current study, we examined the mechanism of peripheral sensitization during imprinting, by exposing juvenile coho and sockeye salmon to L-arginine during several putative imprinting periods. Arginine is a potent salmon odorant for which a candidate olfactory receptor has been identified. We examined full life cycle changes in receptor expression in L-arginine-exposed vs. L-arginine-naïve fish using quantitative PCR. In parallel, we assessed imprinting success of these same exposure groups by behavioral assessments of odorant attraction using maturing adults in two-choice mazes. Fish exposed to L-arginine during appropriate developmental stages demonstrated long-term memory formation for this imprinting odorant ($P \leq 0.05$; two-sample t-tests). Treatment groups that successfully imprinted, as evidenced by adult behavior, also demonstrated increased expression (relative to arginine-naïve fish) of the putative arginine receptor mRNA in the olfactory epithelium during key life stages. Our results suggest that early odorant exposure may affect olfactory receptor

expression levels throughout the life of a salmon. Acknowledgements: Funded by the Bonneville Power Administration and the NWFSC

186 Comparative study of the response of *Aedes aegypti* and *Culex quinquefasciatus* to host odor cues

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The southern house mosquito, *Culex quinquefasciatus,* and the yellow fever mosquito, *Aedes aegypti,* are vectors of numerous human diseases that are among the main causes of human mortality and morbidity worldwide. Carbon dioxide (CO_2), a main component of the host-released odor plume, is a major cue for activation and host attraction in these species. In addition these mosquitoes are behaviourally attracted to 1-octen-3-ol, a key component of human skin and bird emanations. Single sensillium recordings (SSR) were performed on the maxillary palp basiconic sensilla from both *Aedes* and *Culex* to define the sensitivity of olfactory receptor neurons (ORNs) to CO_2 and enantiomers of 1-octen-3-ol. Olfactory receptor neurons housed basiconic sensilla are differentially tuned CO_2 exceeding 600 ppm and 1-octen-3-ol isomers. To understand the kairomonal cues released from the human body, we collected whole-body volatiles from different volunteers. Pooled extracts were used in no-choice behavioural assays to test the attraction of intact mosquitoes, compared with mosquitoes either ablated antennae, maxillary palp or tarsi. Intact *Aedes* and *Culex* were 70% and 50% attracted to human extract respectively. *Aedes* showed 15% attraction with ablated maxillary palp and 5% with ablated antennae. This data support the olfactory organs play a vital role in discrimination of human volatiles, without which mosquitoes are unable to detect these odors. In further studies, we will relate these behavioural studies to gas chromatography coupled electro-physiology investigate which compounds are attractive/repellent to the mosquitoes. Acknowledgements: this work has been supported by the Swedish research council, Formas.To attend this conference, funding was recieved from SLU fund for internationalisation of postgraduate studies.

187 Influence of complex learning contexts on olfactory discrimination abilities and bulbar network

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Discrimination abilities can be modulated by experience through a so called perceptual learning corresponding to an improvement in discrimination between close stimuli following passive exposure. This learning sets the degree of discrimination between stimuli and thus reflects an ongoing process of sensorial environment assimilation. Recently, we have demonstrated that after a 10-day enrichment to a pair of perceptually close odorants, an improvement in discrimination's ability of these odorants can be observed. In real life, the olfactory environment is complex and constantly changing, probably requiring both permanent learning and forgetting of new or obsolete information. To assess how the network handle changing environment, we have investigated the behavioral effect of successive perceptual learning. In this study, animals have been submitted to a 10-day enrichment to a first pair of odorant (+/-limonene) yielding improvement in discrimination between these two odorants. Then, 6, 26, 36 or 46 days later, they were submitted to second 10-day enrichment with another pair of odorants (decanal/dodecanone). With a delay between both enrichments of 6 days, only the decanal/dodecanone is discriminated. When the delay between both enrichments is 16 days, both pairs of odorants are discriminated. Finally, when the delay is superior to 26 days, only the decanal/dodecanone is discriminated. These finding suggest that the neural representation of the learned odorants displays differential time-dependent sensitivity to additional learning. We will further investigate bulbar network responsiveness and neurogenesis to assess how the neural network involved in this learning evolves with complexity of the environment and is changed in function of the discrimination performances. Acknowledgements: CNRS and Marie Curie Foundation

188 Alteration of the scent of age by the xenobiotic citronellal ingestion

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Body odors provide a rich source of chemical sensory information for other animals. In addition to the stable body odor imparted by its genetically encoded odortypes, the odor information on the animal's age including current reproductive situation provides chemosignals which control the behavior of mice. On the other hand, there is considerable evidence suggesting short term fluctuations in body odors due to diet. Our recent study suggested that ingested Mugwort and Mushroom extract reduced the intensity of odors associated with aging in mice, by means of decreasing of endogenous volatiles which increase with age. To investigate whether the ingestion of citronellal which is known as an monoterpene aldehyde that produce some of the most intense aroma for both of human and mice can alter the mouse urinary odor, mice (C57BL6J) were trained in a Y-maze to discriminate urine odors of donor mice which ingested citronellal aqueous solution or control solution. Trained mice could discriminate of male urine odors between citronellal ingestion group and control group. A series of generalization tests revealed that the citronellal ingestion altered mouse urinary odor directly due to citronellal odor, neither the effect of the metabolite of citronellal, nor the alteration of the proportion in the endogenic urinary volatiles evoked by citronellal ingestion. Moreover, the trained mice which had successfully discriminated urine odors of donor mice of different age failed to distinguish the age related change in the male mouse urinary odor by 50 ppm of citronellal ingestion. Thus, this study is the first investigation to show that xenobiotic per os can alter the mouse urine odor by its own odor and confound the behavioral response of trained mice to the scent of age.

189 Lesions of the Medial Amygdala Impair Lordosis And Olfactory Responses to Urinary Volatiles in Female Mice

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Increasing evidence suggests that the main and accessory olfactory systems in rodents, which detect primarily volatile and non-volatile cues, respectively, both may play roles in mate recognition and mating behavior. Recently, we have shown in the mouse that the medial amygdala (Me) receives direct inputs from both systems, and we have begun to examine the role of the Me in mediating behavioral and physiological responses to biologically relevant olfactory stimuli, such as conspecific urine. In this study, we used female mice to examine whether either lordosis or typical preferences of females for urine from gonadally intact vs castrated male mice depends on the posterior Me. Adult females were gonadectomized and given bilateral sham surgeries or electrolytic lesions aimed at the posterior Me. After recovery mice were treated with estradiol benzoate and progesterone and given a series of tests to examine interest in urine derived from castrated or intact males. Lordosis quotients in response to mounts from stud males were also determined in 4 separate tests. Mice with posterior Me lesions showed significantly reduced lordosis quotients in comparison to shams ($p<0.001$). In addition, compared to lesioned mice shams showed a significantly greater investigation time of urinary volatiles from intact vs castrated males ($p<0.05$). However, there was no difference between groups in investigation times when physical contact with the urinary stimuli was permitted. These preliminary results indicate that copulatory behaviors as well as responses to volatile, but not non-volatile, urinary cues from males are compromised by lesions of the posterior Me in female mice. Acknowledgements: NIDCD

190 Butylated hydroxytoluene is a ligand of urinary proteins of female mice

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Male mice secrete substantial amounts of proteins, particularly proteins called major urinary proteins (MUPs) in urine. A recent study showed that although the total protein concentrations of female urine were about 4 times lower than those of male urine, females also produce similar MUP profiles. One function of MUPs has been shown to sequester volatile pheromone ligands, thereby delaying their release and providing a stable, long-lasting signal. Several ligands have been identified for male MUPs. However, no ligand associated with female MUPs has been reported. This raises the question as to whether any volatile ligand is present and binds to female-derived MUPs. In this study, we investigated volatile ligands of urinary proteins in C57BL/6J male and female urines. Our approach was to add guanidine hydrochloride, a protein-denaturant, to urine and identify any volatile compound with increased release from urine upon denaturation as a ligand. Whereas the concentration of 2-sec-butyl-4,5-dihydrothiazole was significantly increased in male urine after protein denaturation as has previously been reported, no change in the level of any endogenous (mouse-derived) compound in female urine was observed following denaturation. However, a substantial increase in butylated hydroxytoluene, a synthetic antioxidant present in the diet, was observed from female urine upon denaturation. Therefore, our data indicate that female-derived urinary proteins can bind, transport and excrete exogenous compounds into urine, and raise the possibility that in addition to the known role in chemical communication, MUPs function as a defense mechanism against exogenous toxins. Acknowledgements: This work was supported partially by ARO contract DAAD19-03-1-0109. Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the United States Government.

191 The role of the neurotrophin receptor, TrkB, in taste system development.

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Brain derived neurotrophic factor (BDNF) and neurotrophin-4 (NT4) are two important regulators with distinctive roles in gustatory neural survival, target innervation and taste bud development. These two neurtrophins activate the same receptors, the tyrosine kinase receptor (TrkB) and the pan-neurotrophic receptor (P75). In this study, we compared $TrkB^{-/-}$ and hybrid mice lacking functional BDNF and NT4 genes ($BDNF^{-/-}/NT4^{-/-}$) to determine if the impact on the taste system is similar. This would indicate that if BDNF and NT4 function via TrkB to regulate gustatory development. We quantified the total number of geniculate neurons at ages E11.5, E12.5, E13.5 in $TrkB^{-/-}$, $BDNF^{-/-}/NT4^{-/-}$ and wild type littermates (n=3/age/genotype). Compared to wild type mice, $TrkB^{-/-}$ mice lost 46%, 81%, and 87% ($P<0.001$) of geniculate neurons at ages E11.5, E12.5, E13.5, respectively, while $BDNF^{-/-}/NT4^{-/-}$ mice lost 32%, 48%, and 80% ($P<0.001$), respectively. $TrkB^{-/-}$ mice lost more neurons than $BDNF^{-/-}/NT4^{-/-}$ mice at age E12.5 ($P<0.001$), suggesting that there might be other neurotrophins contributing to the neural survival at this age. Using DiI labeling of the chroda tympani we found very little gustatory innervation in both $TrkB^{-/-}$ and $BDNF^{-/-}/NT4^{-/-}$ mice compared to wild type mice by E15.5 of development. Almost all the innervation to the tongue surface was lost in both genotypes. We also quantified the number and volume of fungiform papillae in P0 $TrkB^{-/-}$, $BDNF^{-/-}/NT4^{-/-}$, and wild type mice. $TrkB^{-/-}$ and $BDNF^{-/-}/NT4^{-/-}$mice lost 37% ($P<0.001$) and 27% ($P=0.002$) of their fungiform papillae compared to wild type mice, respectively. In addition, the volume of the fungiform papillae in the middle region of the tongue of $TrkB^{-/-}$ and $BDNF^{-/-}/NT4^{-/-}$ mice was 34% and 28% ($P<0.001$) smaller than those in wild type mice. There was no difference in the size or number of fungiform papillae between $TrkB^{-/-}$ and $BDNF^{-/-}/NT4^{-/-}$ mice. Taken together these data indicate that NT4 and BDNF regulate gustatory development entirely via TrkB. Acknowledgements: NIDCD: DC009418

192 Replacement of BDNF by NT4 rescues gustatory neuron targeting but not taste bud number in the tongue

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Taste buds within the anterior tongue are innervated by geniculate ganglion neurons through chorda tympani nerves. The neurotrophin BDNF, but not NT4, regulates the ability of chorda tympani fibers to locate and innervate their correct target, while both BDNF

and NT4 regulate neuron survival utilizing different mechanisms. The different roles of BDNF and NT4 may be due to their different expression patterns in the tongue, or due to different activation of downstream signaling pathways. To distinguish the two possibilities, taste targeting and taste bud number in the anterior tongue were determined in knock-in mice ($bdnf^{nt4\text{-}ki/nt4\text{-}ki}$), in which NT4 gene expression replaces BDNF and is controlled by the endogenous BDNF promoter. Using DiI-labeling, we examined target innervation in the anterior tongue at E16.5. Unlike $bdnf^{-/-}$ mice, in which target innervation is completely disrupted, the innervation patterns in $bdnf^{nt4\text{-}ki/nt4\text{-}ki}$ mice are similar to those in wild type mice, indicating that NT4 expressed instead of BDNF can rescue taste targeting. However, the amount of innervation in $bdnf^{nt4\text{-}ki/nt4\text{-}ki}$ mice appeared to be reduced and fewer specific regions of the tongue surface were innervated in $bdnf^{nt4\text{-}ki/nt4\text{-}ki}$ mice than in wild type mice (68.7 ± 3.2 *vs* 81.3 ± 4.0; $p < 0.01$). Using cytokeratin 8 as a marker of taste buds, we have begun to quantify taste buds in $bdnf^{-/-}$, $bdnf^{nt4\text{-}ki/nt4\text{-}ki}$ and wild type mice at birth. So far, in $bdnf^{nt4\text{-}ki/nt4\text{-}ki}$ mice, we counted 98 fungiform taste buds, which is more than in $bdnf^{-/-}$ mice (54) but fewer than in wild type mice (126). Taken together, these results suggest that replacement of BDNF by NT4 can rescue taste targeting in the anterior tongue, but does not support gustatory innervation to as many fungiform papillae as in wild type mice. Acknowledgements: This work was supported by NIH grant DC007176.

193 Involvement of Wnt/β-catenin signaling in the renewal of mature taste bud of mice

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Renewal of the three different cell types within taste buds occurs about every 10-14 days. The molecular mechanisms responsible for this turnover are not completely elucidated. Embryonic taste bud development has been shown to be controlled by Wnt/β-catenin signaling (Liu et al., 2007). Thus, the present work aims at investigating whether this pathway is involved in taste cell renewal in adult mice. Using the BATGAL strain which expresses β-galactosidase (β-gal) in the presence of nuclear β-catenin, we first examined what cell types are Wnt responsive by performing immunohistochemistry. Quantifying double labelling for cell type markers and β-gal showed that Wnt/β-catenin signaling is mainly active in NTPDase2 (type I) positive cells in circumvallate papillae. Less frequently, β-gal was detected in α-gustducin (type II) and NCAM (type III) positive cells. Then, mice conditionally overexpressing Dkk1, a secreted Wnt inhibitor, were used to explore the functions of Wnt/β-catenin pathway in taste buds. These mice carrying tetracycline-dependent Dkk1 alleles were fed doxycycline chow to induce transgene expression. Two-bottle tests between water and different concentrations of denatonium revealed no difference in the preference ratio curve between control mice and Dkk1-overexpressing mice after 2 and 6 weeks of drug treatment. Nevertheless, because our preliminary data showed a shift in the preference curve after 9 months of diet, we are now continuing drug treatment and two-bottle testing to see when Wnt inhibition begins to affect taste sensitivity to bitter. Identification of Wnt/β-catenin responsive subtypes of taste bud cells and investigating the phenotype of mouse models like the Dkk1-overexpressing mice are necessary to further understand the function of Wnt/β-catenin in taste bud renewal. Acknowledgements: Supported by NIDCD DC008373 to LAB

194 Wnt/β-catenin Signaling within Taste Bud Progenitor Cells Impacts Both Taste bud and Taste Papilla Development.

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Taste receptor cells on the tongue are localized within epithelial-mesenchymal specializations called papillae. Their development initiates with formation of epithelial placodes that invaginate to form papillae. In mice, taste receptor cells differentiate within papillae around birth. Recently we have shown that Shh expressing cells of taste placodes are exclusively taste bud progenitors, which do not contribute to the surrounding papillae (Thirumangalathu et al., 2009). We have also demonstrated that increased Wnt/β-catenin signaling broadly yet exclusively in lingual epithelium induces de novo additional taste progenitors and papillae from what would otherwise be non-gustatory epithelium (Liu, Thirumangalathu et al., 2007); however, these findings do not distinguish between a role for Wnt in progenitor versus papillary development. Here, we test if increased Wnt/β-catenin within Shh-expressing taste bud progenitors impacts taste bud development directly, and if taste papillae are also affected, presumably indirectly by signals downstream of Wnt. Forced activation of β-catenin within Shh expressing placodes as these structures first form results in enlarged taste organs due to increases in both taste progenitor and papillary epithelial cells; papillary mesenchyme appears unaffected in the mutants. Mitotic activity within these developing taste organs is also increased in mutant tongues, and may account for the increase in progenitor pool and/or papillary size; we are currently testing both hypotheses. As Wnt/β-catenin signaling is active within the taste epithelium throughout embryonic development, we are continuing to use conditional molecular genetic approaches to temporally and spatially dissect the function(s) of this pathway in taste patterning, morphogenesis, and differentiation. Acknowledgements: Supported by DC008373 to LAB

195 Adult Mice with Genetic Deletion of SHH in Tongue Epithelium Have Fungiform Taste Buds and Papillae with Aberrant Morphology.

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Sonic hedgehog (SHH) is a secreted factor, which regulates embryonic taste development in rodents (Hall et al., 2003; Mistretta et al., 2003). SHH is also expressed in a subset of intragemmal basal cells in adult mice (Miura et al., 2001), but its function in mature taste buds has not been assessed. Here we used 2 strains of genetically

engineered mice to specifically knock out SHH in basal keratinocytes of the lingual epithelium. These cells have been shown to give rise to differentiated taste bud cells and taste papilla epithelial cells in adult mice (Okubo et al., 2009). Using taste cell type specific immunomarkers, we have found that fungiform taste buds are distorted in postnatal mice assessed at 3 weeks of age. Overall, the shape of mutant taste buds is narrower and more elongate than that of control littermates; gustducin-ir type II cells are also deranged in the mutants. The morphology of fungiform papillae is frequently distorted, and in many cases is distinctly filiform, despite the presence of differentiated taste buds within them. Importantly, these aberrant taste buds and papillae are innervated, in that antisera against PGP9.5 and neurofilaments reveal extensive neurites within mutant papillae and taste buds. Expression of Sox2, a transcription factor associated with stem cell function and expressed in and around adult taste buds (Suzuki 2008) is also disturbed in mutants compared with control littermates. Our data suggest that SHH may function to maintain adult taste buds via its classic role as a mitogen. Thus, we are currently testing the hypothesis that SHH positively regulates taste cell renewal from adjacent Sox2-expressing taste progenitors. Acknowledgements: Funded by DC003947 and DC008373 to LAB

196 Gli Transcriptional Activity in Hedgehog Signaling Regulates Tongue Epithelial Integrity and Postnatal Papilla and Taste Bud Support

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Sonic hedgehog (Shh) is a principal molecule regulating taste papilla development and differentiation. Gli2 is a transcriptional activator of Hedgehog signaling and in postnatal rodent tongue Gli2 is expressed throughout basal cells of the lingual epithelium, whereas Shh is produced within taste bud cells. To test whether Gli2 might participate in postnatal maintenance of tongue epithelium and fungiform papillae and taste buds, we used transgenic mice with conditional activation of *Gli2* under control of keratin promoters active in basal cells of skin and tongue epithelium. In tongues from 7 to 12 week mice with doxycycline-regulated *Gli2* activation during a period of several days, the lingual epithelium was profoundly altered. Filiform papillae had blunted tips, lacking the typical sharp, keratin spines of postnatal tongue. Whereas fungiform papillae in control mouse tongue typically have one taste bud per papilla, in epithelium from *Gli2* activated tongues, large numbers of papillae were misshapen and had no taste bud. Gli2 also is important in early development of lingual epithelium. In late embryonic mice with reduced *Gli2* function, achieved by keratin promoter driven expression of a dominant-negative form of *Gli2*, the tongue epithelium was thin and undifferentiated, with no filiform or fungiform papillae. Our results strongly suggest that Gli transcriptional activity is an important regulator of tongue epithelial integrity. Uncontrolled Hedgehog signaling by *Gli2* activation disturbs support and maintenance of postnatal tongue epithelium and fungiform papillae and taste buds. Acknowledgements: Supported by NIH NIDCD Grant DC000456 (CMM) and NIAMS AR045973 (AAD).

197 Peripheral taste system morphology in taster and non-taster mice

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In humans, taster and non-taster classification is based on an individual's ability to detect/taste blindness to phenylthiocarbamide. It has been proposed that taste receptor density on the anterior portion of the tongue is related to supertasting in humans. Mouse strains are divided into taster and non-taster groups based on their relative preference/avoidance for bitter and sweet tastants. Genetic composition in different mouse strains predicts the taster/non-taster properties in mice. However, whether or not genetic background is reflected in the morphology and number of taste buds and papillae is not clear. Due to the extensive use of transgenic mice in developmental and biological studies of the peripheral taste system, it is imperative to have better understanding of possible variations in the peripheral taste system in different background strains. The majority of the transgenic mice using homologous recombination in the past were generated using 129 mouse embryonic stem cells. To gain better understanding about the effects of background strain on morphological appearance of the peripheral taste system, we studied taste bud and papillae morphology, number and innervation in two taster strains (C57BL/J and FVB) and two non-taster strains (Balb/C and 129). 129 strain had the lowest number of fungiform papillae and Balb/C mice had the smallest fungiform surface area among the strains studied. Multiplying fungiform papillae number by the papillary surface area might be used as an indicator for the size of the receptor field in different mouse strains. If so, our results indicate that non-taster strains had a smaller receptor field area than taster strains. Thus, our study shows that the taster/non-taster phenotype is reflected in the tongue surface morphology among the strains studied. Acknowledgements: R01-RDC007628 from NIH-NIDCD

198 Mosaic Analysis with Double Markers (MADM) as a method to map cell fates in adult mouse taste buds.

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The differentiation pathway(s) leading from epithelial progenitor cells to mature mammalian taste cells function not only during development, but also throughout life as taste cells are continuously replaced. These pathways, however, are not yet clearly understood. In the present study, we have applied a new fate mapping technique to trace taste cell renewal at single-cell resolution in normal mouse circumvallate papillae (CV). For MADM analysis, two mouse lines with chimeric genes containing partial coding sequences for green and red fluorescent proteins (GFP, RFP) separated by a LoxP site, are interbred with Cre recombinase-expressing strains (Zong et al., 2005, Cell 121, 479-80). Occasionally in these crosses, Cre-mediated *interchromosomal* recombination events during mitosis reconstitute functional GFP and RFP genes, with one of the proteins expressed in each daughter cell and its subsequent progeny. To date, we have examined CV taste buds in mice resulting from crosses with two Cre-expressing lines, Hprt-Cre (Cre ubiquitously expressed) and Krt14-Cre (Cre expression targeted to epithelial progenitor cells). In serial

25 μm frozen sections visualized by confocal microscopy, sparse, discrete and well-separated groups of labeled cells were evident in the CV and lingual epithelium from both lines. Within the CV, we noted groups of elongate cells within taste buds, as well as cells associated with the taste pore. In the lingual surface epithelium, stacks of ovoid cells spanning the width of the epithelium were seen. Experiments to identify cell types represented within these putative clones in the CV and to determine lineage relationships are ongoing. Acknowledgements: 1 R15 DC006888 1 R15 EY017997

199 Oxytocin Receptor Is Expressed In A Subset Of Glial-like Cells In Mouse Taste Buds

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We have shown that OXT receptor (OXTR) is expressed in mouse taste buds. Mouse taste buds include three distinct classes of cells: Glial-like (Type I), Receptor (Type II), and Presynaptic (Type III) cells. Because these classes of cells have markedly different functions, we asked whether OXTR expression is restricted to any one of these classes. Using taste tissue from mice in which yellow fluorescent protein is knocked into the OXTR gene (OXTR-YFP mice), we immunostained for marker proteins for each cell type: Nucleoside Triphosphate Diphosphohydrolase-2 (NTPDase2) for Glial-like, PLCβ2 for Receptor, and ChromograninA (ChrA) or Amino Acid Decarboxylase (AADC) for Presynaptic cells. YFP was not co-expressed with either PLCβ2, ChrA or AADC. In contrast, most YFP-expressing cells expressed NTPDase2 and showed the typical ensheathing morphology of glial-like taste cells. Single-cell RT-PCR confirmed that OXTR was expressed primarily in Type I cells. OXT peptide has been reported to affect development in bone and heart. To assess if loss of OXTR also affects the differentiation of taste buds, we examined taste buds from OXTR-YFP heterozygous and homozygous mice (the latter are OXTR knockout). We did not notice any differences in the shape, size, or number of taste cells or buds when comparing OXTR +/+, OXTR+/y and OXTRy/y siblings. Finally, to evaluate the source of OXT peptide that might influence taste buds *in vivo*, we performed RT-PCR and immunofluorescence. We found no evidence of expression of OXT in taste buds, nontaste epithelium or in nerve fibers that approach or penetrate taste buds. Thus, we infer that OXT is delivered to taste buds via the circulation, and may serve to integrate central and peripheral mechanisms for energy balance and appetite. Acknowledgements: Supported by NIH/NIDCD grants R01DC6021, R21DC10078 and American Heart Association Predoctoral Fellowship 0815215E.

200 Glutamatergic and Catecholaminergic Markers are Present in Fibers Innervating Mouse Taste Buds.

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Several neurotransmitters are found in taste buds, including serotonin, norepinephrine (NE), acetycholine and ATP. Previous studies (Stone et al, 2009) and preliminary data from our lab have shown that glutamate may also be a transmitter in taste buds, possibly released from neuronal fibers innervating taste buds. In the present study, we investigated the presence of catecholaminergic and glutamatergic markers in fibers that innervate taste buds. Specifically, we used immunohistochemistry in mouse lingual tissue to test for the expression of vesicular glutamate transporters 1,2, and 3 (VGlut1,2,3), markers for glutamate neurotransmitter release, and for tyrosine hydroxylase (TH), a marker for catecholaminergic neurotransmission (dopamine, NE). Immunostaining revealed VGlut1 and VGlut2 in fibers innervating taste buds of the circumvallate and fungiform papillae, while VGlut3 was absent. VGlut2 also co-expressed with Pirt, a marker for peripheral sensory fibers (Kim et al, 2008), including fibers innervating taste buds. These findings demonstrate that VGlut1 and 2 are specifically found in neuronal fibers innervating taste buds, but not in taste bud cells. Surprisingly, VGlut2 also co-expressed with tyrosine hydroxylase (TH), suggesting that taste fibers might co-release both glutamate and catecholaminergic neurotransmitters such as dopamine or NE. Consistent with these findings, we observed that petrosal and geniculate ganglia expressed Pirt, VGlut2, and TH. Collectively, the findings indicate that sensory cells that innervate taste buds extensively co-express markers for glutamatergic and catecholaminergic transmitters. Further experiments are required to determine the functional implications for the possible co-release of neurotransmitters in terms of taste bud signaling and function. Acknowledgements: Supported by NIH/NIDCD grant 5R01DC000374 (SDR).

201 Orally Administered Capsaicin Reduces Taste Bud Volumes in Rats Treated as Adults, but Not Those Treated as Neonates

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In rats, the earlier in development that gustatory or associated somatosensory nerves are transected, the greater the gustatory losses (e.g. presence of disfigured papillae, reduction of fungiform papillae, lower taste bud numbers or volumes; Gomez & Sollars, 2006; Sollars, 2005). Based on this, it was of interest to see if a more commonly encountered form of potential nerve damage, orally administered capsaicin (the spicy chemical found in peppers), would yield a similar pattern of results. Rats were given either a sucrose solution containing capsaicin (CAP) or a plain sucrose control solution (SUC) beginning at postnatal day 5 for the neonate group or postnatal day 44/45 for the adult group. Rats were given solutions every day for 35 days, after which half of the animals were sacrificed 2 days post treatment, while the other half were given a period of time for possible recovery to occur (i.e. 50 days) before sacrifice. Tongues were analyzed for fungiform papillae morphology and taste bud volume. Unlike previous research, adult, not neonatal, taste buds were more negatively impacted by the capsaicin treatment; adults treated with capsaicin and sacrificed 50 days post treatment had smaller taste buds than age-matched controls. This counters previous research that finds greater susceptibility in neonates than

adults. No differences between the CAP and SUC animals were found in papillae morphology for either age group at either sacrifice point. The discrepancy between the results of the current study and past literature likely derive from the various differences between the two treatments (i.e. transection vs. chemical), including the duration, severity, and location of treatment, as well as interactions with other physiological processes. Acknowledgements: NIDCD

202 Lipopolysaccharide-Induced Inflammation Attenuates Taste Progenitor Cell Proliferation and Taste Bud Cell Renewal

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Taste buds undergo constant cell turnover, and their structural homeostasis is maintained by balancing cell proliferation and death. Whether inflammation, an underlying condition in a number of diseases associated with taste disorders, interferes with taste cell renewal is unknown. Here we report the effects of lipopolysaccharide (LPS)-induced inflammation on taste progenitor cell proliferation and taste bud cell renewal in mouse taste tissues. Intraperitoneal injection of LPS rapidly induced expression of several inflammatory cytokines, including tumor necrosis factor (TNF)-a, interleukin (IL)-6, and interferon-g, in mouse circumvallate and foliate papillae. LPS-induced inflammation significantly reduced the number of 5-bromo-2'-deoxyuridine (BrdU)-labeled newborn taste bud cells 1-3 days after LPS injection, suggesting an inhibition of taste bud cell renewal. Quantitative real-time RT-PCR revealed that LPS markedly reduced mRNA levels of Ki67, a cell proliferation marker, in the circumvallate and foliate epithelia. Immunofluorescent staining using anti-Ki67 antibodies showed that LPS decreased the number of Ki67-positive cells in the basal regions surrounding circumvallate taste buds, the niche for taste progenitor cells. In addition, PCR array experiments showed that mRNA expression of cyclin B2 and E2F1, two key cell cycle regulators, was markedly downregulated by LPS in the circumvallate and foliate epithelia. Together, our results show that LPS-induced inflammation inhibits taste progenitor cell proliferation and interferes with taste cell renewal. Acknowledgements: Supported by NIH/NIDCD grants DC007974 and DC007487.

203 Distinct GABA synthesizing enzymes and GABA receptors in each cell type of mouse taste buds

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Taste buds are reported to contain several neurotransmitters. Cao et al. (2009) recently showed electrophysiological responses to GABA in some rat taste cells. To understand the significance of GABA signaling within mouse taste buds, we asked which cells synthesize GABA, and which may respond to GABA via membrane receptors. RT-PCR showed that both GABA-synthesizing enzymes, GAD67 and GAD65, are expressed in vallate, foliate, fungiform and palatal taste buds, but not in nerve fibers or non-taste epithelium. Single-cell RT-PCR and immunofluorescence data showed that GAD65 is expressed only in NTPDase2-expressing Type I /Glial-like cells, while GAD67 is expressed only in Presynaptic (Type III) cells (Tomchik et al., 2007). Next, using anti-GABA immunofluorescence, we detected GABA in many cells in each taste bud. GABA-accumulating cells expressed either GAD65 (Type I cells) or GAD67 (Type III cells). However, ~37% of GAD67-expressing cells did not appear to accumulate GABA. We also analyzed the distribution of GABA receptors. Taste buds abundantly expressed mRNAs for ionotropic GABA-A receptor subunits, β1, -2 and -3, δ and π, and for metabotropic GABA-B receptor (R1). Using pools of PLCβ2(+) cells and SNAP25(+) cells, we found that Receptor cells express GABA-A β2, δ and π while Presynaptic cells express GABA-A β3. In contrast, mRNA for GABA-B R1 was detected in both Type II (Receptor) and Type III cells. In summary, GABA is synthesized by GAD65 in Type I cells and GAD67 in Type III cells of mouse taste buds. Receptor cells and Presynaptic cells express different sets of ionotropic GABA receptors and may thus produce very different responses to secreted GABA. What role GABA plays in communication between taste cell types and whether it modulates taste-evoked signals remains to be shown. Acknowledgements: Supported by NIH/NIDCD R01DC6308

204 Expression patterns of adrenergic receptors in rat posterior taste buds.

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Cell-to-cell communication within the taste bud is thought to be an important aspect of the transduction process. A large number of neurotransmitters, neuropeptides and their corresponding receptors allow the bud to process information prior to the afferent neural discharge. Among neurotransmitters, multiple lines of evidence suggest that norepinephrine plays a yet unknown role in the taste bud. Here, the expression pattern of adrenergic receptors in the rat posterior taste bud was investigated. Subsets of cells were observed to express the α1A, α1B, α1D, α2A, α2B, α2C, β1, and the β2 receptor subtypes as observed with whole bud and single cell RT-PCR and with immunocytochemistry. Unexpectedly, cells lacked expression of dopamine β-hydroxylase though did express adrenergic transporters (NETs) suggesting cells concentrate rather than synthesize norepinephrine. Further, expression of the PMNT, the synthetic enzyme for epinephrine, was observed. Double label phenotyping studies of adrenoceptors with gustducin, SNAP-25, or NCAM suggest they are prominently expressed in differing subsets of type II cells and segregated from type III cells. Alpha receptor expression segregated into three groups: cells expressing α1A, α2A or co-expressing α1B/α1D/α2C. Some overlapping expression of beta receptors was observed with all three groups. Almost all α2A cells and a majority of β cells co-expressed gustducin. Collectively these data strongly suggest that adrenergic signaling occurs in the taste bud through complex pathways that include a presynaptic and postsynaptic array of adrenoceptor subtypes. These pathways likely play modulatory roles in processing of gustatory information similar to other peripheral sensory systems such as the retina, cochlea, and olfactory bulb. Acknowledgements: NIH NIDCD R01 DC00401

205 Serotonin acts to facilitate tastant responses in the rat chorda tympani nerve.

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Our previous work has demonstrated that serotonin (5HT) and the $5HT_{1A}$ receptor are expressed in a paracrine manner in rat posterior taste buds and that activation of this receptor results in inhibition of several ionic currents in taste receptor cells (TRCs). Recent work suggests that 5HT acts to inhibit ATP release from posterior TRCs. Here we explore the role of 5HT at the neural level. Chorda tympani recordings were conducted before and after single bolus jugular injection (300 µl of 100 µg/kg) of WAY100635, a specific antagonist of the $5HT_{1A}$ receptor. Reversible inhibitions of tastant responses to NaCl, sucrose, and quinine were observed compared to preinjection response magnitudes. Inhibitions reached a maximum effect within 5 – 10 minutes after drug infusion and showed signs of recovery within about 30 minutes and were not observed with injection of a saline bolus. WAY100635 produced no significant change in blood pressure or heart rate over the course of the experiment. These observations suggest that $5HT_{1A}$ activation potentiates taste responses. To further verify the whole animal chorda tympani preparation, jugular injection of ATP and a purinergic receptor antagonist were tested. Single bolus jugular injections of varying doses of ATP produced concentration-dependent excitatory responses that displayed quick desensitization and were reversibly suppressed by PPADS, a non-selective P2 purinergic antagonist. PPADS administration also strongly and reversibly inhibited tastant responses on a time scale comparable to WAY100635 injections. In conclusion, we propose that serotonin acts to modulate purinergic transmitter release from the taste bud preventing rapid desensitization of postsynaptic purinergic receptors and thus overall acts to facilitate the neural response. Acknowledgements: NIH NIDCD R01 DC00401

206 Paroxetine, a selective serotonin reuptake inhibitor, does not alter concentration-dependent licking of prototypical taste stimuli by rats.

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Serotonin (5HT), which is found in taste buds, is implicated in dysgeusias accompanying depression and was shown to decrease human taste thresholds to sucrose and quinine. Here the effect of paroxetine (PAR), a 5HT reuptake inhibitor, on licking responses to water, sucrose, NaCl, citric acid, and quinine was assessed. Rats were tested 3x a week while either partially food and water restricted or 23-h water-deprived (to generate stimulus sampling) in 30-min sessions during which licks were measured to taste solutions presented in 10-s trials. Rats were tested for 6 weeks with an array of concentrations of a different taste stimulus each week and were injected with 1 of 4 doses of PAR (0.3-10mg/kg ip) or vehicle (DMSO 1ml/kg) 1-h prior to the third test session (n=5-8). During week 6, sucrose testing was repeated and a vehicle of 10% DMSO (1 ml/kg) was used (PAR 0.3-3mg/kg). Other rats (n=8) were injected with either 5mg/kg PAR or vehicle (10% DMSO 2ml/kg) on all 3 days of testing with sucrose. PAR at the doses used had no effect relative to vehicle on interlick intervals during continuous water access or on licking responses to the tastants. PAR at 5mg/kg reduced the number of trials taken to sucrose and at 10mg/kg reduced the number of trials taken to NaCl, citric acid, and quinine. PAR at 5mg/kg decreased licks to continuous water and at 5 and 10mg/kg decreased short-term feeding during an intake test after 23-h food deprivation. Collectively, the findings suggest that broad-spectrum modulation of 5HT signaling can, under certain circumstances, decrease feeding and appetitive behavior (eg, trial initiation), but does not ostensibly alter the affective processing of taste stimuli. Whether PAR at the doses used affects performance in other taste-related tasks remains to be tested in rats. Acknowledgements: This work was supported in part by NIDCD NRSA 1F32DC010517-01 to CMM.

207 Activation of Synaptic Glutamate Receptors Stimulates Mouse Taste Cells and Induces Serotonin Release

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Studies have shown that the neurotransmitter glutamate evokes responses in isolated taste buds and taste cells at concentrations well below those that evoke umami taste. Additionally, axons innervating taste buds express vesicular glutamate transporters, suggesting that these fibers release glutamate. However, it is not known what specific cell types in taste buds respond to glutamate as a possible neurotransmitter and what effect synaptically released glutamate may have on taste bud function. To answer these questions, we isolated single taste cells, loaded them with the calcium-sensitive dye fura-2-AM (5 µM), and tested their responses to synaptically relevant concentrations (≤ 100 µM) of glutamate. We found that a subset of taste receptor (Type II) cells (~20%) and presynaptic (Type III) cells (~50%) responded to glutamate at these concentrations. We also measured transmitter release (serotonin, 5-HT) from isolated taste buds and taste cells in response to glutamatergic stimulation, using 5-HT biosensors (Huang and Roper 2005). Bath-applied glutamate (100 µM) stimulated taste buds and isolated presynaptic cells to secrete 5-HT. The ionotropic glutamate receptor agonists NMDA (100 µM) and kainic acid (100 µM) also induced 5-HT release from isolated taste buds and this release was blocked by specific NMDA and AMPA/Kainate receptor antagonists (15 µM DL-APV and 30 nM CNQX, respectively). These data demonstrate that glutamate may have an important regulatory role within the taste system via activation of ionotropic glutamate receptors, especially those on presynaptic (Type III) taste cells. Further studies are required to determine the source of synaptically released glutamate and to examine how synaptic glutamate affects taste signaling as a whole. Acknowledgements: Funded by NIH/NIDCD grants 5R01DC000374 and 5R01DC007630 (SDR).

208 The amiloride-insensitive component of the chorda tympani response to NaCl is larger in A/J than in C57BL/6J mice.

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Our recent study has shown that mouse strains vary in their behavioral NaCl taste thresholds, and mice from the C57BL/6J (B6) and A/J strains have low and high thresholds, respectively (Ishiwatari and Bachmanov, 2007). We performed multiunit electrophysiological recordings from the chorda tympani (CT) nerve in these two strains, in order to examine whether these behavioral differences are associated with variation in peripheral taste responsiveness. Mice were anesthetized, the CT was accessed by exposing the nerve's passage through the inner ear, and NaCl and other solutions were flowed over the anterior tongue in order to measure the integrated whole-nerve response. NaCl was applied before, during, and after application of 100 micromolar amiloride. Responses to low concentrations of NaCl were significantly larger in A/J than B6 mice, despite the relatively high behavioral threshold for NaCl in the former strain. This difference in NaCl response magnitude arose due to a larger amiloride-insensitive NaCl response in A/J mice, whereas the amiloride-sensitive component of the NaCl response was similar in the two strains. These data support a role for peripheral taste mechanisms in causing the strain differences in behavioral taste thresholds for NaCl. However, the results suggest that detection of NaCl by mice depends on the ratio of amiloride-sensitive and –insensitive responses in the CT, rather than on the absolute level of the whole-nerve response to NaCl or on the size of the amiloride-sensitive component alone. Ishiwatari Y, Bachmanov AA. NaCl taste thresholds in 13 inbred mouse strains. 29th AChemS annual meeting, Sarasota, Florida. April 25-29, 2007. Chem. Senses 32: A26, 2007.

209 Differential regulation of chorda tympani (CT) taste nerve responses to sweet, salty, bitter and umami taste stimuli by phosphatidylinositol 4, 5-bisphosphate (PIP_2)

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To investigate the regulation of taste responses by changes in membrane PIP_2 levels in taste receptor cells, we monitored rat CT responses to sweet, bitter, umami and salty stimuli before and after topical lingual application of a short chain synthetic PIP_2 (diC8-PIP_2; 250 μM) dissolved in dimethyl sulfoxide. Tonic CT responses to 500 mM sucrose, 5 mM SC45647, 250 mM glycine and 10 mM quinine were enhanced after lingual application of diC8-PIP_2 relative to control. DiC8-PIP_2 treatment inhibited the CT response to 100 mM NaCl relative to control. The decrease in the NaCl CT response was due to a decrease in the putative benzamil (Bz)-insensitive TRPV1t-dependent NaCl component of the CT response. DiC8-PIP_2 had no effect on the Bz-sensitive ENaC component of the NaCl CT response. DiC8-PIP2 also did not alter the CT response to 100 mM monosodium glutamate (MSG)+5 μM Bz+1 μM SB-366791 (SB) relative to control. However, diC8-PIP_2 inhibited the increase in the CT response to MSG+Bz+SB+1 mM inosine 5'-phosphate (IMP) relative to MSG+Bz+SB observed under control conditions. Bz and SB were added to the stimulus solution to eliminate the contribution of Na^+ to MSG CT response. We conclude that PIP_2 is a common intracellular effector involved in the transduction of sweet, bitter, umami and TRPV1t-dependent salt tastes. While PIP_2 enhances CT nerve responses to sweet and bitter stimuli, it inhibits the synergistic effect of IMP on MSG CT responses and the putative Bz-insensitive TRPV1t-dependent component of the NaCl CT response. An increase in PIP_2 inhibits salt taste by directly interacting with TRPV1t and enhances sweet and bitter CT responses by modulating $PLC\beta_2$ activity. Alternately, PIP_2 may compete with the IMP binding site on the umami receptor and inhibit CT responses to MSG+IMP. Acknowledgements: Supported by grants DC-000122 (VL) and DC-005981 (VL) from NIH/NIDCD.

210 PKD2L1 is required for normal chorda tympani nerve responses to acids

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Recent studies have suggested that the polycystic kidney diseases-like ion channel PKD2L1 and its associated partner PKD1L3 are potential candidate for sour taste receptors. PKD2L1 and PKD1L3 are shown to be coexpressed in a subset of taste cells in the posterior tongue innervated by the glossopharyngeal (GL) nerve, while only PKD2L1 is expressed in the anterior tongue innervated by the chorda tympani (CT) nerve. In a heterologous system, PKD1L3-PKD2L1 channel complex responds to acids with an off-response property. *In vivo*, genetic elimination of cells expressing PKD2L1 substantially reduces CT nerve responses to sour taste stimuli. However, *in vivo* function of PKD1L3 and PKD2L1 remains unclear. In this study, we measured CT and GL nerve responses to various taste stimuli in PKD1L3 knock-out (1L3KO), PKD2L1 knock-out (2L1KO), PKD1L3 and PKD2L1 double knock-out (DKO) mice in comparison with wild type (WT) mice. We found that 2L1KO and DKO mice showed significantly decreased responses of the CT nerve to acids (HCl, citric and acetic acid) without affecting responses to salty, bitter, sweet and umami compounds, while no such difference in acid responses was observed between 1L3KO and WT mice. In the GL nerve, magnitudes of responses to acids in the 3 KO strains were not significantly different from those of WT mice. In single CT fiber response measurements, we found that spike frequencies of acid-responsive fibers to acids were significantly lower in 2L1KO mice than WT mice. These results suggest that PKD2L1 is essential for normal sour taste transduction, at least in the anterior region of the tongue innervated by the CT nerve.

211 Comparative analysis of ENaC and TRPV1-mediated NaCl responses of the rat chorda tympani nerve

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NaCl taste transduction consists of two putative components: the first a highly selective cation epithelial channel for Na^+ (ENaC) that can be blocked pharmacologically by amiloride or benzamil; the second a non-specific cation channel of the TRP family (TRPV1t) that is broadly responsive to Na^+ as well as K^+, NH_4^+, and Ca^{2+} salt solutions. We examined the ENaC-mediated portion by comparing

the relative effectiveness of amiloride and the more selective amiloride analog, benzamil in suppressing NaCl responses of the chorda tympani nerve (CT) in male Sprague-Dawley rats. In addition, we assessed the nonselective pathway by recording responses to 100mM NaCl mixed with 1μM SB-366791, a TRPV1 antagonist. We recorded whole nerve activity from the CT in response to lingual application of NaCl (100, 300, 600mM) and all NaCl concentrations mixed with either 100μM amiloride or 4μM benzamil in artificial saliva (15mM NaCl, 22mM KCl, 3mM $CaCl^2$, 0.6mM $MgCl^2$). All taste stimuli were delivered for 10 s at a constant flow rate of 50 μl/s and temperature of 35°C and were preceded and followed by 60-s rinses with artificial saliva. Preliminary data (n = 3) indicate that amiloride was more effective than benzamil in suppressing CT responses to NaCl. Amiloride suppressed CT responses by 54% across all NaCl concentrations, while benzamil suppression decreased with increasing NaCl concentration (44% at 100mM NaCl and 26% at 600mM NaCl). Benzamil suppression may be less than that for amiloride because the effective concentrations were not matched or because amiloride affects more than ENaC. Furthermore, SB-366791 suppressed 100mM NaCl responses by only 10%, suggesting that the nonselective cation pathway of NaCl taste transduction may involve mechanisms other than TRPV1. Acknowledgements: NIH grants R01 DC04785, T32 DC00044

212 Anion Size Attenuates Summated Epithelial Potentials of Tongue and Single-cell Responses of Geniculate Ganglion Neurons to TRPV1-mediated Salt Stimulation in Rats

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In anesthetized male rats, we recorded stimulus-evoked summated lingual epithelial potentials (Electrogustogram; EGG) from the anterior two thirds of the tongue simultaneously with single-cell responses in the geniculate ganglion (n = 17). Artificial saliva (15mM NaCl, 22mM KCl, 3mM $CaCl^2$, 0.6mM $MgCl^2$) at 35°C served as the rinse solution and solvent for all taste stimuli. Each stimulus was applied 3-9 times. The average responses to 0.5 M sucrose, 0.1 M NaCl, 10 mM citric acid, and 20 mM quinine hydrochloride, as well as 0.1 M KCl separated neurons into 2 Sucrose-specialists, 7 NaCl-specialists, and 8 Acid-generalists. EGG and single-cell responses were also measured to ascending concentrations of NaCl and NaGluconate (0.03, 0.1, 0.3, and 0.5 M). We used 1 μM Benzamil (Bz) and 1 μM SB366791 (SB) to distinguish 0.1 M NaCl evoked neural responses mediated via ENaC and TRPV1 channels, respectively. EGG amplitude and neural spike frequency increased, while neural response latency decreased with increasing NaCl and NaGluconate concentration. EGG amplitude to NaCl stimulation was 3X greater than that to NaGluconate at each concentration. Sucrose-specialist neurons were virtually unresponsive to Na^+ salts, even at the highest salt concentration. For NaCl-specialist neurons, response latency and frequency was similar to stimulation with both Na^+ salts. In contrast, response frequency was less and response latency longer to NaGluconate than to NaCl in Acid-generalist neurons. In NaCl-specialists, Bz decreased NaCl responses by 39%; in Acid-generalists, SB decreased NaCl responses by 20%. This latter effect is in concert with integrated chorda tympani nerve recordings from our lab. Together, these data support the notion of independent processing pathways for Na^+ in fungiform papillae. Acknowledgements: Supported by NIH grants RO1 DC004785, F31 DC009920

POSTER SESSION IV: CHEMOSENSORY TRANSDUCTION AND SIGNALING

213 Estrogen Modulates Excitability and Olfactory Responses in Mouse Vomeronasal Neurons

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Aggression and parental care are behaviors that are often mediated by chemical cues. Many of these chemical responses occur in the vomeronasal organ (VNO). Vomeronasal sensory neurons (VSNs) are the chemical sensors of the VNO and express specific odor receptors that are G-protein coupled and linked to a PLC pathway. While hormonal modulation of neurons in the CNS by steroids has been well established in mammals little is known about such modulation in VSNs especially rapid (non-genomic) effects of estrogen. To determine if estrogen plays a role in the VNO in mice, RT-PCR was used to determine that the mRNA for two estrogen receptors GPR30, a G -protein coupled estrogen receptor and the classical α-estrogen receptor were present in the VNO but not the β isoform estrogen receptor. Immunocytochemistry revealed that it was the VSNs that labeled for GPR30. Recording from isolated VSNs, using perforated patch clamp, showed that physiological concentrations (1nM) of 17β-estradiol can hyperpolarize the membrane by 3-5mV (n=5 out of 6 cells) and decrease the spontaneous firing of action potentials (n=3 out of 6 cells). VSNs respond to urine, a natural stimulus that is a complex mixture of pheromones, metabolites and salts. Estrogen was found to inhibit urine-induced current responses by 50% or more (n=16 out of 17 cells). Also in 3 out of 5 cells urine-induced action potentials decreased in the presence of estrogen. By studying such effects and dissecting the pathways behind it, a better understanding of how behavior is modulated by the every-changing concentration of hormones that occurs in response to internal and external cues may be possible. Acknowledgements: NIH-DC006939 & NIH-P20RR16435

214 Variation in vomeronasal receptor expression in a terrestrial salamander

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Individuals vary in their physiological and behavioral responses to sensory information. It is unknown whether individual differences at the level of the peripheral sensory organ contribute to individual variation at the organismal level. We studied pheromone detection by the vomeronasal system in a terrestrial salamander (*Plethodon shermani*). In this species, the volume of the vomeronasal organ (VNO) varied both within and between males and females. Using degenerate primers, V2R fragments have been isolated in *P. shermani*. The DNA sequences clustered into 3 subfamilies that are less than 80% similar in sequence identity. *In situ* hybridization (ISH) using RNA probes revealed 2 different patterns of V2R RNA expression in the VNO. In one pattern, the number of cells labeled by the RNA probe was positively correlated to the volume of the

VNO. In other words, the density of cells expressing a particular class of V2R was similar across individuals. In the other pattern, the number of labeled cells was unrelated to the volume of the VNO. In this case, individuals with a larger VNO had proportionally fewer labeled cells than did individuals with a smaller VNO. Thus, individual differences in V2R expression may contribute to differences in sensory function. Since males typically have a larger VNO than females, both in absolute size and relative to body size, this may translate into sex differences in responses to sensory information. Acknowledgements: NSF IOS-0818554 to LDH, NSF IOS-0808589 to KMK and LDH, NSF predoctoral fellowship to KMK.

215 Molecular characterization and localization of olfactory-specific ionotropic glutamate receptors in lobster olfactory receptor neurons

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The molecular basis of olfaction in crustaceans, a major group of arthropods, is still uncertain. While there is accumulating evidence that G protein activation of metabotropic signaling pathways is involved in crustacean olfactory signal transduction, a lobster olfactory-specific ionotropic glutamate receptor, OET07, appears to be an ortholog of the recently discovered *Drosophila* olfactory variant of ionotropic glutamate receptors (IRs). These results suggest that crustacean olfactory transduction mechanisms are at least in part similar to those of insects. As a first step towards understanding the role of IR-mediated signaling in crustacean olfaction, we have begun to characterize the expression of lobster olfactory IRs. We have cloned two full length lobster IR orthologs, including that of OET07, as well as partial sequences from other additional potential IRs, and demonstrated that all of the putative IRs can be detected in lobster olfactory tissue by RT-PCR. The lobster ortholog of OET07 can be detected in most, if not all, olfactory receptor neurons by *in situ* hybridization, and can be localized to the transduction compartment (outer dendrites) by western blot and immunocytochemistry. These results support a role for IR-mediated signaling in the lobster olfactory transduction mechanism. Using heterologous expression, we are currently attempting to determine whether the lobster IR orthologs can function as ionotropic receptors. Acknowledgements: Supported by grants from the National Institute on Deafness and Other Communication Disorders.

216 Measuring Ensemble Activity in Lobster ORNs through Calcium Imaging

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Lobster ORNs can be imaged in the olfactory organ in situ, thereby maintaining the normal polarity of the cells and the ionic environment of the olfactory cilia. The preparation gives simultaneous access to hundreds of ORNs that are viable for hours, thereby allowing rigorous characterization of their steady-state and dynamic properties. Odorants change the level of cytoplasmic Ca^{2+} in a dose-dependent manner in ORNs loaded with Ca^{2+}-sensitive indicator either through bath application or via a patch electrode. The kinetics and amplitude of the odorant-evoked Ca^{2+} signal correlate with the excitatory inward current, the degree of membrane depolarization, and the number of evoked action potentials, thereby establishing the physiological relevance of the Ca^{2+} signal. Spontaneous periodic Ca^{2+} transients in many ORNs correlate with spontaneous bursts of action potentials measured in single cells in the same cluster. We are using signal processing algorithms to analyze the level of correlated activity between these ORNs and the extent to which periodic calcium oscillations in different ORNs are synchronized by common intermittent excitatory input to test the predictions of our computational model for ensemble burst coding in these cells and the potential relevance of bursting input to olfactory scene analysis. Acknowledgements: Supported by the NIDCD (DC001655, DC005995)

217 Ca Imaging of Response Properties of Olfactory Receptor Neurons of Spiny Lobsters, *Panulirus argus*

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The spiny lobster, *Panulirus argus*, is an established model for studying olfaction. Its olfactory organ consists of tufts of specialized sensilla – aesthetascs – on the lateral flagella of the antennules with each aesthetasc containing a cluster of > 300 olfactory receptor neurons (ORNs). Although transduction mechanisms in aesthetasc ORNs have been analyzed in detail with patch clamp electrophysiology (Ache and Young, Neuron 48:417-430, 2005), very little is known about their basic response properties such as sensitivity and spectral tuning. To study these questions, we established Ca imaging of ORN responses in an *in vitro* 'slice' preparation similar to that used for patch clamp recordings. Short segments of lateral flagella were incubated in the Ca indicator Fluo-4 AM, mounted in an experimental chamber perfused with *Panulirus* saline, and imaged with an epifluorescence microscope equipped with monochromator and CCD camera. Our initial results showed that within each cluster several ORNs took up Fluo-4 and responded with a robust increase in fluorescence to global depolarization with high K^+ saline. Some of the labeled ORNs showed spontaneous rhythmic changes in fluorescence intensity. Puffing mixtures of food-related chemicals onto the aesthetascs with a multibarrel pipette caused a stimulus-coupled and transient increase in fluorescence intensity in some ORNs and a decrease in fluorescence intensity in others. Based on previous electrophysiological results (Michel et al., J.Neurophys. 65:446-453, 1991; Bobkov and Ache, J.Neurophys. 97:1052-1057, 2007), we interpret the rhythmic changes in fluorescent intensity as representing spontaneous bursting activity and the transient increases or decreases in fluorescent intensity as representing excitatory or inhibitory responses in ORN firing. Acknowledgements: Supported by NIH grant DC00312 and a GSU Brains & Behavior grant

218 Evolution of haematophagy: what one moth species can teach us.

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The vampire moth, *Calyptra thalictri*, provides an interesting model through which to study the evolution of haematophagic behavior. Contrary to the well-characterized model systems in mosquitoes, blood feeding in this moth appears to be a trait recently acquired. The morphological and physiological evidence we present supports the hypothesis that, in blood feeding male *C. thalictri*, a reduction in the number of antennal *sensilla coeloconica* shown to respond to vertebrate host-related compounds appears to result in the ability to overcome behavioral repulsion and/or acquire attraction to vertebrates, thus providing a new opportunity for these males to land on and feed from vertebrate hosts. Male moths were collected from two sites north of Vladivostok in Far Eastern Russia and from one site in Rotskär, Sweden. The antennae of these moths were examined using scanning electron microscopy, which revealed a lower number of *sensilla coeloconica* in blood feeding compared to non-blood feeding males. The response spectra of these sensilla were described using the single sensillum recording technique. These sensilla responded to a variety of vertebrate-related volatile compounds such as short chain carboxylic acids, phenolics, ketones and aldehydes, as well as ammonia. Acknowledgements: This work was supported by the Linnaeus-program Insect Chemical Ecology, Ethology and Evolution IC-E3.

219 Sex Pheromone Receptor Specificity in the European Corn Borer Moth, Ostrinia nubilalis

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Female moths (Order Lepidoptera) produce and release a mixture of related fatty acid derivatives from their pheromone gland to which males respond from long distances. In many cases, subtle changes in carbon chain length, double bond location and isomer blend differentiate the pheromones of closely related species. The origin and mechanism of the variation in male detection that enables the evolution of new pheromone blends is not known. The European corn borer is used as a model system to study the evolution of sex pheromones among closely related races and species. It exists as two separate sex pheromone races: ECB(Z) females produce a 97:3 blend of Z11- and E11-tetradecenyl acetate whereas ECB(E) females produce an opposite 1:99 ratio of the Z and E isomers. Males of each race respond specifically to their conspecific female's blend. The Asian corn borer, a closely related species, uses a 3:2 blend of Z12- and E12-tetradecenyl acetate. We used homology-dependent (degenerate PCR primers designed to conserved amino acid motifs) and homology-independent (pyrophosphate sequencing of antennal cDNA) approaches to identify five candidate sex pheromone transcripts (OnOr1 & 3-6) from ECB(Z). OnOr1 & 3-6 were expressed 14-100 times more abundantly in male compared to female antennae. OnOr6, characterized in *Xenopus* oocyets, was highly selective for Z11-tetradecenyl acetate (EC_{50} = 0.86 +/- 0.27 microM). OnOr6 was 1000 times less sensitive to the E11 isomer. Surprisingly, OnOr1, 3 and 5 responded more broadly to all four pheromones tested (Z11, E11, Z12 and E12 components). Receptors broadly-responsive to a class of pheromone components may provide a mechanism for variation in the male moth response that enables population level shifts in pheromone blend use.

220 Molecular characterization of accessory proteins mediating sexual selection in two *Ostrinia* species

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Sexual selection and mating in moths is mediated by olfactory sensing of pheromone blends. Differences between pheromone blends are detected at the periphery of the olfactory system by receptors and accessory proteins expressed in trichoid sensilla on the male antennae. Pheromone binding proteins (PBPs) and sensory neuron membrane proteins (SNMPs) are involved in pheromone detection, and may play a role in discrimination of pheromone blends. The European corn borer (*Ostrinia nubilalis*) (ECB) exists as two different races, the Z-race uses (Z)-11-tetradecenyl acetate as the main component of its pheromone blend while the E-race uses (E)-11-tetradecenyl acetate. The closely related Asian corn borer (*Ostrinia furnacalis*) (ACB) uses a slightly different pheromone, (E) and (Z)-12-tetradecenyl acetate. We hypothesized that changes in the pheromone components are accompanied by changes in the sequence or expression level of the genes involved in their detection. Partial transcripts of 5 PBPs and 2 SNMPs from the ECB Z-race were identified by pyrosequencing antennal cDNA. Complete cDNA sequences were obtained by rapid amplification of cDNA ends. Primers designed to untranslated regions were used to amplify open reading frames from both ECB races and ACB. Evidence of positive selection within predicted ligand-binding sites was analyzed by calculating the ratio of synonymous and non synonymous substitutions. Expression levels of PBPs and SNMPs in the antennae were measured using quantitative real-time PCR. We found that there was no evidence for positive selection in the sequences or expression levels of the PBPs and SNMPs, and therefore these proteins are not likely to be involved in the discrimination of the ECB and ACB pheromones. This is the first report of multiple PBPs and SNMPs in ECB and ACB.

221 Behavioral and Olfactory Consequences of Slipping Imaginal Discs Between Two Moth Species.

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Two heliothine moths, *Heliothis virescens* (Hv), and *Heliothis sublfexa* (Hs), are sympatric species in North America and have a close phylogenetic relationship. Both species use the same chemical substance as a key component of their respective sex pheromone blends and show homology in their pheromone-related antennal lobe structures, the macroglomerular complex. Despite the significant overlap in the sex pheromone communication systems of these two species, interspecific attraction is avoided by the presence of different behaviorally essential minor pheromone components that can also serve to inhibit males of the

other species. Since the olfactory receptor neurons (ORNs) for these minor pheromone components are functional on the male antennae of both species, further processing in the brain must play a critical role in precise pheromonal perception and is reliant upon establishment of the correct wiring connections between ORNs and second-order neurons, the projection neurons (PNs), in the antennal lobe. Forced wiring of heterogeneous ORNs to the indigenous PNs in the brain can be accomplished through interspecific transplants of male antennal imaginal discs. The behavioral responses of male Hv to Hs transplants were assayed in a wind tunnel. Males exhibited a strong preference (56%) for a blend intermediate between Hv and Hs. Electrophysiological recordings from central PNs (N=47) revealed that 32% responded to Z9-14:Ald, as a result of the presence of donor-type antennal ORNs. Both PNs (N=3 out of 5 stains) and ORNs (N=3) exhibited unusual patterns of arborization in the antennal lobe. These results suggest that the predicted spatial relationships between specific peripheral inputs and second-order olfactory neurons were not necessary for successful odor discrimination and behavior to occur. Acknowledgements: Supported by NSF-IOS 0641014 to NJV and CEL.

222 Modulation of pheromone responses by cyclic nucleotides and DAG in antennal trichoid sensilla of the hawkmoth *Manduca sexta.*

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Manduca sexta males detect sex pheromones with antennal trichoid sensilla. One of the two olfactory receptor neurons which innervate the pheromone-sensitive sensilla always responds to bombykal (BAL), the main pheromone component. The pheromone-dependent signal transduction cascade in insects is still under debate since evidence from different investigators indicated the involvement of several different signal transduction cascades. In tip recordings of pheromone-sensitive trichoid sensilla of the hawkmoth antenna we investigated BAL responses with a non-adapting stimulation protocol (dosage 10 or 1 µg BAL, duration 50 ms, interstimulus interval 5 min) for 3 hours at Zeitgebertimes (ZT) 1-4 (beginning of day = end of activity phase) and 8-11 (rest phase). Perfusion of the sensillar lymph with 100 µM 8bcAMP, a membrane-permeable cAMP analog, increased the sensillar potential (SP) amplitude but did not significantly affect the initial action potential (AP) frequency at ZT 1-4. In accordance, the perfusion with 50 µM forskolin increased the SP amplitude but did also increase the AP frequency at the beginning of the recordings. However, perfusion with 100 µM of a diacylglycerol (DAG) analog inhibited the BAL-dependent AP response and decreased the SP amplitude at ZT 8-11. Our results are consistent with our previously posted hypothesis of pheromone transduction suggesting that BAL activates a phospholipase C-dependent signal transduction cascade, with IP_3-dependent activation of Ca^{2+}-permeable ion channels. The increase of intracellular Ca^{2+} and DAG is suggested to decrease the sensitivity of the signal transduction cascade via activation of protein kinase C. Furthermore, time-dependent sensitization of the pheromone transduction is suggested via octopamine-dependent rises of cAMP. Acknowledgements: [Supported via DFG grant STE 531/20-1 to MS]

223 Subunit Contributions to Insect Olfactory Receptor Function

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Insect olfactory receptors (ORs) are heteromeric ligand-gated ion channels consisting of at least one common subunit (OR83b in *Drosophila*) and at least one subunit that confers odorant specificity. We expressed several ORs in *Xenopus* oocytes to investigate features of the receptor complex and odorant-binding site. First, we examined the sensitivity of *Drosophila* ORs to inhibition by ruthenium red (RR), a cation channel blocker. Inhibition was non-competitive and the reduction in receptor current amplitude by 50 µM RR varied among the ORs, ranging from 40.6 ± 3.1% inhibition for OR35a/83b to 105.4 ± 7.1% inhibition for OR67a/83b. Since OR83b is common to each receptor, these results suggest the odorant specificity subunit in each receptor contributes to the structure of the site of RR action, the ion pore of the receptor. Next, we found that ORs formed by *Drosophila* OR35a and an OR83b from either *Drosophila melanogaster*, *Apis mellifera*, or *Ostrinia nubilalis*, displayed highly similar odorant response profiles, suggesting that the OR83b subunit does not contribute to the structure of the odorant-binding site. Finally, a rational expansion of ligand structures based on known activators of *Drosophila* OR67a/83b enabled us to identify new odorant ligands for this receptor. Comparing the structures of full agonist, partial agonist, and antagonist structures allowed prediction of odorant-binding site requirements, including accommodation of a ring structure and an electronegative functional group on the odorant. Hydrophobic pockets available in either active or inactive conformations of the receptor may provide steric buttressing support and help explain activation or inhibition of the receptor by particular odorants. Acknowledgements: This work was supported by grants from the USDA (2008-35302-18815) and the NIH (DC008119). A.S.N. was supported, in part, by T32 HL07188.

224 Enzymatic conversion of odorants in nasal mucus affects olfactory glomerular activation patterns and odor perception.

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Odor information is decoded as a combination of olfactory receptors, and thus transformed into discrete spatial patterns of olfactory glomerular activity, which reflect differences in the quality of odorants. We previously found that there were differences between the ligand specificity of an olfactory receptor in vitro and of its corresponding glomerulus in vivo for some odorants. These observations led us to hypothesize that there existed pre-receptor events that affected the local concentration of a given odorant in the nasal mucus, which causes the apparent specificity differences. Here we show that odorants with functional groups such as aldehyde and ester are targets of metabolic enzymes secreted in the mucus, resulting in the conversion to corresponding acid and alcohol. Using in vivo imaging, comparison between the activation patterns in the olfactory bulb in the presence or absence of an enzyme inhibitor in the mucus suggested that the spatial glomerular activity pattern elicited by an enzyme-targeted odorant, acetyl isoeugenol, for example, was not purely the representation of the receptor code for acetyl isoeugenol but for the mixture of acetyl isoeugenol and the enzymatically converted odorant, isoeugenol. Importantly, olfactory discrimination tests revealed that the mice behaviorally trained to associate acetyl isoeugenol to sugar rewards could not

discriminate acetyl isoeugenol after the treatment with the enzyme inhibitor, suggesting that they perceive acetyl isoeugenol as a different odor from the odor of acetyl isogenol during training. These results reveal that the enzymatic conversion of odorants in the nasal mucus appear to affect the odor quality at the level of perception, shedding light on a unappreciated role of nasal mucosal enzymes in odor sensation.

225 PI3K-dependent Inhibitory Signaling in Mammalian Olfactory Receptor Neurons

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Phosphoinositide-3-kinase (PI3K)-dependent signaling can modulate the response of mammalian olfactory receptor neurons (ORNs) to complex odorants. We now extend this observation to single odorant pairs. Citral inhibited the response to octanol in 13 of 91 octanol-responsive acutely dissociated rat ORNs measured with calcium imaging. Citral itself was not an effective ligand for these ORNs while inhibiting the response to octanol in a concentration-dependent manner. Blocking PI3K relieved the citral-dependent inhibition but had no effect on the response to octanol. Citral also inhibited in a PI3K- and concentration-dependent manner the response to IBMX/Forskolin (IF) in about 1% of IF-responsive ORNs. We argue that citral affects PI3K-dependent signaling being otherwise weak agonist in these ORNs. The PI3K-dependent enhanced response to citral was blocked by MDL12330A and SQ22536, implicating cyclic nucleotide-dependent signaling in the output. Similar results were obtained with lilial-dependent inhibition of the response to bourgeonal, suggesting the finding can generalize to other odorant pairs. Collectively, our findings raise the interesting specter that either PI3K constitutively modulates OR binding, or ligand-directed binding to the OR targets both cyclic nucleotide and phosphoinositide signaling in mammalian ORNs. Acknowledgements: NIH NIDCD DC001655, DC005995

226 Functional implication of PI3K beta and gamma in rodent olfaction

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Phosphatidylinositol 3-kinase (PI3K)-dependent signaling couples to receptors for many different ligands in diverse cellular systems, including rat olfactory receptor neurons (ORNs). Here, we generalize the latter finding to mice by showing PI3K-dependent inhibition of the calcium response of mouse ORNs to odorant stimulation as well as odorant-dependent increase in phosphoinositides in the mouse olfactory epithelium (OE) measured in ELISA. Like rat ORNs, mouse ORNs express two known GPCR -activated isoforms of PI3K, PI3Kβ and PI3Kγ. Both isoforms are expressed in many, if not most ORNs. Isoform specific blockers, TGX-221 (PI3Kβ) and AS252424 (PI3Kγ), are equally effective in reducing both the odorant dependent rise in phosphoinositides as well as the PI3K-dependent inhibition of the calcium response to odorants. ORNs from transgenic mice deficient for PI3Kγ show a residual response to pan-specific PI3K blockers, suggesting that PI3Kγ plays a role but is not exclusively responsible for PI3K mediated signaling in murine ORNs. Collectively, our results suggest that PI3Kβ and γ may have redundant function in rodent ORNs, as known to occur in bone marrow-derived macrophages and blood platelets. Further studies using mice deficient for PI3Kβ alone and double deficient mutants are targeted to help resolve the relative roles of the two isoforms of PI3K in rodent olfaction. Acknowledgements: Supported by the National Institute on Deafness and Other Communication Disorders (DC001655, DC005995) and a Feodor Lynen Research Fellowship from the Alexander von Humboldt Foundation

227 REGULATION OF SODIUM CALCIUM EXCHANGER (NCX) ACTIVITY BY CALMODULIN OR OMP IN THE OLFACTORY SIGNALING TRANSDUCTION CASCADE.

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Dynamic changes in the concentrations of Na^+ and Ca^{2+} modulate signaling in the olfactory sensory neuron (OSN). Na/Ca exchanger (NCX) activity plays an important role in this process and its activity is modulated by different proteins. We reported reduced efficiency of Ca2+ extrusion by NCX in OSNs of the OMP-KO mouse. Here we address the molecular interactions between NCX and olfactory marker protein (OMP) or calmodulin (CaM) and their functional consequences in the regulation of intracellular Ca. We assayed reverse-mode NCX activity to determine Na dependent Ca influx in CHO cells stably expressing NCX1 protein. Pretreatment with CaM antagonists e.g. Ophiobolin-A or W7 significantly inhibits NCX activity, suggesting a modulatory role for CaM. The NCX -CHO cells were transiently transfected with pCMV-OMP-IRES-GFP or vector, and NCX1 activity was determined. In cells expressing OMP, NCX activity was significantly inhibited compared to control, indicating OMP modulates NCX1 activity. Addition of OMP to the membrane lysates obtained from porcine heart or from NCX1-CHO cells inhibited NCX1activity, as determined by solid support membrane based electrophysiology (Iongate) confirming our cell-based findings. By contrast, in SGLT1-CHO cells (sodium-dependent glucose *transporter*1) OMP had no effect, demonstrating the selectivity of OMP for NCX1. Interaction of OMP and/or CaM and NCX activity will be tested in freshly isolated rat cardiac myocytes (a rich source of endogenous NCX1). Although our prior analyses of the OMP-KO mice indicated that NCX activity was reduced in the absence of OMP, these findings suggest that OMP inhibits NCX activity. This apparent discrepancy may relate to the differential interaction of NCX with CaM and OMP to modulate NCX activity. Acknowledgements: NIH Grant RO1 DC003112

228 Inhibition or Loss of Plasma Membrane Calcium ATPases Prolongs Desensitization In Mouse Olfactory Sensory Neurons

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Olfactory sensory neurons (OSNs) respond to odorant stimuli with increases in intracellular Ca^{2+}. Termination of the Ca^{2+} signal is achieved through removal from the cell by Na^{+}/ Ca^{2+} exchanger (NCX) and plasma membrane calcium ATPases (PMCAs) or through sequestration by the SERCA pump or binding proteins. Since Ca^{2+} is involved in feedback regulation of the olfactory signal transduction pathway, its clearance also contributes to recovery from desensitization. We have previously demonstrated that the inhibition of PMCAs significantly slows down Ca^{2+} clearance from mouse OSN knobs after odor stimulation. In order to understand the effects of PMCA inhibition and the subsequent slower Ca^{2+} clearance on OSN functionality, we examined the adaptation properties of wild type (WT) and PMCA2 knockout (PMCA2KO) OSNs in response to two successive 8s IBMX/Fsk stimulations. While WT OSN knobs showed significantly attenuated second Ca^{2+} responses when stimulated at 150 and 200s after first stimulation, in the KO, the attenuation was larger and significant even at 250s interval. Comparison of the reduction in Ca^{2+} amplitudes between the WT and PMCA2KO showed that the attenuation was stronger in the KO at 150 and 200s. When WT OSNs were treated with the PMCA blocker carboxyeosin, there was a further reduction in the second Ca^{2+} responses. These results indicate that slower Ca^{2+} clearance prolongs desensitization in PMCA-inhibited OSNs. We also show that the desensitization observed with our choice of stimulus (8s application of IBMX/Fsk) is mediated through CaMKII. Inhibition of CaMKII with an inhibitory peptide (AIP) caused the Ca^{2+} responses to two successive IBMX/Fsk stimuli to be similar in amplitude in both WT and PMCA2KO at 150 and 200s intervals. Supported by R21 DC006643. Acknowledgements: R21 DC006643

229 Exogenous Odorant Receptor Suppresses Endogenous Receptor Expression in Cultured Olfactory Sensory Neurons

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Discrimination of odorants is achieved by a large number of odorant receptors (OR) expressed by olfactory sensory neurons (OSNs). Each OSN expresses only one OR gene among the repertoire of about 1000 genes in the mouse genome. ORs are believed to play an important role in the maintenance of the selected OR expression. The regulatory mechanisms of OR selection and maintenance in OSN are still unclear. We established a dissociated OSN culture system, which allows efficient genetic manipulation of gene expression *in vitro*. In the OSN culture, endogenous OR expression was assessed by real-time RT-PCR. Among 30 selected OR genes, we detected the expression of 18 ORs in cultured OSNs. Lentiviral vectors expressing a selected OR can be infected into cultured OSNs efficiently and functional exogenous OR expressions are detected. Exogenous I7 was expressed in OSNs for 5 days *in vitro* (DIV). Levels of endogenous OR transcripts in exogenous I7 expressing culture were compared with that of the control GFP expressing culture. We observed reduced expression levels for all tested endogenous OR genes in exogenous I7 expressing OSN culture. We further rested this phenomenon by introducing either exogenous P2 or MOR118 to the culture. Consistently, exogenous OR expression suppress the transcript levels of endogenous ORs at 5 DIV. Furthermore, we observed that exogenous MOR118 expression suppresses the expression of endogenous MOR118 as well. Whether other exogenous OR expressions also suppress their endogenous OR transcripts is being investigated. Acknowledgements: NIH/NICDC DC010237

230 Heterologous Expression of Mouse Pheromone Receptors Identifies Cognate Ligands

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Pheromones are chemicals from conspecifics that affect innate behavior or hormonal changes. In mammals, the vomeronasal organ (VNO) is thought to play a prominent role in detecting pheromones; the vomeronasal sensory neurons (VSNs) express three families of seven-transmembrane G-protein coupled receptors (GPCRs): the V1Rs, V2Rs, and FPRs, in two molecularly and spatially-distinct regions. In mice, VSNs that express the V2Rs are thought to detect peptide cues, including MHC-presenting peptides, major urinary proteins (MUPs), and exocrine gland-secreting peptides (ESPs). They are thought to be involved in various pheromone-mediated behaviors and physiological changes, such as mating, aggression, and selective pregnancy block. In order to understand how pheromones are detected by the vomeronasal receptors, it is essential to know which receptors are activated by a given chemical. However, identifying cognate ligands for the V2Rs has been challenging, partly because they are poorly localized to the surface of heterologous cells. Here, we show the establishment of heterologous cell system to functionally identify the V2Rs and demonstrate that the ESP ligands can differentially activate the V2Rs. We also show the large extracellular domain of the V2Rs plays a critical role in ligand selectivity. Our results provide a platform to characterize ligand selectivity of the V2Rs and suggest that a unique mechanism that regulates the functional expression of the V2Rs.

231 Muscarinic Receptor M3 Potentiates the Function of a Broad Range of Mammalian Odorant Receptors

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Mammalian olfaction begins with the binding of odorant molecules to specific odorant receptors (ORs), which are expressed on the surface of olfactory sensory neurons (OSNs) and make up the largest family of G-protein coupled receptors (GPCRs). Here we show that type 3 muscarinic acetylcholine receptor M3, a non-OR Class A GPCR expressed in OSNs, enhances odorant-specific response of a large set of ORs. Coexpression of M3 in heterologous cells produces an ~10-fold leftward shift in EC_{50} values and increases the maximum response by 25-1000% for a broad range of ORs in the cAMP-mediated luciferase reporter gene assays for OR activity. Coexpression of M3 does not enhance the cell-surface expression of the ORs. Thus, the action of M3 is distinct from that of known accessory factors, such as the RTPs. The M3-dependent potentiation of OR activity is significantly enhanced in the presence of RTP1, indicating that the effect of M3 is synergistic and increases the response of ORs

already at the cell surface. M3 potentiation of OR activation is further enhanced by muscarinic agonist carbachol, but inhibited by the muscarinic inverse agonist atropine. Conversely, OR activation by cognate odors causes the activation of M3 downstream signaling by eliciting a Ca^{2+} response, an effect that is inhibited when cells are simultaneously exposed to odor and atropine. The interaction and crosstalk observed between ORs and M3 in heterologous cells is likely dependent on the ability of these GPCRs to form stable heterometric complexes, as ORs and M3 coprecipitate each other in HEK293T cells. Our study suggests that OR and M3 heteromers functionally couple with one another, and will serve as an effective screening tool to identify active ligands for the ORs. This work was supported by NIH-NIDCD. Acknowledgements: NIH-NIDCD Duke Undergraduate Research Support Grants

232 The OR37 subfamily: establishment of the clustered expression pattern

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In contrast to most other olfactory sensory neuron (OSN) populations, cells expressing a member from OR37 subfamily of odorant receptor (OR) genes are restricted to a small central patch of the mouse olfactory epithelium (OE). To obtain insight into the regulatory mechanisms which determine this unique spatial organization, a lineage tracing approach (mOR37C-IRES-Cre) was performed to visualize all cells that transcribed OR37C at any time during differentiation. As expected, labelled OSNs were found in the typical central patch; surprisingly, however, numerous additional OSNs were found which were broadly dispersed throughout the OE. Using in situ hybridization, the mRNA for OR37C could only be detected in those cells located in the typical patch, suggesting that all ectopic OSNs had ceased OR37C expression. The question whether these cells may undergo premature apoptosis was addressed by analysis for active caspase-3; none of them, however, expressed this pro-apoptotic marker. A close examination of the ectopically positioned OSNs revealed that they all extended an axon towards the olfactory bulb (OB), and indeed many glomeruli could be detected which contained a few labelled fibers. The location of these glomeruli in the medial and lateral domains of the bulb indicated that these represented glomeruli that receive input from OSN populations expressing other ORs than OR37C. Altogether, these data indicate that OSNs which initially express OR37C outside the typical patch do not continue, but switch to the expression of a different OR gene, suggesting the involvement of a mechanism downstream of gene choice that restricts OR37C expression to the central patch. Acknowledgements: Supported by the Deutsche Forschungsgemeinschaft

233 Expression of odorant receptor genes on the olfactory epithelium following olfactory nerve transection

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Objective To construct unilateral olfactory nerve transection model of rats and observe the change of expression quantity and position of odorant receptors in regenerated olfactory epthelium. **Methods** Experimental group and control group consisted of 20 and 12 rats respectively. The left olfactory bulb of the rats in the former group was exposed under microscope and the olfactory nerve was transected along cribriform plate; the rats in control group didn't accept any treatment. The change of cell morphology, and quantity and thickness of epithelium were observed by HE staining. The expression pattern of olfactory receptor genes Olr287, Olr226, Olr1493 and Olr1654 in olfactory epithelium and the distribution and quantity changes of each gene during regeneration process of epithelium were observed by gene probe in situ hybridization(ISH). **Results** HE staining showed that 5 days after the operation cell quantity and thickness of the olfactory epithelium decreased obviously. After 6 weeks' recovery, the thickness of the epithelium could reach the control level. The gene probe in ISH showed that the expression pattern of the receptor during epithelium regeneration after olfactory nerve transection was unchanged. Five days after surgery, the average number of Olr1493 gene receptor positive cells in the operated side of experimental was significantly less than that of the control side of experimental group. Until 6 weeks the cells expressing Olr1493 gene receptor in regenerated epithelium of the olfactory transected side was basically reached the same level as the control side and the difference was statistically insignificant. **Conclusion** The regeneration of the sensory neurons and receptors, both the number and the distribution pattern, can recover to normal after olfactory nerve transection. Acknowledgements: Source of support: China Natural Scientific Foundation under project No. 30740052, Beijing Natural Scientific Foundation under project No. 5072018, Capital Medical

234 Olfactory Detection of Aldehydes: Comparison of Dose-Response Functions at the Behavioral and at the Cell/Receptor Levels

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Within the wider goal of understanding the physicochemical basis for the olfactory potency of odorant vapors towards humans, this study presents dose-detection functions for the odor of propanal, butanal, hexanal, octanal, nonanal, and helional. Subjects ($16 \leq n \leq 18$) comprised young adults (18-37 years old) from both genders, all normosmics and non-smokers. Odorant stimuli were quantitatively generated, controlled, and presented via an 8-station vapor delivery device. Gas chromatography served to calibrate and quantify vapor concentrations. Methods included a three-alternative forced-choice procedure against carbon-filtered air blanks and an ascending concentration approach. Group and individual psychometric functions were modeled by a sigmoid (logistic) equation from which an odor detection threshold (ODT) can be defined. ODTs decreased from propanal to octanal: 2.0, 0.46, 0.33, and 0.17 ppb, then increased for nonanal: 0.53 ppb. The potent olfactory-receptor ligands helional (ODT=0.14ppb) and octanal had the lowest ODTs. Inter-individual variability (ODT ratio between most and least sensitive subject) ranged from 10 to 50 times, and was highest for octanal and hexanal. The behavioral

functions showed an olfactory sensitivity 2 to 5 orders of magnitude higher than that shown by cell/receptor functions tracing the activation of specific human olfactory receptors by the same aldehydes, after all functions were expressed as vapor concentrations. The difference between the two types of functions was smallest for the very specific olfactory-receptor ligand helional. The outcome suggests that the sensitivity gap favoring behavioral odor detection functions over cell/receptor olfactory functions might reach a minimum for odorants narrowly tuned to one (or very few) specific olfactory receptor(s). Acknowledgements: Supported by grant R01 DC 002741 from the NIDCD, NIH.

235 Retronasal but Not Oral-Cavity-Only Identifications of Isointense TRPM8 Agonists

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Six TRPM8 agonists were presented retronasally or oral-cavity-only (OCO) for identification as isointense vapor-phase odorants. Participants 1st selected, retronasally or OCO, with reference to undiluted geraniol, isointense vapor-phase concentrations from separate series of sunflower-oil-dilutions of l-carvone, eucalyptol, isopulegol, linalool, and dl-menthol. Next, participants chose identifications for their 5 selected vapor-phase dilutions, and undiluted vapor-phase geraniol, all randomized, from 9 identification choices on digital computer displays under forced-choice conditions, retronasally or OCO. No training for 'correct' identification was done. RESULTS: Overall, the 13 retronasal participants chose different identifications across the 6 isointense odorants and 9 identifications (ANOVA, $p < 0.0001$). Retronasal l-carvone identifications (modal identification = spearmint) differed from geraniol (lemon), linalool (cleaner), dl-menthol (ointment) ($p < 0.002$), and isopulegol (vapor-rub) ($p = 0.01$), but not from eucalyptol (vapor-rub), $p = 0.07$. In contrast, the 7 OCO participants showed no overall difference in identifications chosen (ANOVA, $p = 0.58$). CONCLUSIONS: Isointense vapor-phase TRPM8 agonists, presented retronasally, do not share a common identification, suggesting that mechanisms in addition to TRPM8 channels are stimulated. The lack of OCO identification differences suggests that vapor-phase TRPM8 agonist may not elicit a sufficiently differential oral cavity trigeminal response. Support from a Susan Linn Sage Professorship.

236 Characterization of Ca^{2+} currents in identified subpopulations of rat geniculate ganglion neurons

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Calcium channels play an important role in synaptic transmission and excitability of sensory neurons. Voltage-gated Ca^{2+} channels are classified into high- (HVA; N, L, P, Q and R-type) and low-voltage-activated (LVA; T-type) subtypes. We now report on the subtypes of Ca^{2+} currents expressed by subpopulations of geniculate ganglion (GG) neurons innervating different receptors. Under brief anesthesia, 5% Fluorogold was injected into the anterior tongue, the soft palate and the inner surface of the ear in 32 to 46 day-old rats. The rats were allowed to recover and 3 to 12 days later the GG removed and the neurons dissociated using enzymatic digestion and trituration. Whole-cell recordings were performed on the labeled neurons innervating the anterior tongue via the chorda tympani (CT neurons, $n = 60$), the soft palate via the greater superficial petrosal nerve (GSP neurons, $n = 39$) and the inner surface of the ear via the posterior auricular branch of the facial nerve (PA neurons, $n = 29$). Ca^{2+} current subtypes were identified using voltage step pulses and selective channel blockers. T-type Ca^{2+} currents were observed in 65% of CT neurons, 72% of GSP neurons and 97% of PA neurons. The T-type Ca^{2+} current density in PA neurons was significantly larger than in the CT or GSP neurons. CT, GSP and PA neurons had nimodipine-sensitive L-type, w-conotoxin GVIA-sensitive N-type, and w-agatoxin IVA-sensitive P/Q-type Ca^{2+} currents and there were significant differences in the expression of L-, N- and P/Q-type Ca^{2+} currents between the CT, GSP and PA neurons. These results indicate that subpopulations of GG neurons have distinct biophysical properties of Ca^{2+} currents, which possibly relate to the types of receptors innervated by these neurons. Supported by NIDCD grant DC-000288 (RMB).

237 TEMPERATURE ALTERS SUMMATED EPITHELIAL POTENTIALS OF TONGUE AND SINGLE-CELL RESPONSES OF GENICULATE GANGLION NEURONS TO CHEMICAL STIMULATION IN RATS

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Temperature is important for chemical sensitivity of taste receptors and responsiveness of peripheral gustatory neurons. In anesthetized male rats, we recorded stimulus-evoked lingual potentials (electrogustogram; EGG) simultaneously with single-cell 2.5-s neural responses from 8 narrowly tuned (4 NaCl-best; 3 MSG-best; 1 sucrose-best) and 5 broadly tuned (5 citric acid-best) neurons. We recorded EGG and single-cell responses to 100 mM NaCl, 100 mM MSG, 500 mM sucrose, 10 mM citric acid, 20 mM quinine HCl, and 100 mM KCl at least 3 times each between 23° - 41°C in both ascending and descending temperature steps of 3°C. Artificial saliva (15mM NaCl, 22mM KCl, 3mM $CaCl^2$, 0.6mM $MgCl^2$) served as the rinse solution and solvent for all taste stimuli. Our preliminary findings show that temperature influenced the average EGG response amplitude the most for NaCl and KCl and the least to citric acid. The EGG response amplitude to NaCl and KCl was optimal to 3 highest temperatures (35, 38, 41°C) and declined progressively with decreasing temperature to no response at 23°C. Temperature also influenced breadth of tuning of neuron types. For example in narrowly-tuned cells, NaCl-best neurons responded selectively to NaCl across all temperatures between 41 - 28°C, but also responded to KCl at 25 and 23°C. MSG-best neurons responded selectively to MSG between 28-35°C, but also responded to sucrose at 38 and 41°C. In contrast, broadly-tuned citric acid neurons responded to all chemical stimuli at 35°C, but selectively to citric acid at 23 and 25°C. Thus at lower and higher temperature extremes, narrowly-tuned neurons became more broadly responsive, while broadly-tuned neurons became more narrowly responsive. Acknowledgements: Supported by NIH grant R01 DC004785.

238 Primate Sweet taste is caused by impulses in a dedicated group of taste fibers

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The division between a sweet and bitter taste quality is evident already in the newborn. The discovery of a unique set of taste receptors for the sweet and bitter taste quality provides an answer to how sweet and bitter taste is created, but the question how this information is coded in taste nerves remains an enigma. For many years we have studied taste fibers in primates and found that their single taste fibers cluster according to human taste qualities. Comparisons of effects of the sweet taste modifiers gymnemic acid and miraculin on behavior and taste fiber activity demonstrate that liking of sweet correlates only with changes of activity in fibers clustered as S (sweet best) fibers. Here we test the validity of this theory with lactisole. It suppresses the human sweet taste. We found that the intake of sweeteners by *Macaca fascicularis* diminished significantly after 1.25 mM lactisole had been added. The fact that the animals did not discriminate between lactisole alone and water suggests that lactisole per se did not influence preference. Recordings of 40 single taste fiber showed that lactisole suppressed the response to sweeteners in fibers responding best to sweet, the S-cluster, but had no effect on the responses in fibers that responded to sour, bitter and salt, the H, Q- and N-clusters. Lactisole had been reported to block the T1R3 monomer of the sweet taste receptor T1R2/R3. Consequently, these results suggest, not only that the perception of the sweet taste quality is linked to activity in fibers of the S-cluster, but also that the fibers affected conveyed taste from T1R2/R3 receptors, while the source of the impulses in non-S fibers are other kinds of receptors. Acknowledgements: R01DC06016,

239 Taste-location generalization as a novel tool to study rodent taste and flavor perception

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In order to understand the perception of taste in rodents we can use the established paradigms of conditioned taste aversion (CTA) -generalization or the Morrison task. Our research into the neural bases of taste-odor integration, has lead us to develop a new technique to directly evaluate the on-line expression of taste perception and taste-acquisition of odors by rats. This system has been optimized to be compatible with extracellular recordings. It is similar to the paradigm established by Youngentob, et al. (Phys & Behav, p1053-1059, 1990) for odorants. Water-restricted rats are taught to lick a central gustometer manifold presenting one of five stimuli (water and four prototypical tastants), and subsequently move to and lick water from one of five surrounding spouts. Only licking the correctly mapped spout results in presentation of water. Stimuli and responses are measured throughout the typically 40-minute session of 200-400 trials. After acquisition of this task novel stimuli are also presented at the manifold after which rats are rewarded at any spout of their choice. The spatial response pattern directly reflects the similarity of the novel stimulus to the acquired training set of tastants. Four rats were successfully trained, achieving 60-80% accuracy after 130 days of training. Controls confirmed rats used taste cues to guide their responses. One of the rats showed deviant responses, which ultimately converged with those of the other rats. All rats showed clear concentration-response functions (0, 3,10, 33, 100 and 300% of conc. of train stimuli), which were stable across test sessions. Generalization to novel tastants was also evaluated. Rats can learn to associate tastes with locations, which can be used to investigate their perception of taste quality and intensity of novel stimuli. Acknowledgements: Supported by R01 DC009994-01

240 Behavioral and anatomical characterization of sucralose preferring and avoiding rats

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Rats display a bimodal preference for the non-nutritive sweetener sucralose. While some prefer sucralose over water across a range of concentrations, others avoid sucralose at concentrations above 0.1 g/L. While this phenomenon has been studied primarily in males, there is some evidence that females may differ from males in their preference for sucralose. Currently, the mechanism underlying individual differences in sucralose preference in either sex is poorly understood, but some data suggest that it may be related to individual differences in sensitivity to a bitter taste quality of sucralose. In humans, increased sensitivity to bitter substances appears to be positively correlated with the number of fungiform papillae counted on the surface of the tongue. The goal of the present study was to determine whether preference for sucralose is influenced by sex and/or the number of fungiform papillae on the rat's tongue. Male and female rats (n=22/sex) were given access to ascending concentrations of sucralose (0.0001 - 2.0 g/L) and water in two-bottle, 24-h preference tests. While a greater proportion of males than females were characterized as sucralose preferrers (40% vs 30%), once categorized, we found no sex difference in their preference curves. The tongues of a subset of these rats (10 preferrers; 11 avoiders) were incised just anterior to the median molar eminence, stained with methylene blue, and the number of fungiform papillae with clear taste pores was counted. Sucralose avoiders had significantly more papillae than did sucralose preferrers (109±4 vs 95±5, respectively, $t(19)=2.18$, $p<0.05$). We conclude that sucralose preference curves among preferrers and avoiders are not sexually dimorphic and that preference for sucralose is positively correlated with the number of fungiform papillae. Acknowledgements: Supported by NIH grant DK73936.

241 Natural Variation in Sucralose Drinking Patterns in Rats.

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Rats display variability in their preference for the artificial sweetener sucralose over water. To explore this variability, we recorded the licking behavior of rats on water and increasing concentrations of sucralose via 2-bottle, 24-h preference tests. Rats that preferred sucralose to water at all 7 concentrations were classified as preferers

(n=9). Rats that displayed a preference at the lowest concentrations (0.0001 - 0.01 g/L) but avoidance (preference scores ≤ 10%) at the highest concentrations (0.25 - 2.0 g/L) were classified as avoiders (n=14). At low concentrations (≤ 0.01 g/L), neither intake, preference, rate of licking, bout size (i.e. licks/bout), nor bout number were predictive of the rats' preference/avoidance classification. Similar comparisons could not be performed at high concentrations (≥ 0.25 g/L) since preferers consumed almost all of their fluid as sucralose (~90% preference) and avoiders consumed water almost exclusively (~ 4% preference). However, bout analysis of sucralose vs. water intake revealed that preferers drank more bouts of sucralose than the avoiders drank bouts of water. While no group differences in bout size were evident, the rate of drinking was higher in the preferers drinking sucralose than in the avoiders drinking water. At an intermediate concentration, 0.1 g/L of sucralose, avoiders consumed less sucralose than the preferers, but intakes in both groups exceeded the threshold for examining bout structure. The avoiders' reduction in intake was due to a decrease in bout number, not bout size. These data compliment previous reports that another artificial sweetener, saccharin, also increases bout number compared to water. However, these findings are novel as they describe variation within the population across sucralose concentrations. Acknowledgements: NIH 5 T32 DC000044 & DK73936

242 Experience induced changes in sugar taste sensitivity take place in or before the sugar taste receptor cell of Drosophila melanogaster

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Experience with sweeteners and monosodium glutamate (MSG) increases taste sensitivities for sugars and MSG in humans (Eylam & Kennedy, 1998; Kobayashi & Kennedy, 2002; Gonzalez et al., 2007, 2008), and human psychophysical and hamster chorda tympani data suggest a peripheral nervous system mechanism (Faurion et al., 2002; Hassan et al., 2006). To further explore the locus of the mechanism, we conducted behavioral and receptor cell neurophysiological studies in Drosophila melanogaster. This species avoids stimuli that humans perceive as bitter and has similar sweet taste preferences to humans (Gordesky-Gold et al., 2008). Experience induced changes in sugar taste sensitivities occurred in both Oregon R and Canton Special strains of D. melanogaster. Flies raised on a fructose based medium chose to eat significantly more glucose or fructose of mid-range concentrations than flies raised on a glucose based medium did, in both two-choice and multi-choice behavioral tests. Likewise, the sugar taste receptor cells of fructose reared flies responded to fructose or glucose of mid-range concentrations with significantly greater firing rates than the receptor cells of flies reared on glucose medium. A significant positive correlation between the behavioral and neurophysiological data suggests that at least a portion, if not all, of the changes in behavior resulted from the changes in sugar receptor cell firing. These are the first data to show that experience induced changes in taste sensitivity take place in the receptor cells. They indicate a mechanism for the experience induced changes in or before the taste receptor cells. Acknowledgements: KMG was supported by a NSF Graduate Research Fellowship. The work was supported by NIH NIDCD R15 DC/OD0266 and R15 DC009042 to LMK and Clark research and travel funds to KMG.

243 Taste aversion to quinine in mosquitoes

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Quinine is avoided by many animals (e.g. mice and fruit flies) because it elicits a bitter taste, indicating it is a potential toxin. Despite its aversive taste to humans, it has been used effectively to treat malaria (caused by *Plasmodium* parasite infection) for many decades. However, whether vectors of *Plasmodium*, mosquitoes, avoid the taste of quinine has not been reported. In this study, we investigated taste aversion to quinine in an Anopheles gambiae strain responsible for much of the malaria transmission in Africa and elsewhere. A sucrose solution dyed with either red or blue food colorings was given to each tested mosquito and its intake measured by the color change appearing in the mosquito's abdomen. The head of each mosquito was placed in contact with the sucrose solution and then the mosquito tarsi were stimulated by either water, quinine or ATP (adenosine triphosphate). Quinine clearly inhibited ingestion of the sucrose solution, whereas ATP tended to enhance it. Our results suggest that (1) mosquitoes taste with their tarsi, (2) this input modifies their ingestion, and (3) the anti-malarial drug quinine inhibits ingestion due to its aversive chemoreceptive input. Acknowledgements: The work was funded by a grant from the Gates Foundation, Grand Challenges Explorations to PASB.

244 Plant Root Exudates as Chemoattractants for *Paramecium.*

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Paramecium tetraurelia congregate in bacterial populations by manipulating their membrane potential to bring about changes in swimming behavior. *P. tetraurelia* feed on bacteria and research has suggested that these ciliates use bacterial metabolites to locate populations of their food source. Attractants such as biotin, folate, glutamate, cAMP, and acetate bind to extracellular receptors, which hyperpolarize the cell. Hyperpolarization causes cilia to beat faster and reorient power stroke less frequently, resulting in accumulation in attractant solutions. The bacterial metabolites that generate chemoresponse behavior have not been measured in the required concentrations in pond water. This could be a reflection of the behavioral assays employed or the result of an artificial stimulation of chemoreceptors due to an overwhelming concentration of low affinity interactions. We asked whether plant compounds (specifically root exudates), which occur in higher concentrations than bacterial compounds in pond water, might serve as the required bait to place these organisms in the appropriate location so that they can feed. We discovered that the two root exudates we initially tested, gamma-aminobutyric acid and betaine, both stimulated *Paramecium* at concentration levels appropriate for pond water in our ciliary beat frequency assay. Calcium imaging confirms the results of the behavioral assay. We plan to screen more compounds commonly secreted by plant roots to determine if these are the physiological cues that *Paramecium* utilize in their natural environment. Of significant interest are compounds involved in the development of the bacterial rhizosphere since signals that drive

bacterial colonization may attract predators like *Paramecium*. Funded by a VMI Research Grant in Aid.

245 Sensory mechanisms of chemical deterrence by sea hare ink against predatory blue crabs

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The sea hare *Aplysia californica* releases a purple ink when attacked by predators, and this ink is a powerful feeding deterrent against the blue crab *Callinectes sapidus*. This deterrence is expressed either by rejection of food or an increase in the time the animals handle the food with their mouthparts before ingesting. Towards understanding the mechanism of this chemical deterrence, we used behavioral and electrophysiological techniques to identify the location and characterize the sensitivity of the chemoreceptors responsible for ink deterrence. Using selective ablation of mouthparts and behavioral assays, we showed that the chemoreceptors mediating deterrence are not located on the legs or restricted to a specific mouthpart. We then focused our electrophysiological efforts to record single-unit responses from mouthpart chemoreceptor neurons using as a chemical deterrent a fraction of ink highly enriched in aplysioviolin and phycoerythrobilin (= APV-PEB fraction). Our results indicate that there is not a population of cells specific for these chemical deterrents. Single-unit chemoreceptor responses are neither intense nor specific to this fraction; instead APV-PEB evokes a unique across fiber pattern of activity. Additionally, cross-adaptation experiments revealed no effect of the APV-PEB fraction on the responses of mouthpart chemoreceptors to other chemical stimuli. We conclude that the deterrent effect of APV-PEB is mediated, at least partially, through chemoreceptor neurons in the mouthparts that encode these signals not via deterrent-specific neurons but through a distributed neural code. Acknowledgements: NSF IBN-0614685

246 THE TASTE OF SALICIN IN HAMSTERS

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Salicin is a β-glucopyranoside derived from willow tree bark. The taste of salicin, bitter to humans, is thought to be mediated by the TAS2R16 receptor, which has orthologs in rats and mice with ~50% amino acid identity. In order to examine the behavioral taste of salicin in the golden Syrian hamster (*Mesocricetus auratus*) we conditioned 7 animals to 10 mM salicin (experimental group) and 7 animals to deionized water (control group). After a single conditioning trial, all animals were tested twice with the following test stimuli (TS): 10 mM salicin, 2 analogs of salicin: 10 mM phenyl-β-D-glucopyranoside (P-β-D) and 10 mM phenyl-α-D-glucopyranoside (P-α-D), 0.3 mM quinine•HCl (QHCl), 3 mM caffeine, 1 mM sucrose octaacetate, 1 mM saccharin, 100 mM sucrose, 100 mM NaCl and deionized water. P-β-D tastes bitter to humans, but P-α-D does not. The TS were presented in a counterbalanced order. Because the limited amount of P-α-D available was insufficient to complete 2 test trials in all animals, it was omitted from the ANOVA. The aversion learned to salicin (60% suppression, $p < 0.001$) generalized only to P-β-D (36% suppression, $p < .01$) and QHCl (36% suppression, $p < .01$). A *t*-test revealed no difference between experimental (n=5) and control (n=6) intake of P-α-D. Thus, in hamsters the taste of salicin shares a perceptual quality with QHCl and, as in humans, the P-β-D analog of salicin, but not the P-α-D isomer. Acknowledgements: NIH R01 DC004099-09 and T32 DE0073-14.

247 IS THERE MORE THAN BITTER TO THE TASTE OF SALICIN IN HAMSTERS?

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Salicin and an analog, phenyl-β-D-glucopyranoside (P β-D), activate the human taste receptor TAS2R16. Salicin, a β-glucopyranoside derived from willow bark, tastes bitter to humans. To behaviorally evaluate the complexity of the taste of salicin in the golden Syrian hamster (*Mesocricetus aratus*) we *reconditioned* 23 animals to 10 mM salicin (n=7), 1 mM quinine•HCl (QHCl) (n=7) and deionized water (n=9). After the second conditioning trial, all animals were tested 3 times with the following test stimuli (TS): 3 and 10 mM salicin, 10 mM P-β-D, 0.3 and 1 mM QHCl, 100 mM sucrose, 100 mM L-phenylalanine, 10 mM D-phenylalanine, 100 mM NaCl and deionized water. TS were presented in a counterbalanced order. One animal did not demonstrate an aversion to salicin was excluded from analysis. The aversion learned to salicin (57% suppression compared to water control animals, $p < 0.0001$) significantly generalized to 1 mM QHCl (46%, $p < 0.001$), 3 mM salicin (42%, $p < 0.001$) and P-β-D (44%, $p < 0.001$). In contrast, the aversion learned to QHCl (70% suppression, $p < 0.0001$) generalized significantly only to 0.3 mM QHCl (41%, $p < 0.01$). These results imply that salicin activates a quinine ''receptor,'' but quinine may not activate a salicin receptor in hamsters. Thus, there may be more to salicin taste than bitter if all hamster TAS2R receptors reside in bitter-dedicated taste receptor cells. Acknowledgements: NIH R01 DC004099-09

248 Perceptual Mapping of Cooling Ingredients – The Role of Ethnic, Biological and Product-Use Variables.

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Individual differences in the perception of cooling are poorly understood. We previously reported that Asians perceived more heat/burning from derivatives of l-menthol than Caucasians. For this analysis, data from East Asians (n=94) and Caucasians (n=102) were extracted from three studies in which subjects rated cooling, heat/burning, bitterness and tingling from: the novel ingredients, Coolact® 5, 10 and 38D; mono-(l)-menthyl succinate (SUC), mono-(l)-menthyl glutarate (GLU) and (l)-menthyl lactate (LAC); Coolact 38D/LAC blend; and Coolact 5/Coolact 10 blend. Subjects indicated the areas in the mouth and throat where they perceived each sensation. They also completed a questionnaire on habitual use of products with cooling ingredients. Principal component analysis was used to model the data with separate models for East Asians and Caucasians. For both groups, two dimensional spaces fit the data best; Dim 1 was related to product attributes

(cooling, heat/burning and tingling); and Dim 2 was related to product use (gum, mints and mouthwash). Dim 1 accounted for 52% of the variance in both models, and Dim 2 accounted for 28% and 34 % of the variance in the East Asian and Caucasian models, respectively. In both spaces, the attributes cooling, tingle and heat/burning were associated with the tongue and gums but not other locations (cheeks, throat or roof of the mouth). Cluster analysis revealed 3 groupings for the samples in Caucasians (LAC; SUC + GLU; all others) and 4 groupings for East Asians (LAC; SUC + GLU; Coolact 38D + Coolact 5/Coolact 10 blend; Coolact 5 + Coolact 10 + Coolact 38D/LAC blend). These results suggest that East Asians perceive some cooling ingredients differently than Caucasians which might be related to experience or typical use patterns for products with cooling ingredients. Acknowledgements: Supported by Takasago International Corp. (USA)

249 Both Warming and Cooling Enhance the Bite of Carbonation

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Carbon dioxide, which gives carbonated beverages their distinct bite, probably stimulates through transient acidification of the mucosa. Work by Barry Green and colleagues showed that cooling carbonated water to below tongue temperature enhanced carbonation bite, suggesting that cool-sensitive fibers play a role. Other work suggests that TRPV1-expressing fibers, which are sensitized by warming, account for some of the response to carbon dioxide. Accordingly, we tested the hypothesis that warming carbonated water above tongue temperature would also enhance carbonation bite. Carbonated water was presented at four nominal concentrations (0.0, 2.0, 2.8, and 4.0 v/v) X five temperatures (18.3, 33.9, 38.95, 44.9, and 48.2 °C). Temperature spanned a range from well below to well above tongue temperature. Subjects dipped the anterior portion of their tongues into cups of carbonated water, and rated carbonation bite. Each subject received all combinations of temperature and carbonation level (within-subjects design). Rated carbonation bite followed a U-shaped (significant quadratic) trend with respect to temperature. Thus, we replicated the finding that cooling enhances carbonation bite, and extended previous results to show that warming also enhances bite. Measured carbonation levels decreased somewhat as temperature increased, perhaps from more rapid loss after opening sample bottles. However, the degree of CO_2 loss was relatively small, and would be expected to undermine carbonation bite at higher temperatures rather than enhance it. Accordingly, we conclude that fibers sensitive to both cooling and warming (or both cool- and warm-sensitive fibers) play a role in carbonation sensation. Acknowledgements: Supported in part by The Coca-Cola Company

250 EXPRESSION, SOLUBILIZATION, PURIFICATION AND RECONSTITUTION OF THE HUMAN EPITHELIAL SODIUM CHANNEL INVOLVED WITH SALTY TASTE

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Human fungiform taste bud cells express the heterotrimeric epithelial sodium channel (ENaC), of subunits delta, beta and gamma (d,b,g). Single taste cell PCR rarely showed complete alpha (a) transcript. In rodents, the d gene is an unexpressed pseudogene and the tongue ENaC is a heterotrimer of a b g. The Na response in rodents is sensitive to amiloride while that of human is (generally) not. In other body tissues, the ENaC containing delta is known to be less sensitive to amiloride than that containing alpha, but is, nevertheless, still demonstrably sensitive to amiloride. To investigate this disparity, we expressed and purified the a, d, b and g units through Sf9 cells and reconstituted these into a lipid bilayer. We co-expressed Sf9 insect cells with three baculoviruses, each encoding individual human ENaC subunits, a, b, g, and d, b, g. The cells were lysed into detergent buffer, the subunits purified by affinity chromatography, and the detergent removed by dialysis out of a lipid-containing cassette. For characterization of each heterotrimer channel, the ENaC-containing liposomes were fused with a lipid bilayer. Recombinant a, b, g displayed single-channel Na conductance of 21 pS in 200 mM NaCl, and was blocked by low concentrations of amiloride (apparent K_i amiloride, 0.5 uM). Reconstituted d,b,g-ENaC protein resulted in smaller single channel conductance of 17 pS and higher K_i amiloride of 5 uM, consistent with expected activity of d versus a. These observations were similar to those observed from reconstitution of membrane vesicles prepared directly from d-b-g or a-b-g transfected Sf9 cells. Successful reconstitution of purified subunits means we can study the channel in isolation, modifying its environment with impunity.

251 Taste-evoked chorda tympani responses to $CaCl_2$ are larger in PWD/PhJ than in C57BL/6J mice.

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PWD/PhJ (PWD) mice show preferences for calcium-containing compounds in two-bottle tests with water, whereas C57BL/6J (B6) mice avoid them. It is not known whether this behavioral difference depends on taste sensation, although taste plays an important role in guiding calcium intake in general. We measured taste-evoked chorda tympani (CT) responses in male B6 and PWD mice in order to examine whether peripheral gustatory events differ between the strains. Each mouse was anesthetized and its right chorda tympani nerve was accessed through the ear in order to record whole-nerve responses as solutions were flowed over the tongue. Responses were significantly higher in PWD than B6 mice for $CaCl_2$, $MgCl_2$, citric acid and quinine, but did not differ between the strains for sucrose and NaCl. These findings were consistent with results from two-bottle choice tests; relative to the B6 strain, the PWD strain had higher preferences for $CaCl_2$, $MgCl_2$, citric acid and quinine, but not for sucrose or NaCl. These data suggest that differences in peripheral events, such as taste transduction, contribute to the differences between B6 and PWD mice in preferences for taste solutions. Acknowledgements: Funding provided by NIH RO1 DK-46791

252 Calcium sensing receptor agonists induce response in taste cells

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Many researches have identified the signaling of basic tastes such as sweet and umami. However, the mechanisms underlying the generation of palatability are not clear. Taste enhancer (is called "*kokumi*") for salty, sweet and umami is one of the important factors for palatability of foods, and we recently reported that calcium sensing receptor (CaSR) is a *kokumi* receptor (Ohsu *et al.* J. Biol. Chem. 2010). Interestingly, CaSR agonists enhance the basic tastes, although they do not induce any taste when they are applied alone. In this study, we figured out the receptor cells for taste enhancers, and their physiological properties. For this purpose, we used Calcium Green-1 loaded mouse taste cells in lingual tissue slice and confocal microscopy. Taste enhancers applied focally around taste pore induced increase of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in a subset of taste cells. These responses were inhibited by pretreatment with CaSR inhibitor, NPS-2143. Taste enhancer-induced responses did not require extracellular Ca^{2+}. In addition, a part of taste enhancer-responding cells also responded to depolarizing stimulation with 50 mM KCl. These observations indicate that CaSR-expressing taste cells are primary detector of taste enhancers, and these cells are type III and non-type III taste cells.

253 Expression and characterization of ligand-binding domain of T1R1 taste receptor

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Umami is the typical taste induced by monosodium glutamate, which is thought to be detected by a heterodimeric G-protein coupled receptors, T1R1 and T1R3. The most unique feature of umami taste is its potentiation by purine nucleotide monophosphates (IMP, GMP), which also elicit umami taste by their own. Zhang et al. (Proc. Natl Acad. Sci. USA, 2008) have recently proposed a cooperative ligand-binding model involving T1R1 N-terminal domain (NTD), where L-glutamate (L-glu) binds close to the hinge region, and purine nucleotides bind to an adjacent site close to the opening of the Venus flytrap domain. To further understand the structural basis of umami stimuli recognition by T1R1, a large amount of purified T1R1 NTD is suitable for biochemical and structural studies. Here, we report the successful expression and purification of the soluble NTD of human T1R1. The protein was expressed as insoluble aggregated protein (inclusion bodies) expressed in high level in *Escherichia coli*. The protein was solubilized and in vitro refolded using suitable buffer and additives. We then measured the binding of umami stimuli to T1R1 NTD using fluorescence spectroscopy. Fluorescent assay demonstrated that T1R1 NTD is properly refolded and able to bind L-glu with physiological relevant affinity. The mode of interaction was specific since T1R1 NTD did not bind sweet stimuli like fructose, sucralose or glycine. In addition, we observed that L-glu binding affinity is enhanced in presence of IMP. To further validate our results, we have generated single amino-acid changes in L-Glu and IMP binding sites. In summary, our expression system will allow large scale production of active protein suitable for structural and functional studies. Acknowledgements: This work was supported by INRA and Burgundy council.

254 The interaction between PKD1L3 and PKD2L1 through their transmembrane domains is required for localization of PKD2L1 protein at taste pore in taste cells of circumvallate and foliate papillae

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Polycystic kidney disease 1 like 3 (PKD1L3) and polycystic kidney disease 2 like 1 (PKD2L1) have been proposed to form heteromers to function as sour taste receptors in mammals. Here we show that PKD1L3 interacts with PKD2L1 through their transmembrane domains, but not through the coiled-coil domain, by co-immunoprecipitation experiments using a series of deletion mutants. The deletion mutants lacking the region critical for the interaction were not transported to the cell surface but retained in the cytoplasm, whereas PKD1L3 and PKD2L1 proteins are expressed at the cell surface when both are transfected. Calcium imaging analysis revealed that neither the coiled-coil domain nor EF-hand domain located in the C-terminal cytoplasmic tail of PKD2L1 is required for response upon stimulation with acid solution. Finally, PKD2L1 protein was not localized to the taste pore but robustly distributed throughout the cytoplasm in taste cells of circumvallate and foliate papillae in *PKD1L3* (-/-) mice, in which the genomic region encoding transmembrane 7 to 11 was deleted, whereas it was localized to the taste pore in wild-type mice. Collectively, these results suggest that the interaction between PKD1L3 and PKD2L1 through their transmembrane domains is essential for proper trafficking of the channels to the cell surface in taste cells of circumvallate and foliate papillae as well as in cultured cells. Acknowledgements: Grant-in-Aid for Young Scientists from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, a grant from The Kao Foundation for Arts and Sciences, and a grant from Nestlé Nutrition Council

255 Residual Glucose Taste in T1R3 Knockout but not TRPM5 Knockout Mice

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Deletion of the genes for the sweet taste receptor subunit T1R3 or the taste signaling protein TRPM5 greatly attenuates sweetener preference in mice. However, knockout (KO) mice missing T1R3 or TRPM5 develop preferences for glucose but not fructose in 24-h tests which is attributed to the post-oral reinforcing actions of glucose. The present study probed for residual glucose taste sensitivity in KO mice. Water deprived T1R3 KO, TRPM5 KO and C57BL/6 (B6) control mice displayed similar lick rates for 8% glucose and 8% fructose in 1-min, 2-bottle choice tests. However, when food deprived the KO mice licked very little for either sugar while B6 mice continued to lick for both sugars in 1-min tests. Yet, when the test was extended to 1 h, T1R3 KO mice now displayed a significant glucose preference (66%) while the B6 mice initially preferred fructose (59%). In 1-h, 1-bottle tests, T1R3 KO and B6 mice licked more for glucose than fructose and both groups preferred glucose in a subsequent 1-h, 2-bottle test. However, the glucose preference was greater in T1R3 KO than B6 mice (86 vs. 67%).

The TRPM5 KO mice remained indifferent and licked very little for either sugar in these 1-h tests. In 24-h tests, however, the TRPM5 KO mice licked much more for glucose than fructose. The 1-h data suggest a residual glucose taste sensitivity in T1R3 KO mice which may be mediated by their intact T1R2 sweet receptor or a glucose polymer (polycose) taste receptor. TRPM5 KO mice lack this residual glucose taste but learn to prefer glucose to fructose in 24-h tests based on post-oral glucose reinforcement. Post-oral reinforcement can also explain the preference B6 mice develop for glucose over fructose. Acknowledgements: NIH grants DK031135 (AS), DC03055 and DC03155 (RFM)

256 Herbicides and Antilipid Drugs Block Human T1R3 Receptors

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We have found that ubiquitous phenoxy-auxin herbicides and lipid-lowering fibrate drugs are potent inhibitors of the human T1R3 receptor, but not of the rodent form. T1R3 is expressed in taste cells, endocrine pancreas and enteroendocrine cells of the gastrointestinal tract. In the taste system it forms distinct receptors for sweet compounds and amino-acids. In the intestine and endocrine system it is functionally engaged in the regulation of glucose metabolism and hormone release. Fibrates and phenoxy-herbicides inhibit human T1R3 with a potency comparable to that shown by fibrates acting on their known target, the peroxisome proliferator-activated receptor alpha (PPARalpha). T1R3 thus may be a primary target of fibrates, underlying certain of their beneficial effects in treating hyperlipidemia and type II diabetes. Likewise, phenoxy-herbicides' effects on T1R3 may underlie certain of their side effects in humans that due to the species differences in T1R3 would have gone undetected in studies on rodents. This study was supported in part by NIH grants DC007984 to E.L.M., DC008301 to R.F.M, and DC007399 and DK073248 to B.M.

257 Allosteric regulation of taste chemosensors: insights from molecular modeling and docking

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Modulation of GPCR activity by a ligand binding to an allosteric site, which is topographically distinct from the active site, is a well-studied mechanism in the context of pharmacologically-relevant GPCRs. An allosteric modulator can enhance or reduce receptor response to its endogenous agonist, without changing receptor-agonist interaction directly. In the context of taste response, allosteric regulation has tremendous potential. Natural sugars could be enhanced to provide more sweetness without the need to increase the amount of sugar. The flavor of protein-rich foods could be enhanced without the need to resort to unhealthy strategies such as increasing salt content. Here we present a molecular-level view of allosteric regulation for class C GPCR chemosensors. Comparative modeling and molecular docking were used to investigate potential regulatory sites for atomic ions, amino acids and nucleosides in these receptors. We discuss the physicochemical nature of these sites and suggest scaffolds for potential modulators of human sweet and umami taste response.

258 Direct NMR measurement of ligand binding to the human sweet taste receptor domains

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The human sweet taste receptor is a class C G-protein coupled receptor (GPCR). The receptor exists as a heterodimer and composed of two subunits, T1R2 and T1R3. Each subunit contains three subdomains: amino terminal domain (ATD), cycteine rich domain (CRD) and transmembrane domain (TMD). We have studied the binding properties of small molecule sweeteners and the sweet tasting protein, brazzein to subdomains of the sweet receptor. For this purpose we have cloned and purified different domains of the receptor protein. For the T1R2 subunit, we constructed the ATD whereas for the T1R3 subunit we made three constructs, ATD+CRD, TMD and the full-length subunit. The proteins were expressed by bacterial expression and by cell-free translation systems. We used Saturation Transfer Difference (STD) NMR to monitor direct binding of a panel of small molecule sweeteners, neotame, lactisole, cyclamate, dextrose, alitame and sucralose to the receptor sub-domains. We monitored brazzein binding to the receptor extracellular domains by two-dimensional ^{1}H-^{15}N HQSC NMR. Brazzein showed binding to both ATD-T1R2 and ATD+CDR-T1R3 while small sweeteners showed differential preferences for T1R2 or T1R3 subunits. Acknowledgements: R01 DC009018, R21 DC008805-02, R01 DC008301-01, R01 DC009451-01A2, and P41 RR02301

259 Suppressing effect of cyclodextrin to taste modifiers

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Gymnemic acid (GA) and Gurmarin (Gur) are a triterpen glycoside and a polypeptide that are isolated from the plant *Gymnema sylvestre*, respectively. These chemical compounds are known to selectively suppress taste responses to various sweet substances. Miraculin (Mir) is a glycoprotein extracted from the miracle fruit plant. After application of Mir, it modifies taste so that sour substances taste sweet. Sweet suppressing effect of GA and sweet modifying effect of Mir are specific to humans, but not to rodents, whereas Gur inhibits the responses to sweet compounds in rodents, but not in humans. It has also been known that sweet suppressing effects of GA and Gur diminished by γ-cyclodextrin (CD) and β-CD, respectively. The molecular mechanisms between GA, Gur and Mir and each CDs are not known. In order to examine these interactions, 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) and Gur (30 μg/ml) inhibited the $[Ca^{2+}]_i$ responses of HEK293 cells expressing human and mouse sweet receptors to various sweeteners, respectively. After application of Mir (10 μg/ml), HEK293 cells expressing human sweet receptor showed the $[Ca^{2+}]_i$ responses to 3 mM Citric acid (pH: 5.0). The effects of GA and Gur rapidly disappeared after rinsing the cells with γ-CD and β-CD, respectively. Interestingly, the taste modifying effect of Mir is diminished by β-CD. Our present study confirmed the previous finding and demonstrated that GA and Gur directly interact with human and mouse sweet receptors on the taste cell membrane and these interactions are inhibited by γ-CD and β-CD, respectively. We also showed that Mir directly interacts with human sweet receptor and this interaction is diminished by β-CD.

260 Characterizing the interaction of miraculin, a taste-modifying protein, with human sweet taste receptor

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Miraculin (MCL) is a taste-modifying protein occurring in miracle fruit, the red berries of *Richadella dulcifica*. MCL is not sweet by itself, but it has an unusual property of modifying sourness into sweetness. This activity holds for 1-2 hours after MCL is held on the tongue. Since acid-induced sweetness of MCL is inhibited by human sweet taste receptor (hT1R2-hT1R3) blocker, lactisole, it has been indicated that MCL interacts with hT1R2-hT1R3. To elucidate how MCL induces such unique sensation, we established a cell-based assay system to measure its taste-modifying activity by calcium imaging analysis. We transiently expressed hT1R2-hT1R3 in HEK293T cells with chimeric Ga-protein. Evaluating the cell responses to MCL under different pH conditions, we found that MCL-induced activation of hT1R2-hT1R3 increased as pH lowered in the range of 7.4-5.0. At pH 5.0, the EC_{50} value was ca. 1 nM, which is less than that of any other sweetener. At neutral pH, MCL inhibited hT1R2-hT1R3 activation induced by other sweet proteins such as thaumatin and neoculin. This suggests that MCL does not activate hT1R2-hT1R3 but that it binds to the receptor at neutral pH. Furthermore, we identified MCL binding region on hT1R2-hT1R3. Accumulating evidence from electrophysiological and behavioral studies has suggested that MCL shows taste-modifying effect in man, Catarrhini and Platyrrhini, but not in rodents. Actually, the mouse receptor (mT1R2-mT1R3) did not respond to MCL *in vitro*. Interestingly enough, hT1R2-mT1R3 expressing cells responded to MCL. This suggests that hT1R2 is required for MCL response. Further experiments using human/mouse chimeric receptors, we demonstrated that a certain particular site in the amino terminal domain of hT1R2 is essential for MCL to interact with hT1R2-hT1R3. Acknowledgements: This study was supported by JSPS Research Fellowships for Young Scientists (to A.K.) and Research and Development Program for New Bio-industry Initiatives.

261 Structural role of the terminal disulfide bond in the sweetness of brazzein

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Brazzein, a 54 residue sweet-tasting protein, is thought to participate in a multi-point binding interaction with the sweet taste receptor. The termini, which are connected by a disulfide bond, and two distantly located surface loops have been proposed to be sites of interaction; however, the relative importance of these sites is not well understood. To characterize the structural role of the termini in the sweetness of brazzein, we altered the conformation of the disulfide bond connecting the N- and C-termini of brazzein by inverting the positions of residues K3 and C4. The activity of the resulting mutant, CKR-brazzein, was measured by a calcium mobilization assay and found to be decreased to half that of wild-type (WT) brazzein. The high resolution structure of CKR-brazzein was determined by nuclear magnetic resonance (NMR)spectroscopy with a backbone root mean square deviation of 0.39 angstroms. The structure alignment of CKR-brazzein with WT-brazzein reveals more extended beta structure in the terminal strands in CKR-brazzein and increased dynamics relative to that found in WT-brazzein. These results support previous mutagenesis studies and further suggest that while interactions involving the termini are necessary for full function of brazzein, the termini are not the primary site of interaction between brazzein and the sweet taste receptor. This research was supported by NIH grants R01 DC009018, R21 DC008805-02, and P41 RR02301-which funds the National Magnetic Resonance Facility at Madison.

262 Expression of GABA receptor subunits and Cl^- transporters of taste buds in mice

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Taste bud cells (TBCs) communicate with sensory afferent fibers and may also exchange information with adjacent cells. Recently, γ-aminobutyric acid (GABA) has been proposed as a candidate neurotransmitter or neuromodulator in mammalian taste buds. However the precise role for GABA in the taste buds remains unclear. In this study, we examined possible expression of GABA receptor subunits by using RT-PCR and potential effect of basolateral GABA application to single fungiform TBCs on their electrical activities. The results indicated that TBCs expressed GABA type A receptor subunits, α1, α2, α5, β2, β3, γ2, γ3, δ, π, and GABA type B receptor R1, R2, but not expressed GABA type A receptor subunits α3, α4, α6, β1, γ1, ε and θ. Application of GABA to TBCs produced both facilitation and inhibition of the spontaneous firing rates of TBCs; facilitation in some cells, and inhibition in the other cells. Moreover, firing rates in response to sweet taste stimuli were increased by the GABA application in some of sweet-responsive cells, while those in response to bitter taste stimuli were decreased by GABA in some of bitter-responsive cells. Furthermore, Cl^- transporters (NKCC1 and KCC2) that mediated GABA actions were detected in TBCs by using RT-PCR. These results suggest that GABA may participate in modulation of spontaneous firing rates and gustatory signaling in taste bud. The Cl^- cotransporters may be involved in the GABAergic signaling.

263 GABA Inhibition in Mouse Taste Buds

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Cell-to-cell communication in taste buds is an important component of signal processing in taste buds. For example, ATP secreted from Receptor (Type II) cells during gustatory stimulation activates adjacent Presynaptic (Type III) cells to release serotonin (5-HT). Subsequently, 5-HT feedback inhibits ATP secretion from Receptor cells (Huang *et al.*, J Neurosci, 2009). γ-Aminobutyric acid (GABA) has been proposed as a candidate neurotransmitter or paracrine hormone in taste buds. For instance, taste cells synthesize GABA and express $GABA_A$ and $GABA_B$ receptors. Here, we tested the actions of GABA in taste buds during gustatory stimulation. Specifically, using cellular biosensor techniques (Huang *et al.*, PNAS, 2007), we studied whether GABA affects taste-evoked ATP secretion from taste buds and taste cells. As previously shown, taste buds isolated from mouse vallate papillae secrete ATP in response to stimulation with a mixture of bitter and sweet compounds (cycloheximide, 10 μM; saccharin, 2 mM; denatonium, 1 mM; and SC45647, 0.1 mM). Bath-applied GABA (10 μM) reduced taste-evoked ATP secretion from isolated taste buds. Muscimol (1 μM) and baclofen (1 μM), $GABA_A$ and $GABA_B$ receptor agonists, respectively, similarly inhibited taste-evoked ATP secretion. Bicuculline (10 μM), a $GABA_A$ receptor antagonist, and CGP55845 (10 μM), a $GABA_B$ receptor antagonist, restored ATP secretion inhibited by muscimol and baclofen, respectively. In sum, these findings suggest that during gustatory stimulation, GABA inhibits Receptor cells by activating $GABA_A$ and $GABA_B$ receptors and thereby decreases ATP secretion. Experiments are underway to identify the source of GABA during gustatory stimulation and to determine whether GABA affects 5-HT release from Presynaptic cells. Acknowledgements: Supported by NIH/NIDCD grant 5R01DC007630 (SDR).

264 Intracellular Ca^{2+} and TRPM5-mediated membrane depolarization are required for taste cells to secrete ATP

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ATP is a transmitter secreted from taste bud Receptor (Type II) cells through ATP-permeable pannexin 1 gap junction hemichannels. The elevation of intracellular Ca^{2+} and membrane depolarization are both believed to be involved in transmitter secretion from Receptor cells. However, the specific roles for Ca^{2+} and membrane potential have not been fully elucidated. In the present study, we show that taste-evoked ATP secretion from mouse vallate Receptor cells is evoked by the combined actions of intracellular Ca^{2+} release and membrane depolarization. Unexpectedly, ATP secretion is not blocked by tetrodotoxin, a voltage-gated Na^+ channel blocker. This indicates that action potentials, although likely acting to augment ATP secretion (Murata et al 2008), are not necessary for transmitter release from taste Receptor cells. Taste-evoked ATP secretion is absent in Receptor cells from TRPM5 knockout mice or in Receptor cells from wild type mice where current through TRPM5 channels has been blocked. However, ATP secretion can be rescued in these circumstances by depolarizing Receptor cells with KCl. These findings suggest that membrane voltage initiated by TRPM5 channels is required for ATP secretion during gustatory reception. The findings indicate that taste-evoked elevation of intracellular Ca^{2+} has a dual role: (1) to open TRPM5 channels and thereby depolarize Receptor cells and (2) to act in combination with membrane depolarization to open ATP-permeable gap junction hemichannels. Acknowledgements: These studies were funded by NIH/IDCD grant 5R01DC007630 (SDR)

265 Pannexin-1 and Connexin-43 Immunoreactivity in Rodent Taste Buds

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Pannexin and/or connexin hemichannels may be located at sites of ATP release from rodent taste cells. We hypothesize that Type II and possibly Type III cells release ATP at sites containing pannexin and connexin hemichannels. **Pannexin-1:** Our data indicate that Pannexin-1-like immunoreactivity (LIR) is present in a large subset of taste cells in rodent taste buds. Pannexin-1-LIR colocalizes with PLCβ2 and α-gustducin-LIR in a subset of taste cells. Some taste cells, however, display only Pannexin-1, PLCβ2, or α-gustducin-LIR. A subset of TRPM-5-GFP taste cells display Pannexin-1-LIR and some taste cells express only Pannexin-1-LIR. We believe that Pannexin-1-LIR is expressed primarily in Type II cells. Pannexin-1-LIR, however, is present in a small subset of NCAM-, syntaxin-, and/or 5-HT-LIR cells. Thus, a subset of Type III taste cells exhibit Pannexin-1-LIR. **Connexin-43:** Connexin-43 is the most studied gap junction protein and is present in a large subset of taste cells. Connexin-43-LIR cells are spindle-shaped with large nuclei and are most likely Type II cells. Connexin-43-LIR colocalizes with TRPM5-GFP and IP_3R3-LIR in subsets of taste cells. Connexin-43-LIR colocalizes with a large subset of Pannexin-1-LIR taste cells. Based on our preliminary data, most Connexin-43-LIR is believed to be present in Type II taste cells. Although a subset of Type III cells displays immunoreactivity to pannexin-1, we did not observe any colocalization between connexin-43 and the Type III cell marker, 5-HT. Acknowledgements: This work is supported by NIH grants DC00285 and DC007495

266 Potential modulatory effects of serotonin in taste receptor cell excitability.

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Serotonin (5HT) and the $5HT_{1A}$ receptor subtype are thought to play an important role in the processing of gustatory information via cell-to-cell communication among individual cells of the taste bud. Activation of the $5HT_{1A}$ receptor is known to inhibit a number of ionic currents in taste receptor cells; additionally, 5HT inhibits ATP release from type II cells. Here we attempt to address the potential modulation locus of 5-HT on rat taste receptor cell transduction cascades using patch-clamp technique. In the gap-free recording mode, 5-HT did not affect the sustained currents with holding potentials of -50, -20, -10, and 0 mV, respectively (n = 26). This implies 5HT has no significant effect on resting state

but rather modulates the active state of the cell. We further investigated the potential influence of 5HT on PIP_2 since this phospholipid is central to many taste transduction cascades and its resynthesis important for maintaining cellular excitability. Using an electrophysiological assay with m-3M3FBS treatment (a PLC activator) that monitors PIP_2 resynthesis, we observed that 5HT as well as the $5HT_{1A}$ agonist 8-OH-DPAT both were effective in preventing resynthesis of PIP_2 produced by tastant stimulation (cycloheximide n = 32, and SC45647, n = 30). The effect showed strong adaptation. Further this effect was reduced with GDP-βS in recording pipette (n = 24), consistent with a G-protein dependent activation of the $5HT_{1A}$ receptor. We suggest that 5-HT negatively couples with PIP_2 resynthesis whether during tastant challenge or post stimulus recovery. Further, since PIP_2 resynthesis is necessary to restore activation on many ion channels, including hemichannels, this action may provide a mechanism for 5HT suppression of ATP release from taste receptor cells. Acknowledgements: NIH NIDCD R01 DC00401

POSTER SESSION V: CENTRAL OLFACTION; CHEMOSENSORY PSYCHOPHYSICS & CLINICAL STUDIES

267 Co-stimulation with an olfactory stimulus enhances arousal responses to trigeminal stimulation during sleep in humans

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Recent studies have demonstrated that olfactory stimulation does not lead to arousals or awakenings during sleep. In contrast, nasal trigeminal stimulation induces dose-dependent arousal responses comparable to nociceptive stimuli. The interaction of the olfactory and trigeminal system has been demonstrated previously. The aim of the study was to investigate whether olfactory stimulation influences arousal responses to nasal trigeminal stimulation. Five normosmic volunteers were included in the trial and 10 nights of testing were performed. Intranasal chemosensory stimulation was based on air-dilution olfactometry. For trigeminal stimulation 40% v/v CO_2 was administered either with or without simultaneous stimulation with the pure olfactory stimulant H_2S (8 ppm). Stimulus duration was 1s. Arousal reactions were assessed according to appearance and latency during overnight polysomnography in a period of 30 seconds after every stimulus. 200 stimulations were performed on average per subject. Compared to isolated trigeminal stimulation, co-stimulation with H_2S showed an increase in arousal frequency and a reduction in arousal latency. Arousal frequency was 12.1% without and 16.6% with olfactory co-stimulation with the strongest effect seen during light sleep (with: 32.8%, without: 24%). Mean arousal latency was reduced from 5.7s without to 4.4s with olfactory co-stimulation. The present results demonstrate that olfactory stimulation, although not leading to arousal during sleep, influences the frequency and latency of arousals induced by nasal trigeminal stimulation. As arousals are mostly induced by the thalamus the interaction between the two systems does not occur on a cortical but on a peripheral or early level of central processing. Acknowledgements: The study was supported by the German Research Foundation (DFG)

268 Odor fear conditioning and olfactory system slow-wave sleep

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Sleep plays an active role in memory consolidation. Sleep structure (REM/ Slow-wave sleep [SWS]) is modified after conditioning, and in some cortical circuits, SWS is associated with replay of the learned experience. Interestingly, the sleep modifications can be local, only affecting activity in the brain regions active during the training. Here, we wanted to ascertain possible changes in sleep structure within olfactory cortex following odor fear conditioning. We recorded local field potentials (LFP) from the anterior piriform cortex (aPCX) in behaving animals and analyzed odor-evoked changes during fear conditioning and subsequent sleep structure modifications. Long-Evans hooded rats were chronically implanted with telemetry electrodes in the aPCX. Rats were placed in a conditioning box for 30 min on three baseline days, conditioned with ten paired odor-shock stimuli on the fourth day, and tested with five odor pulses on the fifth day. On the conditioning and test days, behavioral (freezing or vocalization), autonomic (heart rate) and LFP responses to the conditioned odor were examined. After each daily session, we placed the animal in a dark, sound attenuating chamber and recorded LFPs and EMG for 4 hours. Preliminary data show that rats learned behavioral and autonomic fear responses to the odor and that aPCX odor-evoked beta (15-40 Hz) oscillatory activity may correlate with the magnitude of the fear response. Furthermore, aPCX SWS increased following odor-shock conditioning compared to baseline days. Unpaired control rats showed neither odor fear responses nor an increase in SWS. Finally, the activity of aPCX single-units during SWS was shaped by recent odor experience and there was enhanced functional connectivity between the aPCX and hippocampus during SWS compared to other period. Acknowledgements: NIDCD to D.A.W. Fyssen Foundation Fellowship to J.C.

269 A neural pathway underlying dynamic control of odor-induced responses to a wide range of odor concentrations

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The olfactory system of any animal species must be able to cope with a wide range of odor concentrations that often fluctuate instantaneously in large magnitude in nature. Little is known on how olfactory circuits at the first synaptic center – the olfactory bulb in vertebrates or antennal lobe (AL) in insects – adjust their sensitivity to quickly encode the concentration fluctuations. We have conducted a series of experiments in the AL of the hawk moth, *Manduca sexta*, to gain insights on this gating mechanism. In moth AL a special set of enlarged glomeruli located at the beginning portion of male AL – the macroglomerular complex or MGC – is devoted to process the conspecific female sex pheromones. The output (projection) neurons (or PNs) of MGC can be readily identified using juxtacellular recording method in conjunction with pheromonal stimulation. Upon stimulation of a series of 5 odor pulses, PNs often produce the strongest response to the 1st odor pulse and then weaker but constant responses to the rest of pulses, suggesting there

is a fast inhibitory feedback pathway regulating the PN's sensitivity, most likely via GABAergic local interneurons (LNs). We therefore used a known GABA-A receptor antagonist, picrotoxin, to manipulate the putative feedback pathway. After the drug was added, the cell activity changed from randomly bursting to non-spiking pattern; response to odors was also much reduced but the after-inhibition period following each response was increased. These results indicate that PNs are tonically inhibited by GABAergic LNs. As a feedback gating mechanism, the same pathway may regulate the output of PNs depending on the activity level of the same PNs, thus expanding the dynamic range of the PNs to respond to wide range of odor concentrations. Acknowledgements: NIH grant R01-DC-02761 to JGH

270 Transformation of olfactory information by neural networks in the honey bee 'olfactory cortex'

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Mushroom bodies (MBs) in the insect brain are higher-order structures involved in integration of olfactory, visual, and mechanosensory information. They receive direct input from the antennal lobes, which are the analogs of the mammalian olfactory bulb. Our objective was to investigate how the neural circuitry in the MB transforms the input from the antennal lobes. Therefore, we recorded simultaneously the spiking activity from neurons providing input to and output from this neuronal structure. Input to each MB is provided by about 800 projection neurons (PNs) of the antennal lobe (AL). PNs send their axons to the input region of each MB, which consists of approximately 170,000 Kenyon Cells [KC]. Output from each MB is carried by around 400 extrinsic neurons (ENs) projecting into different brain areas. We recorded simultaneously single unit activity from PNs and ENs while presenting two single odors (A, B) and their mixture ($X_{A,B)}$. At both neuronal stages the responses of a single unit to $X_{A,B}$ was not a simple liner transformation of the response to A and B. We applied principal component analysis (PCA) to visualize the timing of the establishment of stimulus representations at MB input and output to estimate the latency of transfomation of the signal by the MB network. Furthermore we analyzed the contribution of a single unit to the ensemble representation of odor A, B and $X_{A,B}$ separately for PNs and ENs. Surprisingly at both levels units dominating the ensemble representation of A are not the ones dominating B or $X_{A,B}$. The characterization of these complex responses and the degree of modification after processing in the MB is necessary for further investigation of influence of plasticity at the different stages of odor processing. Acknowledgements: This research was funded by NIH NCRR RR014166 to BHS, and by a subcontract of NIH NIDCD (DC007997) to BHS.

271 An Ih-dependent Switch from Inhibition to Excitation in ET Cells by Co-release of GABA and DA from SA Cells

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Olfactory bulb short axon (SA) cells co-express DA and GABA and form multiglomerular circuits spanning 10's-100s of glomeruli. ET cells receive direct ON input and provide excitatory drive to SA, PG and mitral/tufted (MT) cells. Thus modulation of ET cells significantly impacts glomerular processing and OB output to olfactory cortex. There is evidence that ET cells receive synaptic input from the GABA-DAergic SA cells. How does co-release of DA and GABA influence ET cells? ET cells respond to stimulation of SA cell interglomerular circuit with short latency hyperpolarization followed by a strong rebound depolarization that generates a burst of action potentials. Both the initial hyperpolarization and the rebound spike burst were blocked by gabazine. Brief hyperpolarization of ET cells leads to strong rebound depolarization due to activation of a prominent Ih current. Thus, ET cell hyperpolarization mediated by SA cell GABA release might activate Ih triggering the rebound spike burst. In many CNS neurons DA enhances Ih. Indeed, bath applied DA (in the presence of fast synaptic blockers) increased Ih in ET cells. Thus co-release of DA by SA cells might strengthen the GABA-induced rebound excitation. Consistent with this hypothesis, upon SA stimulation the rebound depolarization, but not the initial hyperpolarization, was significantly attenuated by D1-like antagonists. ET cells drive PG cells, which inhibit MT cells. Thus the net result of SA cell activation may be to generate interglomerular inhibition of MT cells. Further, the present findings demonstrate a novel neural mechanism whereby synaptic inhibition is transformed into strong excitation by target cell intrinsic properties and DA modulation. Acknowledgements: Supported by NIH NIDCD DC005676

272 Olfactory-visual integration facilitates perceptual discrimination of facial expressions

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Multisensory integration markedly improves perception of a stimulus, especially when the sensory input is minimal (known as ''the rule of inverse effectiveness''). Research has largely focused on cross-modal interactions among senses other than olfaction, implicating the thalamus as a primary neural substrate in this process. Given that olfactory input bypasses this structure en route to the primary olfactory cortex, this theory is unlikely to figure well in olfaction-related sensory merging. It thus remains to be explored how olfactory information interacts with other sensory stimulation. In this study, we examined the effect of odor on the discrimination of two highly similar faces using a dot-probe paradigm in combination with brain event-related potentials. On a given trial, we delivered an unpleasant or neutral odor for 2 sec, and then presented a pair of faces simultaneously in the left and right visual fields for 400ms, followed by a dot appearing at the location of either of the faces. In each pair, one face contained a neutral expression and the other face the neutral expression morphed with 12.5% of fearful expression of the same person. Despite that a two-alternative-forced-choice task confirmed that these faces were indistinguishable, a visual ERP component, P1, appearing at 90 ms post-stimulus indicated visual discrimination of the faces in the presence of unpleasant (vs. neutral) odors, varying as a function of the level of anxiety of the subjects (P <0.05). This finding demonstrates the effectiveness of olfactory information in cross-modal perceptual facilitation, accentuating the potency of hedonic tone in olfactory-visual integration.

273 Characterization of somatostatin systems in the mouse olfactory bulb

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The neuropeptide somatostatin is widely expressed in the brain and contributes to neuroendocrine and cognitive functions through its six receptors. It is also detected in most sensory regions. In the mouse main olfactory bulb (MOB), somatostatin is mainly concentrated in local GABAergic interneurons restricted to the external plexiform layer (EPL). These axonless interneurons make dendrodendritic reciprocal synapses with the dendrites of mitral cells, the principal cells of the MOB. Immunohistochemical experiments revealed that sst2, sst3 and sst4 receptors are found in the MOB, selectively addressed to distinct cell layers: sst4 labels a selective population of cells in the peripheral glomerular layer while sst3 is expressed more centrally in the cilia of granule cell neurons. Sst2, the major somatostatin receptor subtype in the telencephalon, is expressed in mitral cells. As odor-activated mitral cells synchronize and generate gamma oscillations of the local field potentials, we investigated whether pharmacological manipulations of sst2 receptors could influence these oscillations in freely-behaving mice. In wild-type, but not in sst2 knockout mice, gamma oscillation power decreased lastingly after intrabulbar injection of a sst2-selective antagonist, while sst2-selective agonists durably increased it. Sst2-mediated oscillation changes were correlated with modifications of the dendrodendritic synaptic transmission between mitral and granule cells. Finally, bilateral injections of sst2 antagonist and agonist respectively decreased and increased odor discrimination performances. This suggests that endogenous release of somatostatin contributes to gamma oscillation modulation and odor discrimination via sst2 activation. Functional roles of sst4 and sst3 are currently under investigation.

274 Glutamate modulates inhibitory inputs of GABAergic interneurons in the superficial EPL of the main olfactory bulb

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The superficial external plexiform layer (sEPL) of the main olfactory bulb (OB) contains GABAergic interneurons (INs), many of which exhibit spontaneous EPSCs mediated by AMPA receptors and modulated by presynaptic NMDA receptors. Here, we show that AMPA and NMDA receptor-mediated excitation also modulates IN inhibition. In OB slices from transgenic mice expressing eGFP under control of the promoter for the GAD65 gene, many $eGFP^+$ sEPL INs exhibited spontaneous IPSCs (sIPSCs) that were blocked by the $GABA_A$ receptor antagonists gabazine (GZ) or bicuculline. In the presence of TTX, glutamatergic modulation of miniature IPSCs (mIPSCs) was observed. The NMDA receptor antagonist APV reduced the mIPSC duration, but it did not affect mIPSC amplitude and frequency. The AMPA receptor antagonist CNQX had similar effects, and it also accelerated the mIPSC rising slope. In the presence of APV and CNQX, high K^+ stimulation could still evoke mIPSCs, suggesting that presynaptic glutamate receptors modulate GABA release. Stimulation of the EPL evoked short-latency IPSCs (eIPSCs), which were blocked by GZ and TTX. Both APV and CNQX reduced the latency of the eIPSCs. Dual-pulse stimulation resulted in depression (PPD) of the paired eIPSC; PPD was not affected by APV or CNQX, but CNQX changed the ratio of rising slope of the paired eIPSC. In Mg^{2+} free ACSF, PPD and effects of APV and CNQX on paired eIPSCs were not observed. These results indicate that glutamatergic synapses modulates GABAergic inhibition of sEPL INs. Acknowledgements: NIH DC007876

275 Ion Channel in the Olfactory Bulb Subserves as a Metabolic Sensor

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Naturally occurring modulators of the Kv1.3 ion channel, as expressed in mitral cells, include the insulin receptor and the TrkB neurotrophin receptor - both of which have been implicated in other parts of the CNS to modulate metabolism. We now demonstrate via slice electrophysiology that mitral cell action potential firing properties are sensitive to another metabolically important substance, glucose. Like the hypothalamus, we have found the olfactory bulb contains two populations of glucose-sensitive mitral cells; glucose excited and glucose inhibited. Gene-targeted deletion or acute suppression of Kv1.3 results in an overall increased mitral cell sensitivity via increased-current evoked spiking frequency, decreased latency to first spike, and a more depolarized resting membrane potential. To explore the 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 and then systems physiological parameters of body weight, respiration, locomotion, and ingestive behaviors were quantified in a custom designed, computer interfaced, metabolic chamber. 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). Kv1.3-null mice were resistant to DIO with a weight gain of only 10% and no change in adiposity as a result of a significant increase in basal metabolic rate linked to the MHF challenge. Bilateral olfactory bulbectomy (OBX) in a Kv1.3-null background yielded mice that were no longer resistant to DIO. The mice exhibited a 30% increase in body weight via preventing the increase in basal metabolic rate in response to the MHF challenge, which resulted in a decreased activity-dependent metabolism. Acknowledgements: NIH NIDCD R01DC003387 & F31DC010097 and the TMH/Robinson Foundation.

276 The Expression Pattern of TrpM5 and NT-3 in the Ventral Main Olfactory Bulb of Mice Reveals Two Distinct Populations of Glomeruli.

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Previous investigations in mice demonstrated that the transient receptor potential channel M5 (TrpM5) and neurotrophin-3 (NT-3) are expressed by a subset of olfactory sensory neurons (OSNs) located in the main olfactory epithelium. OSNs expressing high levels of either TrpM5 or NT-3 project to glomeruli located in the ventral regions of the main olfactory bulb (vMOB). The $TrpM5^+$ glomeruli of this region process semiochemical information including odors of urine. The goal of the current study is to determine whether the OSNs expressing TrpM5 or NT-3 project the same or discrete populations of glomeruli in the vMOB. We bred TrpM5-GFP/NT-3-LacZ mice where GFP is expressed under the control of TrpM5 promoter and the coding region for NT-3 was replaced by *E. coli* lacZ thus the expression of β-galactosidase mimics the expression of NT-3. Standard fluorescent immunocytochemical protocols were utilized to label and visualize both GFP and β-galactosidase. The expression pattern of GFP and β-galactosidase observed in the current investigation was consistent with results reported in previous investigations, i.e. glomeruli in the vMOB are strongly positive for one or the other of the markers. In the current study, preliminary analysis suggests that glomeruli that are strongly labeled for GFP or β-galactosidase constitute two largely separate populations. The results of the current investigation suggests that strongly labeled $NT\text{-}3^+$ glomeruli in the vMOB represent a distinct subset, separate from the $TrpM5^+$ population. Future studies are needed to determine if $NT\text{-}3^+$ glomeruli participate in processing urine or semiochemical odor information. Acknowledgements: Supported by grants from the NIH and BNAT.

277 Expression and function of Rap1gap2 in the developing olfactory system

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Regulation of Rap1 signaling plays important roles in cortical circuit formation and synapse remodeling. To investigate the function of Rap1 regulation in the development of the olfactory system, we cloned and characterized a novel Rap1GAP, also known as Garnl4. *Garnl4* is 95% homologous in amino acid sequence to human *RAP1GAP2*. GTPase Activating Proteins (GAPs) catalyze the inactivation of small GTPases, thereby regulating their multidimensional signaling roles. We validated that Garnl4 *i*s the mouse Rap1gap2 by comparing active Rap1 levels with and without Garnl4 overexpression in a heterologous cell system. Rap1gap2 is exclusively expressed in the central nervous system as detected by western blotting analysis. Immunohistochemistry shows Rap1gap2 expression in olfactory sensory neurons at the olfactory epithelium as well as in OSN axons, which persists to the nerve terminals in the glomerular layer of the olfactory bulb. During development, Rap1GAP2 signal is detectable in all glomeruli at P0; however, by P14 a mosaic glomerular expression pattern emerges and persists into adulthood. We have characterized Rap1-GAP2 function in a neuroblastoma differentiation assay. Rap1gap2 overexpression inhibits Neuro2A neurite outgrowth. In contrast, Neuro2a cells exhibit exuberant outgrowth with Rap1 overexpression, which is eliminated by co-overexpression of Rap1gap2. The function of Rap1GAP2 in the regulation of olfactory axon growth and branching is currently being investigated. Acknowledgements: Supported by: NIH DC06015, NSF IBN0324769, and the NIH T32 Doctoral Training Grant

278 Dishevelled-1 in mouse olfactory system development

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Olfactory sensory neuron (OSN) axons navigate from the olfactory epithelium (OE) to the olfactory bulb (OB), where they coalesce into specific glomeruli. Odor receptor (ORs), a variety of trophic and repulsive molecules, and OSN functional activity have been strongly implicated in the coalescence and targeting of OSN axons. Formerly known as morphogens, there is increasing evidence that Wnt molecules, signaling through Frizzled receptors (Fz), contribute in a variety of processes, including cell proliferation, migration and the development of neuronal circuits. We previously demonstrated that Fz-1 and Fz-3 are expressed in OSNs from early embryonic stages, and that they present specific expression patterns during development and in adult mice. In order to characterize putative Wnt/Fz signaling mechanisms, we began by characterizing the expression pattern of Dishevelled-1 (Dvl-1). Dishevelled plays a central role in many of the proposed Wnt/Fz signaling mechanisms. Expression of Dvl-1 in the OE begins early in development and is restricted to the most dorsal zone of the OE. Dvl-1 expression exceeds that of NQO1, a marker for OSNs located dorsally. OSNs expressing the OR M72, which is a dorsal Class II OR, coexpress Dvl-1. In the OB Dvl-1 is restricted to OSN axons, where it has a punctate distribution and appears later than embryonic day 13. Axons expressing Dvl-1 overlap with NQO1, but also with some dorsal OCAM-glomeruli that are NQO1 negative. Rostal-caudal analyses showed that expression in the OB is lateral in the most rostral part and then shifts to medial-dorsal in the most caudal portion. Both the punctate axonal distribution as well as the relatively late onset of expression in axons suggest a possible role of Dvl-1 in synapse formation/stabilization of OSN in the OB. Acknowledgements: Support In Part By: NIH DC00210, DC006972 and DC006291 to CAG.

279 MMP-2 expression in the olfactory bulb is associated with neuronal reinnervation

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We previously reported that matrix metalloproteinase-2 (MMP-2), an enzyme that degrades the extracellular matrix, is elevated in the olfactory bulb of mice 7 days following olfactory nerve transection (NTx), when newly regenerated axons begin to innervate the bulb. To determine if MMP-2 is associated with the regenerated axons, we inserted a piece of Teflon between the cribriform plate and bulb following nerve transection to block the axons (TB-NTx). We then compared olfactory bulb expression levels of MMP-2 and olfactory marker protein (OMP) following NTx and TB-NTx at different recovery time points using Western blot. Following NTx, OMP expression decreased by day 3 and remained low for a week, demonstrating neuronal degeneration. By day 10, OMP expression returned to control levels, indicating neuronal regeneration and bulb reinnervation. With the Teflon blocker, OMP levels decreased but failed to return to control levels, indicating successful blockage of the regenerated axons. In contrast to the significant increased in MMP-2 observed following NTx, levels in TB-NTx mice remained

low. This finding demonstrates the increased expression of MMP-2 is dependent on the regenerated axons innervating the olfactory bulb. These axons may utilize MMP-2 to penetrate through the extracellular matrix to reestablish connections in the olfactory bulb. Acknowledgements: Supported by R01 DC000165 (RMC) from the National Institute of Deafness and Other Communication Disorders

280 In Vivo Expression of Osterix in Mouse Olfactory Bulb

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Osterix (Osx) is identified as an osteoblast-specific transcription factor, which is required for skeletogenesis. Here, we examined the expression of Osx in non-skeletal tissues. Together with high expression in bones, Osx was moderately expressed in brain. Osx expression in mouse brain was gradually increased during postnatal developmental periods. Osx was highly expressed in olfactory bulb rather than cerebral cortex and cerebellum. To convince Osx expression in mouse olfactory bulb, Osx expression was examined in brain of Osx heterozygous mice with a LacZ knock-in in the Osx locus, resulting in strong X-gal staining in mouse olfactory bulb. X-gal positive cells were located in mitral and granule cell layer of olfactory bulb, confirming by immunohistochemical analysis with anti-Osx antibody. Osx-positive cells were specifically expressed in mature neuronal cells as shown in merged images with NeuN. Morphological difference was not observed in Osx heterozygous compared to wild-type mice by cresyl violet staining. In immunofluorescence using neuronal marker genes, the number, shape, and localization of immature neurons, mature neurons, astrocytes, and proliferating cells were identical in both wild-type and Osx heterozygous mice. Even though there are no big differences in neuronal cells of Osx heterozygotes compared to wild-type, the function of Osx in olfactory bulb would be more studied. Consequently, in this study, *in vivo* expression of Osx was first observed in mouse olfactory bulb and this finding may provide a new function of Osx in olfactory bulb not in only bone. Acknowledgements: NRF 2009-0071230, BK21 Project in 2010

281 Calbindin, Parvalbumin and Calretinin Immunoreactivity in the Medial Amygdala of Male Hamsters

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The hamster vomeronasal organ (VNO) detects chemosensory signals from both conspecific and heterospecific species and has strong direct projections via accessory olfactory bulb (AOB) to anterior medial amygdala (MeA), which has strong projections to MeP, thence to hypothalamic regions implicated in social behaviors. VNO, AOB and MeA respond to natural conspecific and heterospecific social signals, including flank gland secretion (FGS) and vaginal fluid (HVF) from hamsters, and mouse urine (MU). Subsets of these stimuli increase FRAs immediate early gene (IEG) expression in MeP, suggesting selective responses to biologically relevant stimulus categories. GABAergic inhibition from intercalated nuclei (ICN) and interneurons within MeA/P may determine the selective response in MeP. Calcium binding proteins (CBPs), parvalbumin, calbindin, and calretinin, often colocalize with GABA, and have been helpful in distinguishing subgroups of GABAergic cells with different functions. A group of large parvalbumin-ir cells extends from lateral MeA into the anterior amygdaloid area. Calretinin-ir cell bodies and fibers are densely concentrated in dorsal and ventral areas of MeA with few fibers centrally. In MeP, large calretinin-ir cells are concentrated dorsally. Calbindin-ir cells appear to be of two types, distributed throughout MeA and MeP. ICN cells are not CBP-ir. We have evidence for GABA suppression in MeP in response to biologically relevant stimuli so we hypothesized that differences in IEG activation of calcium binding protein-ir cells might reveal details of the MeA/P circuits involved in categorical chemosensory response. Preliminary double-label results suggest that the ICN and calbindin-ir cells are differentially activated by these stimuli, but that calretinin-ir and parvalbumin-ir cells are not. Acknowledgements: Supported by DIDCD grant DC005813

282 Sexually Relevant Olfactory Stimuli Activate the Medial Preoptic Nucleus in an Age-Dependent Manner

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The olfactory system plays a significant role in mediating appetitive and consummatory aspects of sexual behavior in rodents. Chemosensory input from the olfactory bulb and vomeronasal organ project to the medial amygdala. This information is then relayed, directly and indirectly, via the bed nucleus of the stria terminalis to the medial preoptic area (MPOA). The MPOA is a central integrative region in the regulation of male sexual behavior present in most studied species. In this study, sexually experienced adult- (~ 1 year old) and elderly- (~2.5 years old) male rats were exposed to a partially sedated female in estrous in a neutral cage for 10 minutes. An hour later they were sacrificed and their brain immuno-processed for the presence of Fos, the protein byproduct of the immediate early gene c-fos, used here as a measure of cellular activation in the medial preoptic nucleus (MPN), a central nucleus in the MPOA. Analyses revealed no significant difference in the length of time each group spent investigating the female. Analyses did reveal differences in the number of cells containing Fos between the two groups. Specifically, the caudal portion of the MPN in the elderly rats had a higher number of Fos-positive cells when compared with adult rats. There were no differences between the two groups in the rostral and central regions of their MPN. Higher activation in elderly males suggests a compensatory mechanism, whereby more cells must be recruited to elicit equivalent sexual activity as in adult males.

283 Anatomical and Molecular Characterization of Centrifugal Cells Within the Olfactory Cortex

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Centrifugal fibers play an important role in numerous cortical sensory systems, allowing for powerful feedback onto upstream circuitry. The olfactory or piriform cortex (PC), while lacking pre-cortical thalamic processing, has a high density of centrifugal cells sending axons back to the main olfactory bulb (MOB). It has been demonstrated through behavioral studies that these fibers are likely involved in the modification of MOB sensory information, but the identity and ultimate function of these cells remains an open question. Our aim was to study the anatomical and molecular composition of PC cells that send centrifugal axons to the MOB. We injected retrograde dye Fluoro-Gold (hydroxystilbamidine) into the MOB *in vivo*, which led to well-labeled cells within the PC. All of the labeled cells in the PC were found within layers II/III and could be anatomically identified as pyramidal cells based upon the morphology of their soma and initial dendritic segments, and their lack of GABA and GAD67 expression. We measured the density of centrifugal cells as it relates to the total population of excitatory cells. Centrifugal cell density was found to be dependent upon lamination as well as the position along the rostral/caudal axis. Through the combined use of a transgenic mouse strains and immunohistochemical staining, we found that a lower percentage of centrifugal cells express CamKII, and a very high percentage of cells express CamKIV within both layers of anterior and posterior PC. This study provides a first look at the specific molecular and anatomical identity of cortical centrifugal cells and provides quantitative information regarding the density of these cells. Acknowledgements: NCRR

284 Reversible Partial Deafferentation of the Zebrafish Olfactory Bulb with Repeated Detergent Application

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We set out to establish a novel method of examining degeneration and regeneration of the olfactory organ (OO) and resulting influence on the olfactory bulb (OB) in adult zebrafish. Repeated application of the detergent Triton X-100 was used to ablate portions of the OO and temporarily reduce afferent input to the OB. This method also allows for regeneration since the detergent application can be ceased and the OO can repair itself. Adult zebrafish were anesthetized, and 0.7% Triton X-100 was applied to the right nasal cavity every 2-3 days for three weeks. Another group was allowed three weeks of recovery following treatment. Slides of control and treated fish were stained with H&E to observe morphology, anti-calretinin and DiI to examine mature olfactory sensory neurons (OSNs), and anti-tyrosine hydroxylase to analyze activity of juxtaglomerular neurons in the OB. The 3-week treated group showed severe morphological disruption of the OO, although there were small pockets of epithelium that contained mature OSNs. After 3 weeks of recovery, the epithelium was more extensive and more neurons were labeled. The OB was affected by repeated peripheral damage. OB volumes were reduced in response to treatment, but bulb size recovered with cessation of treatment. Anti-tyrosine hydroxylase staining in the OB on the treated side of the 3-week group appeared diminished compared to the control side, suggesting a decrease in afferent input; following recovery, there was little difference in staining, presumably due to a return of afferent input. These results suggest that repeated exposure to Triton X-100 eliminates a substantial number of mature OSNs and reduces afferent input to the OB. However, it also appears that these affects are reversible and regeneration will occur when the OO is given time to recover. Acknowledgements: WMU FRACA Award #09-019

285 Hemi-bulb Organization in the Elasmobranch Brain

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Olfactory cues are detected by olfactory receptor neurons (ORNs). The information is conveyed via the olfactory nerve to the olfactory bulb (OB), the first relay station in the brain. The axons of the ORNs make contact with mitral cells in so-called glomeruli. In teleost fishes, both tracing and electro-physiological studies showed that the teleost OB is divided into separate functional zones that process different types of odorants with no suggestion of somatotopy. While the OB in teleosts has a round shape, the OB of elasmobranchs is a long structure that lies parallel to the olfactory lamellae. In some elasmobranchs, the OB is physically partitioned into ''hemi-bulbs'', either as two distinct hemi-bulbs or as a succession of connected swellings along the OB. The functional significance of these hemi-bulbs is not fully understood. A previous study of three elasmobranch species found that ORNs in the olfactory epithelium (OE) projected immediately posterior to the adjacent group of glomeruli in the OB, suggesting a somatotopic arrangement. The present study examined the organization of the OBs in two elasmobranch species, the Atlantic stingray (*Dasyatis sabina*) and the bluntnose stingray (*D. say*) to test the hypothesis that axons projecting from the OE to the OB in elasmobranchs exhibit a somatotopic arrangement. We injected various fluorescent tracers into the OBs to retrogradely label ORNs in the epithelium; and into the olfactory epithelium to anterogradely label the OBs. Our results suggest that the distribution of glomeruli in the OB is different from that in teleosts and that glomeruli receive projections from three to four olfactory lamellae situated immediately adjacent to these glomeruli. This suggests a somatotopic arrangement of the elasmobranch OB, which may be unique among vertebrates. Acknowledgements: Marsh Scholarship in Marine Biology, Captain Al Nathan Memorial Scholarship

286 Lateral Connections in the Olfactory Bulb: a Transsynaptic Tracing Study

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Previous transsynaptic tracing studies in the rat olfactory bulb (OB) demonstrated columnar organization extending from the glomerular layer to the deep granule cell layer. The columns observed after focal injection of the pseudorabies virus into the OB glomerular layer extended through much of the ipsilateral half of the bulb in a distributed manner. We interpreted that the labeling arises from retrograde virus travel along the mitral and tufted cell lateral dendrites. To further characterize the extent and distributions of lateral connectivity, we performed flat map reconstructions of the PRV labeling patterns through one plane of the granule cell layer after

glomerular layer infection. Results show mosaic patterns generally in the ipsilateral half of the injected side. The degree to which lateral connectivity is dependent on distance is discussed. Acknowledgements: NIDCD R01 DC000086 NIDCD R03 DC008874

287 How stable are olfactory bulb structures in color mutations of *Neovison vison*?

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Gene mutations and transgenic animals are used to specify gene functions, however, genes have multiple effects and even albinism results not just in fur color phenomena but also in structural changes of brain networks. We were interested if the color mutations described for the American mink (*Neovison vison var. spec.*) induce changes in the olfactory bulb. Therefore we investigated different coat color varieties of the American mink: ''standard'' (*Neovison vison var. atratus*), ''silverblue'' (*Neovison vison var. glaucus*), ''pastel'' (*Neovison vison var. suffuscus*), ''wild'' (*Neovison vison var. carinum*) for size and composition of the olfactory bulb. Following histological processing always the right olfactory bulb of adult males and females was analyzed using a morphometric system and weight/volume correction factors. The results reveal, that in all color-varieties of the American mink, the absolute size of the olfactory bulb is statistically significantly different between the sexes, with higher values in males, whereas the size variations within sexes among the color-varieties are small. Analyzing the composition of the olfactory bulb, the neural structure displays the typical appearance with the major portions being fila, external plexiform and granule cell layers. Sexual differences exist in all color-varieties with the females having a thicker granule cell and thinner fila layer compared to males; however, within sexes among the color-varieties, only slight alterations in the composition were observed, which do not show a systematic pattern of changes and no ranking among the color-varieties is obvious. This indicates that the olfactory bulb, probably due to its phylogenetic old age and emphasizing its functional importance, is a very stable structure and less susceptible to gene alterations. Acknowledgements: DFG (SFB 509 /TP C4) FORUM F208/00 M122/13 (2000)

288 Effect of odor exposure on glomerular size in the mouse olfactory bulb

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Glomeruli in the olfactory bulb are formed by the coalescence of olfactory sensory neurons (OSNs) expressing the same receptor in the olfactory epithelium. Glomeruli vary in size depending on the number of OSNs projecting to them. A previous study indicated that pairing odor exposure with aversive stimuli increased the size of GFP-tagged glomeruli with OSNs that were activated by the odor. Shaping the olfactory system in response to changes in the environment could have evolutionary benefits, such as heightened sensitivity to odors of available, palatable foods. In this pilot study, we explored the effects of either eating or simply smelling a scented diet on glomerular size in male and female adult mice. Genetically modified mice expressing GFP in the M71 receptors were exposed to the target odorant, acetophenone, for 3 weeks. The volume of tagged glomeruli (estimated from areas of serial 20 μm sections) revealed significant effects of odor exposure on glomerular size. Medial, but not lateral, glomeruli of exposed animals were significantly larger than those of control animals. Glomeruli were larger in females than males, irrespective of their body weight. A more extensive study will be necessary to determine the perceptual and behavioral implications of these differences. Acknowledgements: National Institute of Health Award nos. F33DC009137 (J.T), DC04657 (D.R.) and DC006070 (D.R.).

289 An Axis-Based Olfactory Neural Code that Predicts Behavior and Perception

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The olfactory system uses a large population of receptors to generate an olfactory image. How the brain reads this high dimensional image is largely unknown. Although there is significant evidence for combinatorial coding in olfaction, we asked whether perceptual and behavioral information can be read out by the brain using simple global features of the olfactory code. We applied standard statistical methods of dimensionality-reduction to neural activity from 12 studies, using 7 species, and found two linear axes of neural population activity that accounted for more than half of the variance in neural response. The first axis was correlated with the total sum of odor-induced neural activity, and reflected the behavior of approach or withdrawal in animals, and odorant pleasantness in humans. The second and orthogonal axis reflected odorant toxicity across species. We conclude that simple global computations can read vital olfactory information from the neural population response. Acknowledgements: This work was supported by an ERC FP7 200850

290 Postnatal Development in Piriform Cortex

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The piriform cortex (PCX) includes 3 layers and the lateral olfactory tract (LOT), which contains mitral/tufted cell axons from the olfactory bulb. Pyramidal neurons located in layers II and III extend apical dendrites into layer I. They receive input from LOT axons in the superficial layer I (layer Ia) and from intracortical associative fibers in the deeper layer Ib. GABAergic interneurons, found in all layers, modulate activity in pyramidal neurons. We examined the development of mouse PCX laminar organization at postnatal days (P) 0, P7, P14, P21, P30 and P60. Layer I was identified using MAP2, which labels dendrites throughout layer I. Layer I was delineated into Ia and Ib using calretinin, a marker of LOT axons. We examined the distribution of inhibitory

interneurons and pyramidal neurons using GAD67-GFP mice and Tbr1, respectively, and NeuN to label all neurons. The thickness of PCX (from the LOT through layer II) increased from P0 to P60. The thickness of layer Ia significantly increased from P0 to P7, while layers Ib and II remained proportional to the rest of the cortex. During the first postnatal week, the segregation between layers Ia and Ib also became more distinct. In layer II, while the number of pyramidal cells was constant throughout development, the number of interneurons decreased significantly from P0 to P7 and remained stable thereafter. A subset of pyramidal neurons expressed Tbr1 but not NeuN during the first postnatal week (8.6% at P7) and almost disappeared by P14. We are now using a BrdU birth-dating protocol and activated caspace-3 labeling to determine if these cells are late-born and immature pyramidal cells, or apoptotic cells. These data reveal important changes in the organization of the PCX with development, especially during the first postnatal week. Acknowledgements: Supported in part by NIH-NIA-PO1- AG028054 to CAG. AAS was supported by the Yale Gershon Fellowship.

291 Oxytocin and vasopressin in the medial amygdala modulate approach/avoidance responses to chemosignals associated with health condition in male rats

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Infected or sick animals are known to release a specific odorant cue, which plays a role in signaling health condition of odor donors to conspecifics as a social communication. Inflammatory response under infection or sickness may have a predominant rule in regulating health-information signals. Both injection of bacterial mimetic, lipopolysaccharide (ip), and pro-inflammatory cytokine, interleukin-1 (icv) induced sickness-like behavior in male rats, and this odor cues from sick animals induced decreased sniffing investigation and increased avoidance in male odor recipients. Given a finding that healthy odor cues induce sniffing in odor recipients, we assessed neural mechanism regulating social approach/avoidance response in odor communication situation using sickness odor paradigm. Real-time RT-PCR analysis of mRNA expression in several brain sites of odor recipient rats indicated that *c-fos* expression was increased in olfactory bulb, the medial amygdala (MeA), the bed nucleus of the stria tarminalis, and the paraventricular nucleus of the hypothalamus, when exposed to conspecific odor. In the MeA, the induction of neuropeptide oxytocin (OT) receptor mRNA was found when rats were exposed to healthy conspecific odor, while inductions of arginine vasopressin (AVP) receptor 1a and 1b mRNA were shown only when exposed to sick conspecific odor. Bilateral infusion of OT receptor antagonist into the MeA blocked approach response to healthy odor exposure, while those of AVP 1a receptor antagonist inhibited avoidance response to sick odor exposure. These findings provide evidence for an essential role of OT and AVP receptors in the MeA in regulating approach/avoidance responses in chemosignal communication. Acknowledgements: National Science Foundation (NSF 0822129), Hope for Depression Research Foundation (HDRF 06-008), and the Center for Development and Behavioral Neuroscience (CDBN) at Binghamton University to TD.

292 A clinical test of gustatory function including umami taste

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The usefulness of the ''taste strips'' for regional testing of gustatory function has been shown in several studies. The aim of the present investigation was to study an extended version of the test including four concentrations of monosodium-glutamate (MSG). 70 healthy subjects (28m, 42f, mean age 30.2/SD 17.2 years) were included in the study. The new test consisted of 22 strips (2 blanks, 20 tastants) impregnated with four different concentrations per quality (umami 0.25, 0.1, 0.04, 0.016 g/ml MSG). To obtain the level of familiarity before testing, a taste strip with 0.1 g/ml MSG was placed at the tip of the subjects' tongue and had to be labeled by the subjects without given descriptors. After advising the subjects of umami taste, the test was performed in a non-forced-choice manner (possible answers were sweet, sour, salty, bitter, umami, or no taste) with a maximum score of 4 for each taste quality and a total score of 20. In order to assess a measure of test-retest-reliability 34 subjects (14m, 20f, mean age 31.2/SD 17.0 years) were retested with a minimum interval of 2 days. Only 10 percent of the subjects spontaneously recognized the first presented strip as umami. The mean taste scores were 3.5 (SD 0.8) for sweet, 3.4 (0.7) for sour, 3.2 (0.9) for salty, 3.1 (1.2) for bitter, 2.8 (0.9) for umami taste, with a total score of 15.9 (2.8). The results of retesting were 3.7 (0.8) for sweet, 3.5 (0.8) for sour, 3.2 (0.9) for salty, 3.3 (1.0) for bitter, 3.4 (0.9) for umami taste, with a total score of 17.1 (3.1). The correlation between test and retest was significant (r_{34}=0.75, p<0.001). Compared to the test with four taste qualities the present investigation suggests the usefulness of the ''taste strips'' with umami as an additional taste.

293 Individual differences in human umami taste perception

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Food is one of the most important sources for good quality of life, but unfortunately also responsible for poor health. The sense of taste plays a special role in our everyday life and our survival. Several of the major health problems challenging the human population today- obesity, heart diseases, hypertension, type-2 diabetes, dental caries and food allergies- are all diet related. Variations in taste perception in healthy individuals might play an important role for the dietary choices made by them and, thus, resulting into diet-related health problems. Umami taste is the taste quality elicited by monosodium glutamate (MSG) and plays a key role in the intake of amino acids. The taste preference for L-amino acids has been suggested as a basic nutritional signal that reflects the amount of dietary protein in the body. In this study, we set out to investigate the inter-individual differences in sensitivity to MSG in the healthy Norwegian and German populations. In addition, we elucidated the extent of familiarity with umami taste in the two European

populations. A survey based on a questionnaire was collected from 105 German and 97 Norwegian subjects to explore the extent of familiarity with the umami taste quality; psychophysical screening for inter-individual variation for MSG sensitivity was conducted on 125 German and 131 Norwegian subjects. The psychophysical tests revealed that 3.2% of the German participants and 4.6% of the Norwegian participants were potential non-tasters of MSG. In conclusion, our study confirms inter-individual differences in sensitivity to MSG in humans. These results suggest that variation with respect to umami perception in the two populations might be a result of umami receptor variants that different populations are equipped with as a result of evolution.

294 CODING MIXTURE COMPONENTS IN SUCROSE-NACL MIXTURES

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Characteristic odors of components emerge from mixtures when selective adaptation and mixture suppression are combined (Goyert et al., 2007). In this study we test whether dynamic chemosensory coding also occurs in gustation by using a binary mixture of equally identifiable sucrose and sodium chloride components. The 2 stimuli have distinctive tastes, do not cross adapt but self adapt within a few seconds. 17 human subjects of average age 25 years volunteered for 4 1-hr testing sessions. Component taste stimuli (labels) were 50 or 100 mM NaCl (salt) and 150 or 300 mM sucrose (sugar). Subjects were seated so they could comfortably rest their chin in order to taste solutions by extending the tongue into a mist sprayed by the experimenter from 2 polyethylene bottles with atomizing spray caps, one after the other. The first stimulus (adapt) lasted for about 5 or 10 sec and the second stimulus (test) lasted about 5 sec. Possible stimuli for the 32 randomly presented adapt-test pairs were the single compounds, the NaCl-sucrose mixture, and water. Subjects, trained to identify water and the single compounds by label, rinsed with water between trials spaced 1 minute apart. Neither concentration nor adapt time affected identification. Within the binary mixture, the 65±12% identification of the suppressed salt taste compared to the 98±2% identification of the sugar taste ($p = .007$). After adapting to sucrose, the salt taste of mixture component NaCl was much better identified (92±5%) ($p=.02$). Tastes of adapted mixture components were poorly identified (35±6%), more so than self-adapted single components (74± 6%) ($p =.003$). We propose that gustation also uses selective adaptation and mixture suppression to dynamically code stimulus quality by adjusting the perceived intensity of the stimulus. Acknowledgements: Supported by NIH grant DC004849.

295 Monell Sucrose Preference Tracking Method: New Findings and Applications

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A brief version of the Monell Sucrose Preference Tracking Method (MSPTM) is currently being evaluated for inclusion in the NIH Toolbox Initiative, which aims to develop brief, comprehensive methods to assess function of people between the ages of 3 and 85 years. Between 2003 and 2007, the two-pass method was used to measure sucrose preferences in a racially/ethnically diverse sample of 356 children (5-9.9 yrs), 169 adolescents (10-19.9 yrs) and 424 adults (20-55 yrs). We established that this method was appropriate for the cognitive limitations of children, with all but 15 children and 1 adolescent completing the two-pass version of the task. In this large sample, we replicated two well-known relationships, supporting the validity of the method. First, there were significant between-age differences, with children and adolescents as a group preferring more intense sucrose solutions than adults ($p <0.0005$). Second, there were significant race/ethnic differences in sweet preferences, such that Black children (identified by mother's report of race/ethnicity) preferred more intense sucrose solutions than White children. A similar difference between White and Black adult participants was no longer significant when age, income, and education were included as covariates. Regardless of race/ethnicity, higher income was associated with lower sucrose preference. The norming phase of the NIH Toolbox initiative will permit these and other associations to be examined in a representative national sample. In preparation for large-scale national testing, a computer program has been developed to aid the examiner in selecting appropriate solutions for testing. Pre-packaged sucrose solutions are also being manufactured by a co-packer and tested for shelf-life. Acknowledgements: The project described was supported by a grant from the Pennsylvania Department of Health, Monell Institutional Funds and by Award Numbers AA09523 from the National Institute of Alcohol and Alcohol Abuse and R01HD37119 from the Eunice Kennedy Shriver National Institute of Child Health and Human Development. The content is solely the responsibility of the authors and does not necessarily represent the official views of the Eunice Kennedy Shriver National Institute of Child Health and Human Development or the National Institutes of Health. The Pennsylvania Department of Health specifically disclaims responsibility for any analyses, interpretations, or conclusions. Data analysis was supported in part with Federal funds from the Blueprint for Neuroscience Research, National Institutes of Health under Contract No. HHS-N-260-2006-00007-C.

296 Individual Differences in Salivary Amylase and the Perception of Oral Viscosity from Starch

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Digestion of starch begins the moment starch enters the mouth. This distinguishes carbohydrates from other macronutrients in humans. Salivary α-amylase initiates the digestion of starches in the oral cavity, prior to swallowing, producing a rapid decrease in polysaccharide chain length and viscosity. Previous research investigating the relationship between amylase levels and starch viscosity perception has assessed oral perception only at a single time point, and so has been difficult to interpret. Thus, we investigated whether individual variation in salivary amylase levels plays a

significant role in the perception of starch viscosity breakdown over time. Individual ratings of perceived viscosity over a specific time-course were analyzed to account for starch-saliva mixing and subsequent amylolytic cleavage of the starch. Salivary amylase levels and enzymatic activity were analyzed using Western blots, enzymatic assays and microviscoamylography. We observed significant individual variation in both salivary amylase levels and starch viscosity perception. When salivary flow rate was taken into account, salivary amylase levels were significantly correlated with an individual's temporal perception of starch viscosity. Our findings suggest that individual variation in oral perception of starch is related to individual production levels of salivary amylase, which in turn is under partial genetic control. Acknowledgements: National Starch Co. NIH

297 The Detection of Free Fatty Acids in Edible Taste Strips

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Recent evidence suggests that humans can detect unsaturated fatty acids as a distinct taste quality. Free fatty acids are hydrophobic compounds that are prepared as mixtures or emulsions for the examination of fatty acid taste. This approach results in tactile sensations when fats are detected in the mouth. However, edible taste strips rapidly dissolve in the oral cavity, and minimize tactile sensations. The goal of this study was to determine whether or not edible strips could measure fatty acid taste in humans. The *cis* unsaturated fatty acid linoleic acid (18:2, n-6) is an essential fatty acid that was used as a representative fatty acid tastant. At present, up to 1.7 nmoles of linoleic acid can be incorporated into a one-inch square, 20 micrometer-thick edible taste strip. Above this amount, linoleic acid undergoes a lateral phase separation as the polymer solution dries to a thin film. Edible strips that contained from 1.1 to 1.7 nmoles of linoleic acid were prepared under an N_2 atmosphere, and used to examine fatty acid taste. Thirty subjects were tested for fatty acid taste function by means of a two-alternative forced choice paradigm. Taste intensity was measured by the general Labeled Magnitude Scale (gLMS). Subjects readily distinguished between control and fatty acid taste strips. Taste intensity values increased with increasing amounts of linoleic acid in the strips, and normalized gLMS values ranged from an average of 7 to 17 in our sample. Subjects described fatty acid taste as oily or greasy. These results indicate that free fatty acids can be incorporated into edible taste strips for the examination of fatty acid taste. Finally, these results suggest that edible strips are a promising method for examining fatty acid taste in humans at both the threshold and suprathreshold levels. Acknowledgements: This work was supported by NIDCD R44 DC007291.

298 The Relative Satiety Value of Candy Bars in American Children

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Objective: Based on 13 Australian adults, Holt (1995), proposed of satiety index, induced by isocaloric consumption of culturally specific foods. We sought to determine if this could also apply to American children using hedonically preferred confectionaries. **Methods:** Twenty-four 12 or 13 year-old seventh graders, self-rated their degree of satiety in response to ingestion of eight different isocaloric (95 +/- 5 Kcal) hedonically positive candies as compared to white bread (Wonder Bread). Confections evaluated were: Tootsie Roll Midgets, Twix, M&M's, M&M's Peanut, Smarties, Star Bursts, Hershey's Kisses, and Gummy Bears. Ratings were performed immediately prior to and after, and at 15 and 30 minutes post ingestion. Using the area under the curve, the satiety index was calculated, and adjusted so that white bread equaled 100%. **Results:** Confectionaries were ranked based on their satiety as compared to white bread (100). Of the candies tested, Star Bursts demonstrated the greatest satiety index (135) being 40% more that the candy showing the lowest satiety index, Tootsie Rolls (179). On casual inspection, these candies seem very similar. Tootsie Rolls and Star Bursts both are individually packaged with multiple packages per portion, and are grossly similar in terms of texture, chewiness, and sweetness. However, Star Bursts, unlike Tootsie Rolls, possess different flavors with each portion, and thus, a more diverse chemosensory experience. This suggests that sensory induced satiety may be an important element in production of the satiety index. **Conclusion:** This validated and extended previous studies on Australian adults and demonstrated that the satiety index could be assessed in American children. **Source of Funding:** None

299 Measuring referral of retronasal odors: The effect of taste

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Referral of odors to the oral cavity is a fundamental phenomenon of flavor perception, yet the mechanism of referral remains poorly understood. A major impediment to understanding has been the absence of a method to measure referral. The aims of the present study were to use a novel psychophysical procedure to measure the perceived location of retronasal odors and to assess the role of taste stimulation in producing or augmenting referral. After sipping and spitting 5-ml aqueous solutions of citral (0.00025%) or vanillin (1.8 mM), Ss breathed normally through the nose and made natural tasting movements as they drew the perceived locations of odors on a diagram of the nasal and oral cavities using Microsoft Paint™. The effect of taste was studied by comparing localization when the odorants were presented alone or with sucrose (0.56 M) or NaCl (0.32 M). With 17 Ss tested the results show that when perceived alone, vanillin and citral are localized about equally often to the nose (61% and 67% of trials) and to the oral cavity above the tongue (52% and 68% of trials), but much less frequently to the tongue itself (29% and 38% of trials). When the odorants were mixed with sucrose, the frequency of localization to the tongue more than doubled (71% and 79%), while the frequency of localization to the nose declined (46% and 57%). Notably, when the odorants were mixed with NaCl, localization was unchanged from the odorants alone. Thus, referral to the mouth can occur, albeit incompletely, in the absence of a specific taste or somatosensory stimulus, but referral to the tongue depends strongly upon the presence of a congruent taste. These findings have implications for the neural mechanism of retronasal referral, particularly for how odors and tastes become co-localized on the tongue. Acknowledgements: Supported by NIH grant RO1 DC005002

300 A Modest Influence of Response Bias on the Enhancement of Taste-Like Properties of Odors

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Prior work has shown that odors come to be rated as smelling more like tastes with which they have been experienced. There is some question, however, regarding whether this effect can be attributed to perceptual learning vs. response bias. For example, one could observe a ''halo effect'' where rating one attribute (e.g. pleasantness) may influence the rating of the subsequent attribute (e.g. sweetness), or a ''dumping effect'' where the absence of a measure for a particular attribute results in the subject ''dumping'' that attribute into another response category. The current investigation aimed to determine the possible roles of these two response biases in the so-called acquisition of taste-like properties by odors. In experiment 1 we examined halo effects. 12 subjects were exposed to a target odor mixed with a sweet solution (0.3 M sucrose) and a non-target odor mixed with water. Subjects rated the odors before and after exposure for intensity, familiarity, sweetness and pleasantness. The order of the ratings was counterbalanced and the effect of order on the predicted increase in odor sweetness rating was examined. We found that sweetness ratings increased for the odor paired with the sweet solution but not for the odor paired with water. Sweetness enhancement was greater when order was included as a covariate. In experiment 2 we are examining dumping by testing whether inclusion of a scale in which subjects can rate ''other'' aspects of the sensation influences the enhancement of taste-like qualities of odors by taste. Preliminary data show that enhancement occurs irrespective of whether the ''other'' category is available. Taken together these findings support the view that the enhancement of taste-like qualities in odors represents perceptual learning, but may also be augmented by halo effects. Acknowledgements: Firmenich

301 Not all Formulas are Alike: Differential Growth Patterns among Infants Fed Protein Hydrolysate or Cow Milk-Based Formulas

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The rate of growth during infancy is a predictor of later obesity risk. Thus, interventions aimed at preventing obesity should begin during infancy when children are fed predominantly milk-based diets. Because of the striking differences among formulas, specifically the higher protein and amino acid content of protein hydrolyzed formulas (PHF) compared to cow milk-based formulas (CMF), we hypothesized that infants fed PHF would consume less formula and show slower rates of growth across infancy. To this end, we conducted a clinical trial in which infants whose mothers had decided to formula feed were randomized to feed either CMF (n=32) or PHF (n=24) from 0.5 to 7.5 months. Infants were weighed and measured monthly, then videotaped feeding their assigned formula under naturalistic conditions. Multilevel linear growth models were employed to compare trajectories for anthropometry and intake. Although there were no group differences at study entry, CMF infants had significantly higher weight-for-age and weight-for-length z-scores across ages 2.5- 7.5 months and significantly greater change in weight-for-age z-scores across ages 1.5-7.5 months compared to PHF infants. Infants fed CMF consumed more formula to satiation than infants fed PHF throughout the study. That there were no differences in infant acceptance or maternal rating of infant enjoyment at any age suggests that the slower growth rate of PHF infants cannot be attributed to rejection of the flavor of PHF. In conclusion, infants fed PHF satiated faster than infants fed CMF and the ensuing decreased intakes resulted in more normative growth trajectories. Whether the enhanced satiating effect of PHF is attributable to its higher protein content, amino acid distribution, or some other metabolic effect is an important area for future research. Acknowledgements: The project described was supported by Award Number R01HD37119 from the Eunice Kennedy Shriver National Institute of Child Health and Human Development. The content is solely the responsibility of the authors and does not necessarily represent the official views of the Eunice Kennedy Shriver National Institute of Child Health and Human Development or the National Institutes of Health.

302 Evaluation of Newborns' Movement by Image Segmentation while They Drink an Infant Formula

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Babies show their comfort or discomfort through facial expressions, vocalizations and body movements. The objective of the present study was to evaluate newborns' movement while they drink an infant formula using a technique called image segmentation. Forty seven newborns were recruited in a local hospital in Xalapa, Mexico. The study procedures were approved by the Ethics Committee of the University of Veracruz, and informed consent was obtained from each mother before the start of the study. All available milk-based and soy based infant formulas for 0 to 6 months old babies were selected. Nine different brands of infant formulas were included in the study. Each baby was randomly given two kinds of baby formula. All sessions were video filmed. Each of the 94 videos were adjusted to 1 min long, and then fragmented into two frames per second. A sequence program was developed in the Matlab R2008a software. The program sets a background that stays constant during the video sequence. The moving object, the newborn in this case, is analyzed. The software identifies the skin color and draws a digitalized figure of the newborn body. The resulting image is processed pixel-based using differences in color between skin and background. Two fundamental variables were measured, the bounding box area and the movement speed. It was determined that newborns moved faster when drinking soy-based formulas, especially during the first 20 seconds of the video. In addition, faster moving infants drank less volume of infant formulas. This technique might be useful to identify emotions in very young individuals, and to be applied in other areas such as the identification of food preferences. Acknowledgements: Thanks to CONACYT, PROMEP and the Government of Veracruz State from Mexico for their support.

303 Factors Influencing Mothers' Perceptions of their Infants' Liking of a Green Vegetable.

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Mothers' perceptions of their infants' liking of foods play an important role in determining the types and variety of foods they feed to their infant. These perceptions may be influenced not only by the infants' responses to foods, but also by various characteristics of both mother and child. The goal of the present study was to determine which factors impact maternal ratings of infants' acceptance of a green vegetable at the time of weaning (5-10 months). To this end, 92 mother-infant dyads were tested under naturalistic conditions in which the infants determined the pace and duration of the feeding. Acceptance and liking of pureed green beans were assessed using a variety of measures, including amount and rate of consumption, and the frequency of facial distaste expressions using the anatomically-based Facial Action Coding system. At the end of each feed, mothers rated their infants' enjoyment of the food. Although maternal ratings were not affected by socioeconomic status (e.g., mothers' income, education level), maternal BMI, or infant temperament, ratings were lower for infants who rejected the food during the first five spoon offers, showed more facial expressions of distaste, and ate less food for a shorter period of time. When these factors were entered into a stepwise multiple regression analyses, we found that mothers' ratings were primarily influenced by how long their infants ate, and by the number of lip raises they made during the feeding session. A better understanding of mothers' interpretations will aid in the establishment of evidence-based guidelines for feeding vegetables during infancy. Acknowledgements: The project described was supported by Award Number R01HD37119 from the Eunice Kennedy Shriver National Institute of Child Health and Human Development. The content is solely the responsibility of the authors and does not necessarily represent the official views of the Eunice Kennedy Shriver National Institute of Child Health and Human Development or the National Institutes of Health.

304 A study examining the incidence of taste disorders in the general population

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Objective: In contrast to olfaction so far little is known about the incidence of taste disorders in the general population. It was the aim of our study to examine the incidence of taste disorders using two different whole mouth tests.**Subjects and Methods:** Altogether 761 subjects (297 male, 464 female, mean age: 36 years, range: 5 -89 years) took part in the study. Whole mouth taste function was tested using both, taste strips (dried filter papers, applied on tongue, four different concentrations of sucrose, citric acid, sodium chloride and quinine hydrochloride) and taste sprays (same substances, but higher (above threshold) concentrations). Moreover, all subjects filled in a questionnaire concerning their general health and evaluated their subjective taste ability on a visual analogue scale. **Results:** Taste sprays: 95% of all subjects correctly identified at least 3 out of 4 sprays, 7 subjects identified only 1 out of 4 sprays, 2 subjects (1.2%) none. Taste strips: 95% identified more than 8 strips correct, 5.3% scored below 8 and 9 subjects (1.2%) scored below 6. All of these were male, 2 in the age of 10 and 12 years, 4 older than 50 years. Subjectively they all rated their taste function as normal. While the two young boys also were unable to identify the sprays correctly, only one of the older males had poor results in both, sprays and taste strips. Subjective evaluation and measured function did not correlate; moreover, regarding sex and age women scored better than men and younger subjects better than older ones. **Conclusion:** Taste disorders are rare. Complete ageusia was not found in the examined population. Similar to the situation in olfactory function subjects are unreliable to correctly rate their taste function.

305 Effects of aging on the injured peripheral taste system

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Peripheral nerve sectioning elicits an immune response to injury that is typically followed by regeneration and functional recovery. Aging can dysregulate these processes resulting in functional deficits. We examined the impact of aging on the injured taste system during the first days after chorda tympani nerve (CT) injury and later during regeneration. Previous work in young adult rats demonstrates that neutrophils rapidly invade both sides of the tongue, followed by a bilateral macrophage response to CT section. Neutrophil invasion of the uninjured side of the tongue also decreases neural responses to sodium in the neighboring, intact CT. We compared leukocyte responses in ''old'' (24 mon old) vs. ''young'' (3 mon old) F344 rats at day 2 after CT sectioning. Activated macrophage levels were not significantly different in old vs. young groups. In contrast, the neutrophil response was elevated and extended on the denervated side of the tongue in old compared to young rats. On the uninjured side of the tongue, neutrophils remained at control-like levels and normal responses were recorded from the intact, uninjured CT nerve. Aging had a more profound effect on taste function at later post-injury periods. Surprisingly, taste responses were absent in all but one old rat even at 85 days post-injury. Thus, aging delayed the recovery of CT function by at least 40 days, even though taste buds are present. The aged taste system appears to be more susceptible to injury and inflammation compared to other peripheral models, though few studies have focused on functional regeneration of both neurons and target cells. Acknowledgements: NIDCD (DC005811)

306 Cigarette Smoking, Obesity and Fat Perception in Women.

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The current study builds upon previous findings that woman who smokes and obese women craved high fats more frequently than women who do not smoke and on epidemiological data revealing that despite different cultures, lifestyles and food habits, smokers and obese individuals consume more fat than non smokers and lean individual respectively. Whether this preference for high fat is due

to a reduced fat perception in these subjects is unclear. This issue was assessed in the present study. To this aim, fat perception was measured via psychophysical measures in four groups of women [Lean Never-smoker (n=12), Lean Smoker (n=10), Obese Never-smoker (n=11) and Obese Smoker (n=14)]. The general Label Magnitude Scale was used to measure perceived intensity of creaminess, sweetness and saltiness and the general Hedonic Label Magnitude Scale was used to measure degree of pleasantness experienced when tasting vanilla pudding and tomato soup prepared with graded amounts of fat (range: 0% - 15.6%). We found that on average, ratings of creaminess significantly increase with increases in fat% (for both pudding and soup) and that the other taste characteristics (i.e. sweetness, saltiness) remained constant for the four groups. However, relative to the other groups, Obese Smoker rated creaminess, sweetness and saltiness of food as less intense. They also reported perceiving less pleasure from these foods. Specifically, Lean Never-smokers preferred the pudding with 3.1% fat content; Lean Smokers the 6.9%, Obese Never-smokers 15.6% and Obese Smokers react with a low flat response not showing a preference for any particular concentration offered. Taken together, the results suggest that, at least in part, the heightened preferences for fat in obese and smokers may be due to alterations in fat perception. Acknowledgements: This project was funded by a grant from the Pennsylvania Department of Health. The Pennsylvania Department of Health specifically disclaims responsibility for any analyses, interpretations, or conclusions. Dr. Pepino is currently a fellow of a NIDA T32 DA07313 at Washington University in St. Louis.

307 Chemosensory Loss: Functional Consequences of the World Trade Center Disaster

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Individuals involved in rescue, recovery, demolition and clean-up at the World Trade Center (WTC) site were exposed to a complex mixture of airborne smoke, dust, combustion gases, acid mists and metal fumes. Such exposures have the potential to impair nasal chemosensory (olfactory and trigeminal) function. The goal of this study was to evaluate the prevalence of chemosensory dysfunction and nasal inflammation among these individuals. We studied 102 individuals who worked or volunteered at the WTC site in the days and weeks during and after 9/11/2001 and an occupationally-matched comparison group with no WTC exposure. Participants were comprehensively evaluated for chemosensory function and nasal inflammation in a single session. Individual exposure history was obtained from self-reported questionnaires. The risk of olfactory and trigeminal sensitivity loss was significantly greater in the WTC-exposed group relative to the comparison group (RR= 1.96 [1.2-3.3] and 3.28 [2.7-3.9] for odor and irritation thresholds, respectively). Among the WTC responders, however, individuals caught in the dust cloud from the collapse on 9/11 exhibited the most profound trigeminal loss. Analysis of the nasal lavage samples supported the clinical findings of chronic nasal inflammation among the WTC-exposed cohort. The prevalence of significant chemosensory impairment in the WTC-exposed group more than 2 years following their exposure raises concerns for these individuals when the ability to detect airborne odors or irritants is a critical safety concern. This outcome highlights the need for chemosensory evaluations among individuals with exposure to acute high or chronic levels o airborne pollutants. Acknowledgements: Supported by a supplement to NIH-NIDCD P50 DC 006760

308 "Gender and Burning Mouth Syndrome"

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OBJECTIVE: To determine if burning mouth syndrome (BMS) in men is similar in presentation to that in women. **BACKGROUND:** BMS affects over one million Americans with overwhelming female predominance. BMS characteristics in men have not been specifically elucidated. **METHODS/RESULTS:** Retrospective chart review of the most recent 22 BMS patients (7 men and 15 women) were compared with statistical analysis using chi-square tests for binary variables and the Wilcoxon rank-sum test for continuous variables. The results from Using Bonforoni corrections, no significant difference was found in the following parameters including gender (male, female): age (59 and 54 years); duration of disease (2 and 3 years); severity of burning on a scale of 1 to 10 (6; 7), presence of complaints of hypogeusia (57%; 67%), dysgeusia (71%; 47%), dry mouth (57%, 20%), changes in taste perception of salt (43%; 50%); sweet (29%; 43%), sour (29%; 50%), bitter (29%; 57%) and presence of complaints of hyposmia or anosmia (43%; 60%), dysosmia (0%; 33%), and phantosmia (0%; 20%). On examination, all patients had normal Romberg testing and vibratory sensation, corrected for age based on Rydel Seiffer Tuning Fork. Olfactory tests including the unilateral University of Pennsylvania Smell Identification Test corrected for sex and age, the unilateral Smell Threshold Test using phenyl ethyl alcohol, Quick Smell Identification Test, the Pocket Smell Test, the Brief Smell Identification *Test corrected for age*, and Alcohol Suprathreshold Testing revealed abnormal olfactory tests bilaterally in at least one test in 100% of males and 87% of females. Taste testing including the Accusens T Taste Test and Taste Quadrant Testing revealed abnormalities (at least in one test) in 100% of males and 90% of females. Significant differences ($p<.05$) included (male; female): phantogeusia (100%; 60%), abnormality of saliva: too much (57%; 14%), and saliva as a bad taste (43%; 7%). **CONCLUSION:** Men with BMS do not have a distinct clinical presentation. **SOURCE OF FUNDING:** None.

309 Surgery for Mucosal Contact-Point Headache

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Headache is a common complaint in many environments. Not infrequently sufferers attribute the cause to inhalation of chemicals. There exists weak rhinological evidence for chemically induced headaches except from strong exposures. One can therefore look into cases where exposure might exacerbate an existing

condition. Headaches attributable to mucosal contact point provide a possible example. Such a headache results from contact between the nasal septum and lateral nasal wall, resulting in trigeminally mediated pain. Distribution of the pain varies. We investigated the location of the headache and also assessed the benefits of surgical correction in patients by use of endoscopic or radiographic evidence. This prospective study included patients who met the following criteria: 1) History of chronic headache, 2) Lack of acute or chronic inflammation, 3) Presence of contact point by nasal endoscopy or CT scan, and 4) Relief after application of topical anesthesia to the contact point. Severity of headache was assessed pre- and post-operatively using a visual analogue scale. Location, duration, and frequency of headache were also assessed using a questionnaire. Headaches occurred in the frontal, temporal, and glabellar area. Those patients whose headache was believed to result from intranasal contact point underwent surgical management. According to the pain questionnaire given pre- and post-operatively, severity, duration, and frequency of headache improved significantly. Contact point headache merits consideration in patients without other obvious causes of headache, including those patients who claim a chemical cause. Significant relief of headache can be obtained by surgery that eliminates the point of contact.

310 Pharyngeal insensitivity in patients with obstructive sleep apnea compared to healthy subjects

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Signs of pharyngeal neurodegeneration have been detected in patients with obstructive sleep apnea (OSA). This degeneration is believed to be due to excessive soft tissue vibration which is typically associated with snoring / apneas. Along with this neurodegeneration, a decreased pharyngeal sensitivity has been described in OSA patients compared to healthy subjects. The decreased sensibility may play a role in the physiology / progression of this disease. Aim of the study was to investigate the chemosensitivity of the pharyngeal mucosa of OSA-patients compared to healthy controls. Healthy controls (c) and patients with OSA (age: 30 till 60 years) were included. Testing of oropharyngeal chemosensitivity was performed with subjective intensity rating (SIR, Visual Scale 0-10) of capsaicin, air puffs (presented with olfactometer) and stimulation with CO_2 at the posterior pharyngeal wall. A two point discrimination test at the soft palate, a SIR of capsaicin at the tongue and a nasal lateralization test were performed. 12 patients with OSA and 6 healthy controls were included. No differences in the SIR of capsaicin on the tongue and in nasal lateralization test were detected. The results demonstrated a decreased pharyngeal sensitivity to capsaicin *(*OSA:7.7±1.2; c:8.3±1.4) air puffs (OSA:4.3±2.2; c:5.8±2.2) and stimulation with CO_2 (*40%*: OSA:2.1±2.2, c:3.2±1.5; *60%*: OSA:3.6±3.0, c:5.6±2.5) in patients with OSA. Two point discrimination at soft palate was reduced in the OSA group (OSA:11.8±6.4mm; c:5.2±2.1mm). The results suggest a reduced pharyngeal chemosensitivity in OSA-patients in addition to the reduced pharyngeal sensitivity as tested with two point discrimination. This supports the idea of a peripheral neurodegeneration in the context of this disease.

311 Intensity of Salt Taste and Hypertension.

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Perceived intensity of the taste of salt and its' relationship with hypertension was investigated in the Beaver Dam Offspring Study, a population-based cohort study of sensory loss and aging. Intensity of taste sensation was measured using filter paper disks and a generalized magnitude scale (gLMS) with a range of 0 to 100. Study subjects were participants who provided information on hypertension history and who used the full range of the gLMS in practice. Hypertension was defined as either a systolic pressure of >140 mmHg, a diastolic pressure of > 90 mmHg, or reported blood pressure medication use. Covariates included age, gender, education, smoking, and obesity (Body Mass Index >30.0 kg/m^2). There were 1753 study subjects (mean age=49.6, range=21-84 years) of whom 662 (38%) were hypertensive. The unadjusted mean salt intensity score did not differ between hypertensives (mean=29.3, standard deviation(s.d.)=19.5) and normotensives (mean=29.2, s.d.=19.2). After adjustment for age and gender, the odds of hypertension in participants rating the intensity as very strong or greater (> 53) were not significantly higher than the odds in participants rating the intensity as less than moderate (<17), (Odds Ratio(OR)=1.24, 95% Confidence Interval(CI)=0.86,1.78). Full covariate adjustment did not appreciably alter this relationship (OR=1.19, 95% CI=0.82,1.74). Restricting the analysis to participants reporting no history of physician diagnosed high blood pressure did not affect the results. In addition, in this restricted sample, no association between hypertension and the frequency of adding salt to food was found (adjusted P=0.82). This study observed no significant cross-sectional relationship between hypertension and the perceived intensity of the taste of salt or the frequency of adding salt to foods. Acknowledgements: The project described was supported by R01AG021917 from the National Institute on Aging, National Eye Institute, and National Institute on Deafness and Other Communication Disorders. The content is solely the responsibility of the authors and does not necessarily reflect the official views of the National Institute on Aging or the National Institutes of Health.

312 Development of an Electronic Tongue (ET) to Evaluate the Bitterness Intensity of Rx and OTC Formulations

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Masking the bitterness of actives to make pharmaceutical formulations more palatable has long been a goal for GSK. To be able to mask the taste of a bitter component, one must be able to evaluate its bitterness intensity first. Sensory evaluation by a human panel is often a difficult exercise due to the lack of a full toxicology profile at early stages of development. Even once this profile is

established, there is a desire to reduce the amount of human tasting to the minimum required. GSK has set up a partnership with the University of St Petersburg to develop an e-tongue with the aim of using the instrument in the evaluation of the bitter taste of pharmaceutical molecules. GSK needs a system with robust sensors to compare data across time, hence the choice to develop its own e-tongue. The bitterness intensity of 8 bitter tasting substances of different nature was evaluated by e-tongue and correlated with the values obtained by a sensory panel by PLS regression. The predicted MRE was 15 % (RMSE=0.72) with an error of only 5% (RMSE=0.27) for the ultra bitter active azelastine HCl . No prediction was possible for caffeine, paracetamol and KNO_3. The first two substances, being non ionic, were hard to detect by the sensors and no other inorganic salts were present in the calibration model for KNO_3. Accuracy in the bitterness prediction was achieved by lowering the original pH 7 to pH 6 and to pH4. The data obtained were merged into a three dimensional set (substance x sensors x pH) and the calibration model was calculated using 3-way PLS regression. The MRE became 12% (RMSE=0.51). The 3D model was not optimal for azelastine HCl (MRE = 22%) or naratriptan (MRE=23%) but it is a promising approach for the prediction of the bitterness intensity using an e-tongue. Further developments are underway.

313 GLMS for Ratings of Taste Intensity by the Elderly: Ready for the Toolbox?

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The General Labeled Magnitude scale (GLMS) is being evaluated as a tool to measure gustatory intensity perception for the NIH Toolbox Initiative, which aims to develop brief methods to assess function of people between the ages of 3 and 85 years. The mandate of a broad age range makes it challenging to choose appropriate Toolbox measures. In an attempt to understand whether older people can use the GLMS to rate the intensity of taste stimuli given a standard set of instructions, we had 30 cognitively competent (as measured by the Mini Mental State Exam) participants ranging from 60 to 100 years of age complete an instruction set for the GLMS developed for Toolbox (including visual and auditory examples) and also rate the taste intensity of water, 0.1M NaCl, and 0.32M NaCl. The averaged data showed the expected types of responses: e.g., the average rating of the brightest light ever seen was 77 on the 100 mm scale and the average rating of the light in the current room was 28. The averaged gustatory stimuli also showed the expected ordinal relationship (water <0.1 M NaCl <0.32 M NaCl). Three participants had ordering errors for the gustatory stimuli. Two of those participants also failed to discriminate between the visual and/or auditory examples in the instruction set. The average duration of the instructions plus the ratings was close to 9 minutes, with a range of 7 to 13 minutes. Modifications such as a shortened set of instructions have been discussed to achieve the stated goal of a five-minute taste measure for Toolbox. Acknowledgements: Supported with Federal funds from the Blueprint for Neuroscience Research, National Institutes of Health under Contract No. HHS-N-260-2006-00007-C

314 How Does Context Affect Taste Intensity?

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Like perception in general, taste perception is contextual. Judgments of taste intensity often show contrast effects: A given taste stimulus is judged less intense when it follows a higher rather than lower concentration of the same tastant (e.g., Riskey et al., 1979; Lawless et al., 2000). Judgments of intensity in other modalities, however, such as loudness, often show an opposite effect, namely, assimilation (e.g., Jesteadt et al., 1977; Marks, 1993). In assimilation, a given stimulus is judged more intense following a stronger stimulus. Judgments of taste intensity rarely if ever show assimilation. This is surprising, given evidence that assimilation reflects processes that should occur in all modalities (Ward, 1979). Assimilation should, therefore, appear in taste. Experiment 1 compared, in the same subjects, judgments of taste intensity (sucrose) and loudness (500 Hz tone), using the same contextual design (Marks, 1993). The judgments of taste intensity showed contrast, whereas the judgments of loudness showed assimilation. The taste and loudness paradigms did differ, however, in one important respect: To allow adequate intertrial rinsing of the mouth, taste stimuli were presented at a rate of about 1 every 30 sec; by comparison, acoustic stimuli were presented at a rate of about 1 every 6 sec. Experiment 2 used the same contextual design but presented, to a subset of subjects from Experiment 1, acoustic stimuli at a rate of 1 per 30 sec. At this slower rate, loudness judgments showed contrast, whereas these subjects' loudness judgments showed assimilation in Experiment 1. It is plausible that assimilation effects can occur in taste too, but only under rapid rates of stimulus presentation. Acknowledgements: Supported by NIH grant R01 DC009021-03 to LEM.

315 Taste Perception and Sensitivity to Emotional Disgust

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Taste perception and the emotion of disgust are both processed by the anterior insular cortex. Propylthiouracil (PROP) sensitivity is an established measure of taste sensitivity. Individuals who are highly reactive to PROP are presumed homozygotes (PAV/PAV) at the TAS2R38 gene locus on chromosome 5 and are labeled "supertasters". Supertasters have a life long history of more intense taste perception than individuals who are homozygous (AVI/AVI) at TAS2R38, "non-tasters" of PROP. As a function of the neural overlap between taste perception, disgust perception and presumed experiential consequences, it was hypothesized that supertasters would have higher disgust sensitivity than non-tasters. To test this proposition, college students completed several questionnaires which varied in the aspects of disgust measured, and a PROP taste test measured by the gLMS. On the basis of their PROP scores, participants were categorized as supertasters, tasters and non-tasters accordingly. Results showed both differences in disgust response as a function of taster status and that PROP sensitivity was positively correlated with disgust reactivity on specific scales. Results are discussed in terms of neural synergy and the underlying components of emotional disgust.

316 Effects of Taste Responsiveness on the Hedonic Reactivity to Sweetness and Bitterness

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Although it has long been suspected, it remains to be demonstrated whether individual differences in general taste responsiveness plays a role in individual differences in hedonic responses to foods and beverages. The present study investigated the hypothesis that higher responsiveness to taste induces stronger hedonic reactivity independent of hedonic valence. This was tested by determining the effect of individual differences in responsiveness to two common taste stimuli, sucrose and quinine hydrochloride, on the hedonic reactivity to the same stimuli. In the first session, subjects (N=40) rated liking/disliking of 4 concentrations of each stimulus on the newly developed Labeled Hedonic Scale. In the second session, they rated the perceived intensity of sweetness, sourness, saltiness, and bitterness for each stimulus on the general version of Labeled Magnitude Scale. The results indicated that individuals who perceived taste more intensely tended to like (for sucrose) or dislike (for quinine) the stimulus more strongly than individuals who perceived the taste as less intense. This tendency was stronger for quinine bitterness (r = -0.47, p = 0.002) than for sucrose sweetness (r = 0.32, p = 0.04). Further investigation showed that the slopes of individual psychophysical functions for sucrose sweetness were also significantly correlated (r = 0.47, p = 0.002) with hedonic range, suggesting that individuals who perceive larger changes in perceived intensity for a given change in concentration also report a wider range of hedonic experiences. The current findings suggest that taste responsiveness, in addition to other factors such as familiarity and cultural differences, may be an important contributing factor to the hedonic responses to foods and beverages. Acknowledgements: Oregon State University Start-up Funds

POSTER SESSION VI: PERIPHERAL AND CENTRAL TASTE; PERIPHERAL OLFACTION

317 Individual Predictors of Oral Free Fatty Acid Detection and Triacylglycerol Response

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Dietary fats provide energy as well as essential fatty acids that serve as precursors for an array of essential bioactive compounds. Depending on their form, they may also contribute to increased risk for a variety of chronic diseases as well as acute malaise. Thus, an orosensory signaling system for fats that aided ingestive decisions would likely hold adaptive advantages. Under appropriate conditions, food fats can be detected by all human sensory systems, with work on taste being the most recent and incomplete. Measures of detection (thresholds) and responsiveness (first phase serum triacylglycerol (TG) concentration (FPTR)) to free fatty acids (FFA) and TG, respectively, are characterized by high individual variability. The basis of which is not known. There are mixed reports from rodent studies and human trials of associations between these sensory measures and age, sex, BMI, PROP-taster status and resting TG concentrations. The present analysis explores these associations (except resting TG and taste thresholds) using a compilation of data from two human studies for taste thresholds and 7 human studies of FPTR. BMI was significantly associated with the mean FPTR (r=0.28, p=0.001) and peak TG (r=0.79, p<0.001) as well as the change of TG (r=0.21, p=0.014). These associations were not significant with matched, fat-free stimuli. Resting TG was associated with the mean FPTR (r=0.911, p<0.001) and peak TG (r=0.80, p<0.001), but not the change of TG. No significant associations were observed between age, sex or PROP-taster status and FFA detection or FPTR. Among the predictive variables tested, only BMI is associated with oral detection of FFA and the FPTR. Acknowledgements: Supported by PHS grant DK45294

318 Measurement of fat perception and electrophysiological assessment of taste function in patients with anterior lingual hemiageusia

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Introduction: Besides basic tastes, it has been speculated that fat perception is mediated via the gustatory fibres rather than by somato-sensory fibres. Basic taste function can be easily measured psychophysically. Gustatory evoked potentials have been described in experimental studies in healthy subjects. Studies including patients with circumscribed lesions have not been undertaken so far. ***Objective:*** To assess fat perception psychophysically in patients with unilateral hemiageusia of the anterior tongue. In the same patients gustatory event-related potentials (GERP) were recoded on the normal and hemiageusic side. ***Methods:*** A total of 21 subjects who had undergone radical mastoidectomy because of cholesteatoma more than two years ago were included in the study. Participants underwent psychophysical taste examination (taste strips) of the anterior and posterior parts of the tongue. Fat perception was also examined psychophysically on each side using a modified three drop method using treated and tastless safflower oil as stimulus. GERP were recorded with a Constant Liquid Flow Gustometer (GU001; Burkhart, Germany). ***Results:*** Psychophysical taste function of the anterior part of the tongue was significantly lowered on the previously operated side (p<0.001). GERP could confirm this unilateral taste deficiency. The psychophysical taste function of the posterior parts of the tongue did not differ between the operated and healthy side. Fat perception was not different between the operated (ageusia) compared to the non operated (normogeusia) side. ***Conclusion:*** The present findings suggest that fat perception is mediated more likely by the trigeminal than by the gustatory system. Furthermore, based on the small sample, we had difficulties confirming the expected release of inhibition phenomenon previously described after injury of the chorda tympani nerve. In contrast, we could successfully use GERP in patients, which should open new perspectives in terms of the clinical workup of patients with taste dysfunction. Acknowledgements: This study was supported by a Grant of the Swiss National Fund for Scientific Research to BNL (SSMBS grant n° PASMA-119579/1).

319 Bitter Taste Receptor Signaling in the Gut Stimulates ABCB1 through a Paracrine Mechanism Involving CCK/Gastrin and its Receptor

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Bitter taste receptors (T2Rs) are expressed in gut derived enteroendocrine cells where they have been hypothesized to play an important role in limiting absorption of bitter tasting, potentially toxic compounds. Because intestinal ABCB1 (P-gp, MDR1) is well known to limit the absorption of xenobiotics, we hypothesized there is a physiological relationship between intestinal bitter taste receptors and efflux transporters. Here, we show that phenylthiocarbamide (PTC), a bitter agonist for T2R38, increased P-gp expression in both Caco-2 human intestinal cells and mouse intestine. We also show that PTC is a functional modulator of P-gp activity as well as an efficient substrate for the efflux pump. In addition, PTC activation of P-gp is decreased by either siRNA targeting T2R38 or treatment with YM022, a gastrin receptor antagonist. These results indicate P-gp in enterocytes is regulated through paracrine signaling by gastrin released in response to PTC signaling in enteroendocrine cells. Thus, intestinal activation of T2R38 and possibly other T2Rs might be targeted therapeutically to upregulate or downregulate intestinal ABCB1 activity to protect against ingestion of dietary toxins or increase the effectiveness of ABCB1 targeted chemotherapeutic agents. Acknowledgements: NIH HL48044

320 TRPM5 is required for fatty acid transduction in mouse taste cells

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A number of studies have demonstrated the ability of free fatty acids (FAs) to activate taste cells and elicit behavioral responses consistent with there being a taste of fat. We have used both molecular and cell-based assays to explore the mechanism of FA transduction in mouse taste cells and found that TRPM5 plays a critical role in this process. Taste cells express several types of putative FA-responsive proteins including the FA-binding protein CD36, FA-sensitive K^+ channels and FA-activated G protein coupled receptors that appear differentially expressed in the various taste papillae. Using whole cell patch clamp recording in taste cells, we found that linoleic acid (LA) depolarized taste cells and this depolarization was significantly reduced in the absence of extracellular sodium ions. LA also elicited rapid, sodium-dependent inward current at −100 mV. Ion substitution experiments revealed that these LA-induced currents were carried by monovalent cations. LA-induced currents were significantly reduced when G-proteins and phospholipase C (PLC) were blocked, suggesting that the inward currents are activated downstream of G-proteins and PLC. LA-induced inward currents were greatly inhibited by the TRPM5 blocker, triphenylphosphine oxide (TPPO), and were virtually abolished in taste cells from mice lacking TRPM5. 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 the FA-induced intracellular calcium rise. These results are consistent with a role of TRPM5 in FA transduction. A model for the transduction of FAs in taste cells consistent with these findings and our previous data will be presented. Acknowledgements: Supported by NIH DK059611, UAES Project 00630 and International Flavors & Fragrances (to TAG) and NIH DC03155 (to RFM).

321 Dietary Modulation of the Fatty Acid Transduction Pathway

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Recently, we have proposed a transduction pathway for fatty acids (FA) in mammalian taste buds that consists of a concerted action of free FAs on FA-activated GPCRs, the FA binding protein CD36 and FA-sensitive delayed rectifying K^+ channels (FA-s DRK) channels. This pathway involves the activation of phospholipase C and TRPM5 channels downstream of the initial receptive proteins. Our previous work has shown that (1) obesity-prone and -resistant rats differentially express FA-s DRK and that this difference is linked with peripheral responsiveness to FAs and (2) that long-term (i.e. weeks) high fat diets lead to alteration in FA-DRK expression and a subsequent reduction in chemosensitivity to FAs. In the present study, we have begun to explore the timing of the high fat diet-induced changes as well as the specificity of these changes. To accomplish these goals, mice and rats are being placed on high fat (60%) or control diets for periods of 3, 7, 14, and 28 days after which time tissues are collected for assay of gene expression changes by quantitative real time PCR using a TaqMan style, multiplexed reaction. At each of these time points, we are measuring changes in CD36, the FA-sensitive GPCRs (GPR40, GPR84, and GPR120) and members of both the FA-sensitive and FA-insensitive DRK channel families. Our preliminary results suggest that the response to a high fat diet occurs as rapidly as 3 days, which may contribute to the alteration of intake seen on this diet during the first week of its administration. Further, the most evident changes in expression occur in the FA-s DRK channels, similar to our previous findings. Taken together, these data point to the ability of FA chemosensory pathways in the taste system to respond to diet in a nutrient-specific manner. Acknowledgements: Supported by NIH DK059611, UAES Project 00630 and International Flavors & Fragrances.

322 Glossopharyngeal nerve transection eliminates preference for a corn oil emulsion but does not decrease high-fat diet intake or the associated acceleration of body weight gain in rats

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In rats, the glossopharyngeal nerve (GL) innervates taste buds in the posterior tongue that express the CD36 fatty acid translocator, as well as the von Ebner's glands, both of which play putative roles in fat taste. In rodents, transection of the GL (GLX) has been shown to eliminate preference for both corn oil and linoleic acid solutions in long-term 2-bottle tests. Here we tested whether GLX would decrease intake of a high fat diet and curtail the

associated acceleration of body weight gain (n = 4–9/group). Rats fed a 60% fat diet (HFD) consumed significantly more calories than rats fed standard rat chow (SD) for ~ 3 weeks, but then stabilized such that there was no difference in caloric intake between the two diet groups. The HFD-fed rats gained significantly more weight than the SD-fed rats over the 10 wk observation period. Histologically confirmed GLX had no effect on these outcomes. In subsequent tests, surgical condition had no effect on interlick intervals to water or licking during 10 s trials to corn oil emulsions (1-32%) in a 30-min test while water-deprived, suggesting that GLX did not cause any obvious motor impairment in drinking. However, GLX (but not diet) eliminated preference for 16% corn oil over water during 48-h two-bottle preference testing (40% vs. 75%). When given both the HFD and SD in 48-h intake tests, GLX rats displayed a slight but significantly higher preference for the HFD compared to SHAM rats. Thus, GLX did not reduce intake or preference for a high-fat diet although it eliminated preference for and decreased intake of a corn oil emulsion. These results suggest that signals from the GL do not substantially influence intake of a high-fat solid maintenance diet, but do contribute to the regulation of intake for a supplemental corn oil emulsion. Acknowledgements: This project was funded in part by NIH R01-DC01628.

323 Insect Olfaction and the Electrostatic Effect

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Laminar flow has been the focus of discussion when investigating the mechanism of odorant deposition on insect sensillae. Although laminar flow is important, electrostatic effects have been reported in the literature, but seldom discussed. Their importance to insect olfaction will be presented. Eric Erickson has shown electrostatic charges to build up on honeybees in flight. These charges range from +10-1470 millivolts. Ulrich Warnke has shown preferential deposition of charged aerosol particles to occur on wing sensillae when he would artificially introduce a charged solution in the hemolytic region of the wing. Philip Callahan exposed insect gustatory chemosensillae to negatively charged aniline blue droplets and found them to migrate to the tip. D.K. Edwards has noted that flies vigorously rub their front legs together and groom their wings when exposed to sudden changes in the electrical field. This author and countless others have noted wing-fanning behavior in moths when abruptly exposed to their own pheromone. Auto-electrification can be achieved by ordinary flight, by wing-fanning behavior, by the rubbing together of leg pairs, and by the self-grooming of their wings. Each of these is known to increase the positive charges found on the surface of an insect's cuticle. Due to the electrical point effect, these charges will be focused on pointed structures or tips such as the antennae, the wings, and the sensillae. Odorant molecules will be preferentially attracted to these regions of the insect body. This will allow charged airborne molecules, especially in low concentrations, to be more easily detected by insects.

324 Preference of the Fatty Acids Linoleate and Oleate during Long-Term 2-Bottle Tests

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In long-term 2-bottle tests, rats preferentially consume fatty acids compared to control solutions. These studies assessed one concentration of a single fatty acid (2% linoleic) and reported only intake and percent preference measurements. This study extends this research by assessing the licking behavior of rats to multiple, lower, physiologically-relevant concentrations of both linoleate and oleate fatty acids using a microanalysis of licking patterns during long-term preference tests. Twelve, adult, male Sprague-Dawley rats had free access to 2 bottles, one containing the fatty acid test solution and the other containing water, for 23hr per day. An AC-108 lickometer recorded each lick for each bottle during the test sessions. The concentrations of linoleate (50, 100, 200 μM) and oleate (100, 200, 400 μM) were presented in randomized order for 2 consecutive days with the left/right position of the bottles reversed on the second day. Licking data was divided into meals (terminated by 600s pause) and a microanalysis of each meal quantified licking patterns. Both session licks and meal licks reveal maximal preference for 100 μM linoleate and 200 μM oleate. Licks decreased for 200 μM linoleate but did not differ between 200 & 400 μM oleate. The number of bursts for 200 μM oleate was greater than both 100 & 400 μM and did not change across linoleate concentrations. Burst size decreased for 200 μM linoleate but increased for 400 μM oleate. Overall, there was little preference for either fatty acid over water suggesting that physiologically-relevant concentrations of fatty acids may be under the threshold for stimulating preferential fatty acid consumption. Currently, we continue to assess preferences of micromolar to 2% (71mM) fatty acid concentrations. Acknowledgements: Community of Scholars at Wofford College, Fullerton Foundation

325 Conditioned aversion to a novel taste infused directly into the mouse gut may be attributed to reflux into the oral cavity

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To determine if information generated by taste receptors in the gut is perceived similarly to sensory information from the oral cavity, we examined preference for a taste infused intragastrically (IG) and paired with a toxin (i.e. LiCl). Mice (n = 5) were given three IG infusions of 5 mM sodium saccharin (1.0 ml over 10 min) immediately followed by LiCl (230 mg/kg i.p.). Four consecutive 23h, two-bottle preference tests (saccharin vs. water) demonstrated a significant decrease in preference for 5 mM saccharin ($p < 0.0001$) in comparison to mice receiving IG saccharin only (n = 5) or i.p. LiCl only (n = 8). This reduced preference indicated that mice ''tasted'' saccharin infused into the gut. However, efforts to replicate this experiment with 10 mM saccharin using a smaller infusion volume (0.50 ml over 10 min) failed. To understand why saccharin was not tasted in the latter experiment, the same mice were infused with 0.25 ml, 0.5 ml, 0.75 ml, 1.0 ml, 1.25 ml or 1.5 ml (n = 3/volume) of 5.4 mM sodium fluorescein over 10 min. Two control mice received neither surgical treatment nor fluorescein. Mice were sacrificed 90 min following infusion and tongues, esophagi and hearts were harvested and stored in 0.1 M PBS for 24 h. Tissue samples were examined under microscope using a fluorescein filter set (4x). Fluorescence was observed

from the tongues and esophagi of mice infused with a volume of 0.75 ml or more. No fluorescence was observed from the heart tissues. These results suggest that infused fluids of sufficient volume can leak through the esophageal sphincter and enter the oral cavity through the esophagus. In the mouse, tastants delivered IG can be detected in the oral cavity and associated with lithium-toxicosis (decreased preference) despite the absence of consumptive behavior during exposure. Acknowledgements: NIDCD 5T32DC000014-30 and USDA CA-09-7442-0585

326 Structural Modeling of the Putative Fat Taste Receptor, CD36

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CD36 has been identified as a possible fat taste receptor (Laugerette et al., J Clin Invest 115:3177, 2005). It is also involved in fatty acid transport (Hajri and Abumrad, Annu Rev Nutr 22:383, 2002). There is currently no three dimensional structure available for this protein or for any homologous protein. The objective of this study is to begin understanding the structural basis for the function of the CD36 protein. Using bioinformatics and molecular modeling, I have modeled the transmembrane helical segments of this protein. Protein-protein docking methods have been used to examine helix-helix interactions, as well as formation of dimers or higher multimers. Acknowledgements: NIH-NIDCD Grant R24 DC008623

327 Does taste determine daily intake of dilute concentrations of glucose and fructose in C57BL/6 mice?

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When a rodent licks a sugar solution, it activates taste circuits in the central nervous system that influence stimulus identification, ingestive motivation and insulin release. Here, we asked whether taste circuits also influence daily intake of sweet solutions in C57BL/6 mice. More specifically, we tested for a causal relationship between taste responsiveness and daily intake of solutions containing saccharin and/or dilute concentrations of sugar. We used dilute sugar concentrations so as to minimize the negative postingestive effects of concentrated sugars on intake. In Experiment 1, we measured both chorda tympani nerve and short-term licking responses to the following solutions: 167, 250 and 333 mM glucose (G), 167, 250 and 333 mM fructose (F), 38 mM saccharin (S), and binary mixtures of G+S and F+S. There was a high correlation between the two measures of taste responsiveness. Both indicated that the mice show higher taste responsiveness to (i) G+S and F+S than any single sweetener, (ii) S than any concentration of F or G, and (iii) F than isomolar concentrations of G. In Experiment 2, asked whether daily intake of these solutions increased with taste responsiveness. No such relationship was observed, however. For instance, the G solutions elicited the weakest taste responses, but the greatest intake. In contrast, the F+S, F and S solutions elicited the strongest taste responses, but the weakest intake. These findings indicate that taste does not determine daily intake of dilute sugar solutions. We propose, instead, that daily intake is controlled primarily by the extent to which a sugar solution stimulates intestinal nutrient detectors. This proposition is based on studies showing that G provides substantially more positive post-oral reinforcement of feeding than F. Acknowledgements: Supported in part by a grant from the HHMI to Barnard College

328 Glucose utilization supports preferences for sugars in mice

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We are investigating the role of glucose utilization as a rewarding postingestive factor influencing carbohydrate intake independently of sweetness perception. C57BL6 mice fitted with gastric catheters were given access to a water-containing recipient such that an infusion pump connected to the gastric catheter was activated whenever licks to water were detected. We first show that in this paradigm mice will consume significantly higher levels of water when gastrically infused with glucose compared to when infused with L-serine, an amino acid known to be a weak promoter of glucose utilization. This finding did not depend on nutrient-specific gastrointestinal absorption since jugular rather than intragastric infusions produced essentially the same effect. We then verified whether these sweet-independent responses to glucose infusion were altered by disrupting glucose utilization with intravenous infusions of 2-deoxyglucose (2-DG, a glucose analogue that does not undergo complete glycolysis). Finally, microdialysis measurements were performed to test whether intravenous 2-DG infusions can alter dopaminergic response patterns in reward circuits that under normal conditions are observed upon nutrient intake. These preliminary findings suggest that preferences for carbohydrates over other nutrients might develop independently of taste quality, and that metabolic signals generated during the catabolism of glucose molecules might reinforce preferences for sugars over other nutrients. Acknowledgements: The J. B. Pierce Laboratory

329 Oxytocin Enhances Brief-Access Taste Preference for Sweet and Umami Stimuli

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The neurohormone oxytocin (OXT), known for facilitating lactation, parturition, and social behaviors, also inhibits ingestion. Mice lacking OXT previously were shown to overconsume sweet and carbohydrate solutions compared to wild-types. The apparent preference for sweet stimuli was attributed to a diminished post-ingestive satiety for carbohydrates (Sclafani et al, 2007). We recently showed that OXT receptor is expressed and functional in some cells in

mouse taste buds. We therefore asked if circulating OXT alters the taste preferences of wild-type mice. We injected 12 water-deprived C57BL/6 mice (6 males, 6 females) intraperitoneally with 10 mg/kg body weight OXT or an equal volume saline. Thirty min later, we tested them in a Davis brief-access lickometer, using concentrations of tastants that give mid-range behavioral responses: 0.3M NaCl (salty), 0.02M citric acid (sour), 0.3mM quinine HCl (bitter), 10mM Na-saccharin (sweet), 0.1M MSG with 500μM inosine monophosphate (IMP) (umami), and water. Animals were tested once daily for 6 days, and given OXT or saline on alternating days. For each tastant, we calculated lick ratios as lick rate for tastant / lick rate for water. OXT significantly increased the lick ratio for saccharin (1.62±0.15 *vs.* 1.08±0.04) and for MSG+IMP (1.44±0.17 *vs.* 1.05±0.03) but not for other tastants. OXT decreased lick rates to all solutions and to water as expected from its effect on ingestion. However, the lick rates to saccharin and to MSG+IMP were decreased less than for water, resulting in higher lick ratios. There were no differences between males and females. Thus, systemic OXT decreases fluid intake overall while enhancing preference for palatable (sweet and umami) tastes. To what extent this is due to action on peripheral vs. central sites remains to be investigated. Acknowledgements: Supported by NIH/NIDCD grants R01DC6021, R21DC10078 and American Heart Association Predoctoral Fellowship 0815215E.

330 Generalization of conditioned taste aversion (CTA) to guanosine 5′-monophosphate (GMP) in C57BL/6 mice

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Guanosine, inosine and adenosine 5'-monophosphates (GMP, IMP and AMP) have synergistic effects on umami taste of MSG in humans (Yamaguchi, 1967), and on taste responses of the rat chorda tympani nerve to various amino acids (Yoshii, 1987). These purine 5'-ribonucleotides however differ in whether they evoke an umami taste without MSG: IMP and GMP alone have a distinct umami taste, but AMP is almost tasteless to humans (Yamaguchi, 1967). Only a few studies have examined taste perception of purine 5'-ribonucleotides in mice. Our previous mouse behavioral study has shown that IMP evokes a taste quality similar to MSG, but there are some perceptual differences between these two compounds (Murata *et al.*, 2009). The goal of this study was to examine taste perception of GMP. We developed LiCl-induced CTA to 10 mM GMP and examined its generalization to 17 taste stimuli in C57BL/6 mice. An aversion to GMP generalized to 10 mM IMP, to a representative umami taste stimulus (a mixture of 50 mM MSG, 2.5 mM IMP and 30 μM amiloride added to block sodium taste) and to NaCl, but it did not significantly generalize to AMP and other taste stimuli. These results suggest that like humans, mice perceive a similar taste quality of GMP and IMP, but AMP does not evoke this qualitative taste sensation. Whether AMP has its own taste and whether it is qualitatively different from that of IMP and GMP will be examined in our future studies. Acknowledgements: Supported by the Fisheries Research Agency (Yokohama, Japan) (YM) and NIH grant DC00882 (AAB)

331 Cyclophosphamide Effects on Umami Taste of Mice

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Cyclophosphamide is one of the most commonly prescribed chemotherapy drugs used in the treatment of cancer. It is a DNA alkylating agent which attacks guanine base pairs and interferes mainly with the S-phase of the cell cycle (Emandi et al., *Nat. Rev. Clin. Oncol*., 2009). As a chemotherapy drug, it's adverse side effects include loss or alterations in taste senstion which can lead to malnutrition and poor patient recovery. However, little is known about how the taste system is altered by cyclophosphamide. We are studying the changes in taste perception produced by this drug and whether there is any change in the number and physiology of taste buds. Taste sensory cells have a high turnover rate, hence are thought to be susceptable to chemotherapy drug treatment. We hypothesize that cyclophosphamide damages the DNA of taste progenitor stem cells as well as transitory amplifying cells, causing their cell cycle to arrest until DNA repair is completed. The taste sensation comes back when the cell cycle resumes and the taste buds are repopulated with functional sensory cells. We tested this hypothesis by training mice to discriminate between MSG and IMP, then injecting the mice with cyclophosphamide and testing their discrimination performance for 16 more days. Performance was disrupted for up to 4 days after injection, then again 9-12 days after injection. This 2-phase disturbance in taste function corresponds to what one would expect when cells are immediately challenged by the toxic effects of the drug (phase 1) and the longer term (phase 2) impact of arresting the normal cell replacement cycle in taste buds. Acknowledgements: This work is supported by Vermont Genetics Network (Grant P20 RR16462).

332 Anatomical dissociation of melanocortin receptor agonist influences on taste- and gut-sensitive feeding processes

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Melanocortin 3/4 receptor ligands such as melanotan-II (MTII) have been strongly implicated in the control of feeding and metabolism. In particular, melanocortins have been suggested to reduce food intake through enhanced postingestive feedback (satiation). We used a microstructure analysis to characterize the effects of MTII delivered to the forebrain (3V) or hindbrain (4V) ventricles on licking for sucrose, saccharin and water solutions in rats. A dose response analysis of licking after 4V infusion of MTII doses ranging from 0.005nM to 1nM revealed that MTII at doses of 0.1nM and 1nM significantly suppressed consumption of 0.8M sucrose solution in 7 rats (ps <0.05). This effect was carried through a reduction of the number of licking bursts and in meal duration; measures commonly associated with inhibitory postingestive feedback (satiation). There was no effect of 4V MTII on measures associated with taste evaluation such as initial lick rate or mean lick burst size. A comparable dose-response analysis after 3V injection of MTII (n=13) revealed nearly identical effects on the consumption of 0.8M sucrose solution, with 0.1nM and 1nM MTII doses significantly suppressing meal size (ps <0.05). Likewise, measures of postingestive feedback (meal duration and burst count) were also

significantly suppressed (ps <0.05). However, one measure of taste evaluation (initial lick rate) was also significantly reduced (p <0.05), suggesting a possible effect on taste sensitivity related to forebrain but not hindbrain melanocortin stimulation. To explore this hypothesis, 1nM MTII was infused into the 3V and the effects on water, 0.1% saccharin, 0.1M and 1M sucrose solutions were evaluated (n=4). MTII significantly reduced meal size for saccharin and 1M sucrose but not for the less preferred 0.1M sucrose solution or water. Initial lick rate was significantly suppressed for saccharin whereas meal duration was suppressed for 1M sucrose, and burst count was reduced for both solutions (ps <0.05). Overall, the results suggest an anatomical dissociation of melanocortin sensitive sites and their respective influences on taste and postingestive feedback sensitive measures of feeding behavior. To further explore the possibility that forebrain melanocortin-sensitive sites have a potential influence on taste evaluation, we are evaluating the influence of 3V MTII infusions on brief access licking for a range of palatable and aversive taste solutions. Acknowledgements: NIH DC 07389 Amherst College

333 Diminished Fat Preference in Preprodynorphin KO Mice

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Opioid signaling plays an important role in modulating taste reward. Signaling through kappa opioid receptors (KOR) increases palatable food intake, while blockade of these receptors attenuates consumption, implicating endogenous KOR signaling in taste-guided behaviors. Competing hypotheses have proposed that opioids increase intake of preferred tastants or, instead, preferentially increase consumption of fat. Presently the role of endogenous KOR ligands in taste-guided behaviors is poorly understood. To understand the contribution of dynorphin (the primary endogenous KOR ligand) signaling to taste processing, we have systematically investigated tastant intake in preprodynorphin KO and WT mice during 48 hour two bottle choice experiments using sweet (saccharin, 0.1 – 100 mM; and sucrose, 0.01-1 M), and fatty tastants (olestra, 0.16-10%; and intralipid, 0.03-10%). To elucidate the contribution of dynorphinergic signaling to fat preference, we performed additional two bottle choice experiments in which sweet and fatty taste options were presented simultaneously (sucrose [3-300 mM] vs. intralipid [0.4, 0.8%] and saccharin [0.1-3 mM] vs. olestra [2.5, 5%]). Intake of fatty tastants was significantly lower for KO mice relative to WT mice for both caloric (intralipid; main effect of genotype, p=0.01) and non-caloric lipids (olestra; main effect of genotype, p=0.04). In contrast, KO mice did not differ from WT mice in overall levels of sweet tastant consumption (all p>0.05). When fat vs. sweet preferences were tested directly, preference in KOs for non-caloric tastants was significantly shifted toward sweet and away from fat tastants (main effect of genotype for preference, p=0.001, and olestra intake, p≤0.01). When tested with caloric tastants, KO mice consumed significantly less lipid than WT mice (p<0.05). These data suggest that 1) endogenous dynorphin signaling promotes intake of reinforcing tastants and 2) under choice conditions, dynorphin signaling preferentially promotes fat over sweet tastant consumption. Acknowledgements: Supported by NIH grant 5R21MH082325-02, NARSAD, University of Utah.

334 The Chorda Tympani Carries Two Anatomically Distinct Inputs to Rostro-Central Subdivision and to Rostro-Lateral and Ventral Regions of the NTS

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The afferent component of chorda tympani nerve (CT) is composed of fibers with heterogeneous morphology: While 75% of the axons are thin and unmyelinated, the remainder are thick and myelinated, implying at least two functionally distinct CT inputs projecting to nucleus tractus solitari (NTS). In the NTS, in addition to the dense projections within taste-responsive rostro-central subdivision (RC), occasional CT fibers were reported in other divisions. In order to explore whether these peripheral CT projections are mere extensions of axons projecting to gustatory subnucleus, we examined the entire extent of NTS in adjacent coronal sections stained for Nissl, myelin and the tract tracer identifiying CT projections. In all cases examined (n=3 adult rats), a dense plexus of CT axon projection was found in the rostral 800 (±150) μm of the nucleus, while less densely packed fibers were present throughout the rostro-lateral (RL) and ventral (V) subdivisions of the NTS. The transition from the dense (core) and the sparse (outside of core) regions was evident as a clear separation of two distinct anatomical structures. At the electron microscope resolution, several morphological differences were present: Peripheral fibers formed larger synaptic boutons, more frequently engaged in glomerular structures, more frequently formed axo-axonal synapses, and emerged from myelinated axon stalks. These differences were also evident in P15 animals (n=4), in which CT projections to NTS was found to occupy a larger area and the RL and V projections were denser than in the adult. These results suggest that CT provides two anatomically distinct projections to NTS, and the CT projections into non-taste subdivisions of NTS may be an important component of developmental reorganization of taste afferents to NTS. Acknowledgements: NIDCD-R56-DC010183

335 Chorda tympani nerve injury initiates a microglial response in the nucleus of the solitary tract (nTS)

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Transection of the chorda tympani (CTx) results in loss of taste buds. Within weeks taste buds recover with CT reinnervation yet taste disturbances may persist for months. Changes in CNS function may underlie this long-lasting sensory disruption given that recovery is not correlated with CT regeneration. Peripheral nerve trauma in other systems induces central activation of microglia which are involved in synaptic remodeling. We sought to test whether microglia respond in the nTS – where CT fibers first synapse – as an indication of central reorganization. In adult mice the CT was cut unilaterally via the middle ear and animals survived for 1-30 days (6 times, n=3). Microglia were detected in the nTS using an antibody to Iba1 (microglial calcium binding protein). The microglia on the injured side had an altered morphology indicative of activation. Activated microglia have short, thick processes and ragged nuclei while resting microglia display thin, highly ramified processes and smooth nuclei. Microglial counts showed an increase

in cells on the injured side already at 2 days post lesion. By 5 days post CTx, the number of microglia on the lesioned side was 300% of control levels but returned to normal by 30 days. There were no changes in numbers on the uninjured side. Possible origins for the increase of cells were assessed. Using chimeric bone-marrow grafted animals, we found no evidence of in-migration from the periphery after nerve injury. There is, however, a clear emergence of mitotic cells within a day of CTx as seen by the presence of Ki-67. All Ki-67+ are also Iba1+ microglia indicating that mature microglia proliferate to generate the additional cells. The potential of microglial migration from nearby brain areas and of quiescent (unlabeled) cells becoming activated are also being studied. Acknowledgements: Grants to DLB, TEF, D Restrepo

336 NaCl- induced c-fos expression in the nucleus of the solitary tract of mice that lack P2X receptor subunits necessary for taste transmission

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The gustatory nerves of mice that lack P2X2 and P2X3 receptor subunits (P2X dbl KO) are unresponsive to all taste stimulus qualities (Finger et al., 2005). Surprisingly, P2X dbl KO mice have residual behavioral responses to concentrated taste solutions, which may reflect non-gustatory or post-ingestive information presumably intact in P2X dbl KO mice. We previously measured brain activation in response to consumption of 150 mM monosodium glutamate (MSG), using the immediate early gene c-fos, in the nuc. of the solitary tract (nTS) - the primary central taste and viscerosensory nucleus. We found significantly less c-fos-like immunoreactivity (cFLI) in rostral (gustatory) levels of the nTS of P2X dbl KO animals as compared to WT controls. In contrast, cFLI did not differ between WT and P2X dbl KO mice in caudal (viscerosensory) nTS levels. However, MSG has a sodium component in addition to its primary glutamate component. Thus, the current study measured NaCl-induced c-fos activation. P2X dbl KO and WT mice were placed on 22 h water restriction 3 days prior to stimulation. On stimulation day, mice consumed water or 150 mM sodium chloride (NaCl) for 30 min. Following taste stimulation, mice were left undisturbed for approximately 60 min, perfused transcardially with buffered paraformaldehyde and then their brains were removed and processed for cFLI. For each genotype, the number of NaCl-induced c-fos-positive cells in the nTS was compared to the number induced by intake of water, yielding a measure of NaCl-dependent cell labeling. NaCl stimulation elicited little NaCl-dependent cFLI in either WT or P2X dbl KO animals, which did not differ between the two groups, and was not different from water- induced cFLI. Thus, MSG- induced nTS cFLI is attributable solely to the glutamate component of MSG. Acknowledgements: NIH and 3ARP grants to T.E.F.

337 Overexpression of BDNF in the Lingual Epithelium Alters Terminal Field Organization in the Mouse NTS

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Brain Derived Neurotrophic Factor (BDNF) is expressed within gustatory epithelia and is required for gustatory neurons to locate and innervate their correct target during development. Genetic deletion of the *bdnf* gene and the BDNF receptor gene, *trkB*, results in a 50% loss of geniculate ganglion neurons and a significant loss of taste buds. Interestingly, when BDNF is overexpressed (BDNF-OE) throughout the lingual epithelium, chorda tympani fibers are misdirected and innervate inappropriate locations in the tongue (non-taste papillae), leading to a severe loss of taste buds. The remaining taste buds are hyper-innervated because of increased numbers of innervating neurons, but not because of increased branching. We sought here to examine the effects of BDNF-OE on central gustatory organization by fluorescently labeling the chorda tympani, greater superficial petrosal (GSP), and glossopharyngeal (IX) nerves in adult BDNF-OE mice and examine their terminal field organization in the nucleus of the solitary tract (NTS). The chorda tympani nerve terminal field volume was approximately 2X greater than in controls, with the greatest expansion in the dorsal zone of the NTS. The volumes of the other two nerves were similar to controls. Furthermore, the overlapping terminal fields that included the chorda tympani nerve were significantly larger than in controls, whereas the overlapping terminal field that did not contain the chorda tympani (GSP with IX) was unaffected. To extend these findings, we found that the chorda tympani nerve in BDNF-OE mice was functional and responded to a variety of taste stimuli and, unexpectedly, the number of geniculate ganglion cells that comprise the chorda tympani nerve was not different from controls. Acknowledgements: NIH grant R01 DC00407

338 Development of intrinsic properties of rostral nucleus of solitary tract (rNST) neurons in embryonic and postnatal rats

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The rNST, the first relay in the central taste pathway, must be functional at birth to guide feeding. However, few details are available on maturational changes that take place in intrinsic physiological properties of embryonic rNST neurons during development. We have characterized the action potential (AP) discharge characteristics and subthreshold membrane currents in rNST neurons at gestational days (E) 14, 16, 18 and 20 and postnatal days 1-2, 6-8, 14 and 19-21. The rNST was identified in trans-illuminated brainstem slices as lying medial to the solitary tract. Neural recordings were made with whole-cell patch-clamp. In response to depolarizing current injections, almost all neurons tested (135 of 141 neurons) generated APs. Only 4 embryonic and 2 postnatal neurons did not generate an AP in response to depolarization. APs were largely suppressed by superfusing 1 μM tetrodotoxin (TTX) over the slices, but in a few neurons at E14 the AP was not significantly diminished by TTX. In all E14 neurons, depolarization elicited only a single AP whereas in older embryos 75% of neurons responded with a single AP. In contrast, 73% of postnatal neurons generated five or more repetitive APs when depolarized (on average, trains of 18 APs). About 80% of neurons had a hyperpolarization-activated, transient outward potassium current (I_{KA}) across all age groups. The proportion of neurons with large amplitude I_{KA} currents (>

200 pA) significantly increased in the postnatal period. The decay time of the I_{KA} current in embryonic neurons (mean = 26 msec, n = 27) was significantly faster than the decay time in postnatal neurons (67 msec, n = 48; Mann-Whitney U test, p <0.001). These results indicate that substantial changes take place in properties of rNST neurons during development and maturation of the nucleus. Acknowledgements: NIDCD, NIH Grant DC009982 to RMB

339 Brainstem Sites Underlying Sucrose-Induced Analgesia in Neonatal Rats

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Taste analgesia in human and rat neonates elicited by nursing involves an opiate receptor-dependent component triggered by sugars in mother's milk. This analgesia is replicated by intraoral infusion of sucrose. Previous work in our lab showed that sucrose-induced analgesia persists in rat pups after mid-collicular transection and thus involves brainstem circuitry. Here, neuroanatomical and microinjection studies were used to further identify the loci involved in sucrose analgesia in P10-13 rat pups. Fos, as a marker for elevated neuronal activity, and tract tracing studies revealed that intraoral infusion of sucrose elicited significantly greater numbers of Fos+ neurons in the parabrachial nucleus (PBN) that project to the periaqueductal gray (PAG) compared to infusion of a salt stimulus, ammonium chloride. Additionally Fos-positive PAG neurons projected to the rostroventromedial medulla (RVM). Intraoral sucrose also elicited significantly more Fos+ PAG neurons that expressed the opioid peptide precursor pro-dynorphin compared to ammonium chloride. These findings disclose a potential circuit underlying sucrose-induced analgesia: (1) sucrose-responsive PBN neurons project to PAG, and (2) opioid (pro-dynorphin)-positive PAG neurons activated by sucrose project to RVM. In parallel experiments, intraoral sucrose, but not saline, produced analgesia. Inactivation of the PAG or RVM via microinjections of lidocaine abolished sucrose analgesia. Similarly, intra-RVM injections of the non-selective opiate receptor antagonist naloxone eliminated sucrose analgesia. Taken together, these studies demonstrate that the PAG and RVM are required for the production of sucrose analgesia, and further, that activation of opiate receptors in the RVM is necessary. Support: PHS Grant HD057601 & UTHSC Neuroscience Institute.

340 Brainstem convergence of efferents from the gustatory and visceral regions of the rat solitary nucleus

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Primary afferent fibers in the 7th and 9th nerves that supply oral taste buds and vagal fibers that innervate visceral organs project to segregated regions of the nucleus of the solitary tract (NST). However, behavioral responses of decerebrate animals to taste stimulation are dramatically altered by satiety signals conveyed by the vagus nerve, suggesting brainstem interactions between gustatory and visceral signals. Previous studies focused on the parabrachial nucleus (PBN) as a site of convergence but there are also hints of medullary interactions. The present study employed single and dual anterograde fluorescent tracers to more precisely identify sites of potential convergence in the medulla. Injections of biotinylated dextran or tetramethyl rhodamine dextran were made into the rostral NST (rNST) at sites that responded to oral stimulation and into the caudal NST (cNST) at the level of the area postrema (an area that receives dense vagal input from the gut). Biotinylated dextran was revealed with fluorescently-labeled strepavidin and sections examined with confocal microscopy to identify putative terminal fields. As observed previously, intermingled varicosities from the rNST and cNST occurred in specific PBN regions, including the waist area. In addition, overlapping terminations were present in the parvocellular and intermediate reticular formation subjacent to NST in an elongated column that extended from rNST to cNST. The most extensive region of potential interaction in the medulla was in NST itself. The rNST projected robustly to cNST and vice versa and there was dense overlap in the medial NST between the injection sites. Finally, there was copious terminal staining for dopamine beta hydroxylase in all these medullary regions suggesting a substrate for catecholaminergic modulation. Acknowledgements: DC00416 (SPT) & DC00417 (JBT)

341 Mapping the tongue onto the brainstem

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Understanding the synaptic organization of neurons innervating sensory organs is fundamental to deciphering their mechanism of information encoding. In the tongue, geniculate ganglion cells receive and encode the information regarding tastant quality. While it is well known that these cells organize their peripheral processes into discrete clusters coinciding with taste-buds on the tongue, due to technical hurdles, little is known about their central termination patterns in the brainstem. We have developed a retrograde labeling method that allows us to view the brainstem termination pattern of geniculate ganglion cells innervating single fungiform papillae from young-adult rats (~P30). The method uses an iterative labeling procedure, spread out over several days, allowing the same individual papilla to be targeted for multiple rounds of iontophoretic injection with retrograde dye. Using this method, we have successfully labeled the central processes of geniculate ganglion cells innervating single fungiform papillae and traced their termination patterns in the nucleus of solitary tract in the brainstem. Our overall aim with this new method is to produce an anatomical map of the output from single fungiform papillae in the brainstem, to determine if any spatial organization exists. The data will be useful in deciphering how information from the tongue is organized in the brainstem, and will help shed light on gustatory function. Acknowledgements: NIH R01 DC00407

342 Competitive Changes in CT Terminal Field Morphology and Taste-Related Behaviors Following GSP and IX Nerve Section

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Competitive processes play a role in the establishment and maintenance of terminal field organization in sensory systems. The rostral

nucleus of the solitary tract contains overlapping terminal fields of three gustatory nerves: the chorda tympani (CT), the greater superficial petrosal (GSP) and the glossopharyngeal (IX). These three nerves all undergo a progressive decrease in terminal field volumes throughout postnatal development, leading to decreases in overlapping fields. We propose that competitive interactions between the three nerves shape terminal field development. To examine the effects of removal of competition from GSP and IX on CT terminal field development, the GSP and IX nerves were sectioned at postnatal day 15 (P15), P25 or P65, representing different stages of terminal field maturation. The terminal field volume of the CT nerve was then assessed 35 days following nerve section. Using an anterograde tracer coupled with confocal microscopy, we found that the CT terminal field volume was five times larger than age-matched controls. This finding was consistent regardless of age of GSP and IX section. We confirmed that there were no changes in taste responses from the CT nerve. The absence of cytokeratin-19-like immunoreactivity in the foliate papillae and in the nasoincisor duct was used to confirm the lack of IX and GSP reinnervation, respectively. Following bilateral GSP and IX section at P65, no behavioral changes were seen in brief-access taste testing responses to a concentration series of NaCl or quinine. These studies highlight the remarkable plasticity of the central gustatory system and provide a basis for future, more mechanistic studies of gustatory competition. Acknowledgements: Supported by NIH Grants R01 DC00407 and R01 DC006938

343 Analysis of functional and anatomical relationships between trigeminal inferior alveolar afferents and gustatory neurons within the nucleus of the solitary tract

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Since a recent clinical study revealed an increase in taste thresholds with dental deafferentation (Boucher *et al.*, 2006), we wanted to investigate the biological basis of this phenomenon. We explored trigeminal inferior alveolar nerve (IAN) innervating mandibular teeth and gustatory interactions within the nucleus of the solitary tract (NST) of rats. We recorded single unit tastant-evoked responses of NST neurons before and after IAN electrical stimulation. Electrical IAN stimulation eliciting a short latency jaw opening reflex resulted in a significant decrease in gustatory NST neuron responses. We furthermore used a double-label strategy with c-Fos mapping of chorda tympani (CT) activated NST gustatory neurons coupled to an anterograde labeling of IAN afferents. We observed labelled IAN boutons "en passant" apposed to CT activated neurons in the gustatory NST. With a complementary triple-label approach using retrograde labelling of solitary-parabrachial neurons coupled to anterograde labelling of gustatory CT and trigeminal afferents, we evidenced NST second order gustatory neurons apposed by CT and IAN afferents. Taken together, our results provide an anatomical and functional basis to support trigeminal dental and gustatory interactions in the brainstem. ref: Boucher Y, Berteretche MV, Farhang F, Arvy MP, Azérad J, Faurion A. taste deficits related to dental deafferentation: an electrogustometric study in humans. eur j oral sci. 2006;114(6):456-64 Acknowledgements: IFRO

344 Repeated Peripheral Nerve Injury Leads to Enhanced Growth of Terminal Fields in the Nucleus of the Solitary Tract of Adult Rat

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Unilateral transection of the chorda tympani nerve (CTX) leads to long-term changes in the peripheral and central taste systems of adult rats. Most notably, there is a loss of about 50% of the CT terminal field volume in the nucleus of the solitary tract (NTS). We found that the injury-induced decrease of CT nerve terminal field volume is not due to cell death and degeneration of central processes, but rather a failure in regeneration of all peripheral processes. Accordingly, the significant reduction of volume occupied by the CT nerve terminal field following CTX may lead to the expansion of intact neighboring nerve terminal fields. The current experiment assessed long-term reorganization of the intact glossopharyngeal (IX) and greater superficial petrosal (GSP) nerve terminal fields following CTX in adult rats. To examine this potential reorganization, we fluorescently labeled the regenerated CT and the GSP and IX nerves 60 days post-CTX and then examined the terminal field organization of these three nerves in the rostral NTS. Unexpectedly, the terminal field volumes of all nerves, including the CT, were greater than controls. This unforeseen increase in CT terminal field indicates that a conditioning lesion effect occurred. That is, the initial CTX served as a conditioning lesion that when followed by triple nerve label, which requires the transection of all three nerves, leads to the rapid expansion of the CT nerve terminal field. It is unclear if the expansion of IX and GSP terminal fields is related to CTX and the subsequent loss of CT terminal field or if this reorganization occurs in the 48 hours following triple labeling along with the rapid expansion of CT terminal field. Acknowledgements: Supported by NIH Grants R01 DC00407 and R01 DC006938

345 Amino acid taste-evoked activity in the parabrachial nucleus of mice

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Objective: The parabrachial nucleus (PBN) is a key interface between medullary and forebrain gustatory areas in rodents. We investigated responses to basic tastants as well as umami-tasting stimuli, including MSG, IMP, and L-type amino acids, in the PBN using both in vivo physiology and taste-evoked c-Fos IHC techniques. We also examined Fos expression in PBN neurons that project to the lateral hypothalamus (LH), an area involved in feeding, via retrograde tracing. Methods: Taste-evoked responses in the PBN of C57BL/6J inbred mice were recorded with in vivo single-unit recording techniques. For Fos studies, injections of the retrograde tracer Fluorogold were made bilaterally into the LH. Several days later, mice were stimulated intraorally with MSG, IMP, MSG+IMP, sucrose or water. Mice were perfused 2 h later, and brain sections through the PBN were processed for FG and c-Fos IHC. Results: Preliminary analysis of 10 recorded PBN taste cells reveals several possessing a robust response to MSG, including a synergistic response to MSG + IMP, which is the hallmark of the umami taste response. In the c-Fos studies, we show that the umami

taste stimuli produced significant activation of taste neurons in the PBN, including a subset of cells in the dorsal lateral subnucleus (dls) of the PBN that project directly to the LH. This pattern of labeling was comparable to that evoked by sucrose, indicating that either sweet or umami stimuli produce activation of a putative ''appetitive'' taste projection from the PBN to the LH. Acknowledgements: Ajinomoto Research Program (3ARP) NIH DC000353

346 Quantification of c-Fos in the PBN reveals that visceral response does not play a role in strain differences observed in conditioned taste aversion between C57BL/6J and DBA/2J mice

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Objective: Previous behavior work has suggested differences between C57Bl/6J (B6) and DBA/2J (D2) mice in the acquisition and extinction of a conditioned taste aversion (CTA). This study was conducted to investigate whether these results may be due to the strength of the visceral response (malaise) resulting from the unconditioned stimulus. **Methods**: B6 and D2 male mice received intraperitoneal (i.p) injections of either a 20 ml/kg or 40 ml/kg dose of 0.15 M LiCl. Two hours following injection, mice were perfused and sections stained for the immediate early gene c-Fos. We analyzed c-Fos expression in the parabrachial nucleus (PBN), as it is suggested to be a site of convergence of visceral and gustatory information, and plays a key role in CTA formation. Strong c-Fos expression was found in the external lateral subnucleus (ELS), which is known to receive primarily visceral information. **Results**: First, we determined that c-Fos activation is correlated to the degree of malaise induced by LiCl, as there were significantly more FLI-positive neurons following the 40 ml/kg dose compared to 20 ml/kg for both strains (B6, $p \leq .002$; D2, $p \leq .0001$). Next we compared B6 and D2 mice at each dose, and found no strain differences in number of FLI-positive neurons following injections (20 ml/kg, $p \leq .48$; 40 ml/kg, $p \leq .75$). **Conclusions**: We conclude that these results suggest that strain differences observed in the acquisition or extinction of a CTA are not likely due to varying degrees of malaise experienced, but rather a result of CNS changes in gustatory or learning response. Acknowledgements: DC000353

347 An Analysis of Spike Timing in Parabrachial Gustatory Neurons

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Recent evidence suggests that in addition to spike rate, spike timing may also help to distinguish among taste quality signals in the brainstem and cortex. For instance, Di Lorenzo and colleagues (2003, 2008) reported that 1/3 to 1/2 of neurons in the nucleus of the solitary tract change best stimulus category over repeated trials. Interestingly, such cells are more likely to show evidence of temporal coding in a metric space analysis and to be broadly-tuned. We recently observed a population of narrowly-tuned, bitter-best neurons in the parabrachial nucleus (PBN) that responded in bursts of spikes, suggesting that other neuron types might also exhibit temporal coding. To determine whether this was the case, we recorded from taste-responsive PBN cells over multiple trials, applied metric space analysis, and examined bursting patterns. These parameters were compared across cells for best stimulus, breadth of tuning and receptive field. Neurons were tested at least 10 times on each of 5 different stimuli (0.3M sucrose, 0.1M NaCl, 0.03M citric acid, 0.03M quinine and 10 μM cycloheximide). Preliminary analysis revealed that best stimulus category changed only rarely over repeated trials, and in contrast to solitary nucleus neurons, the few cells that did switch best stimulus were not more likely to show evidence of spike timing. However, narrowly-tuned bitter-best neurons had a greater percentage of spikes occurring within bursts ($p < .02$) and longer burst durations ($p < .002$) than acid/salt-best neurons, suggesting differing temporal characteristics for the two groups. Acknowledgements: NIDCD grants DC000416 (SPT) and DC008678 (LCG).

348 Spatial differences in molecular characteristics of the pontine parabrachial nucleus

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Various neurons in the pontine parabrachial nucleus (PBN) are involved in signal transduction in the general visceral and somatic sensory, gustatory, and autonomic nervous system; PBN neurons are so intermingled. In this study, we analyzed their gene expression profiles in male Wistar rats to obtain data on gene expression in the PBN and principal sensory nucleus of the trigeminal nerve (Pr5). Using these data in combination with *in situ* hybridization results, we identified genes showing higher expression in the PBN than in the Pr5. In-depth analysis of spatial distribution in the PBN enabled to classify the genes into seven characteristic spatial expression patterns. Expression signatures were significantly different from one another, in the subnuclei of the rostral half, mediodorsal half, and ventrolateral third of the PBN indicating a correlation between the spatial arrangement of the subnuclei and the molecular characteristics of the corresponding neurons. These results provide valuable information for elucidating the role of the subnuclei in the PBN. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

349 The anterior insula drives the insula-opercular taste network during sensation of sweet taste

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The precise location of human primary taste cortex is currently debated. Neuroimaging studies show that response to taste can be observed in three distinct regions within the insula and overlying operculum; (1) the posterior insula and parietal operculum (PO), (2) the mid insula and rolandic operculum (MI), and (3) the anterior insula and frontal operculum (AI). Based on work showing that the parietal operculum is the first region to be activated during sensation of a taste, it has been suggested that taste inputs first reach the parietal operculum. In contrast, anatomical data in monkeys suggests that the primary representation should be in the AI. As taste stimulation is naturally concurrent with oral somatosensory stimulation, we have previously argued that the PO responses represent oral somatosensation. Here we used dynamic causal modeling to compare models of information flow through the insula-opercular network. Tasteless and sweet solutions were presented to 20 subjects and fMRI responses were measured with a 3T Siemens scanner. We predicted that when a tastant is present in a solution, gustatory input will first influence the network through the AI. In contrast, when no tastant is present, the initial network influence will be through the PO. Comparison of Bayesian posterior probabilities showed that the proposed model has a 98% greater probability for generating the observed neural signal than other configurations of inputs into these three areas. These findings are consistent with the view that the AI represents the human primary taste cortex and that the PO contributes to the processing of the somatosensory component of oral signals. Acknowledgements: Supported by NIDCD grant R01 DC006706.

350 Central Amygdala Stimulation Activates Neurons in the Gustatory Brainstem and Increases the Number of Taste Reactivity Behaviors in Conscious Rats

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A role for the central amydgala (CeA) in the control of taste reactivity (TR) behaviors was investigated by electrically stimulating the CeA in conscious rats while monitoring TR behaviors and then mapping active brainstem neurons using Fos-like immunoreactivity (FLI). Following surgery to implant an electrode into the CeA and intra-oral cannulas, and a recovery/adaptation period, 24 male Wistar rats were videotaped for 5 min during intra-oral infusion (0.233 ml/min) of either dH_2O (W), 0.1 M NaCl (N), 0.003 M quinine HCl (Q) or no stimulus. In half of the rats in each group, the CeA was stimulated (40 Hz, 0.4 ms, 0.1-0.2 mA) throughout the intra-oral infusion. One hour after behaviors were videotaped, the rats were sacrificed, perfused, and their brains were removed, sectioned, and processed for FLI. Stimulation of the CeA dramatically increased TR behaviors in the absence of intra-oral infusion ($p<0.05$) but did not significantly alter the overall number of TR behaviors elicited by W, N, or Q. However, CeA stimulation did change the type of behaviors performed to Q such that aversive behaviors no longer outnumbered ingestive ones. The number of FLI neurons in the waist area of the parabrachial nucleus more than doubled during CeA stimulation both without intra-oral infusion as well as during infusion of each tastant ($p<0.05$). In the rostral nucleus of the solitary tract (NST) and the medullary reticular formation, CeA stimulation also increased the number of FLI neurons during infusion of N but not W and Q. The increases in FLI neurons were mainly in the ventral subdivision of the rostral NST and the parvocellular reticular formation. These preliminary data suggest that descending projections from the amygdala alter TR behaviors by influencing the activity of neurons in brainstem taste nuclei. Acknowledgements: Supported by NIH grant DC007854 to M.S.K.

351 Effects of expectation on gustatory processing and multi-area interactions

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Outside laboratory conditions tastes are rarely perceived in complete isolation from other sensory events. Gustatory stimuli are often anticipated by environmental cues and actively tasted on the basis of some expectations. To study the influence of expectation on gustatory processing we performed chronic multielectrode recordings in multiple forebrain areas of rats trained to either passively receive a taste at a random time or to self-administer it following an anticipatory tone. Ensemble responses to unexpected and expected stimuli were recorded from gustatory cortex (GC) and from two high order areas connected with GC and involved in the processing of expectations - orbitofrontal cortex (OFC) and basolateral amygdala (BLA). We found that general expectation optimizes the representation of gustatory information in GC; specifically, self administered stimuli produce taste-specific responses at a latency at which passively delivered tastes do not. The improvement in taste classification correlates with an increase in the number of taste responsive neurons and is related to a subset of cells whose activity is modulated by anticipatory cues. This tone-related activity was further investigated to address its associative nature. Finally, simultaneous recordings in GC, OFC and BLA were used to track the flow of bottom-up and top-down information related to expectation. The results of this analysis show a strong flow of information that ascends from GC to both AM and OFC in the case of passive deliveries and a robust top-down flow to GC that occurs in the case of expected tastes. These results emphasize the importance of behavioral states, and specifically expectation, in modulating sensory responses and the balance between bottom-up and top-down dynamics. Acknowledgements: Supported by NIDCD R01-DC010389 and the Klingenstein Fund to AF

352 Interaction Between Top-down and Bottom-up Synaptic Potentials in the Insular Cortex of Anesthetized Rats

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Basic taste stimuli have intrinsic psychological dimensions that are coded in the time-course of neural responses in insular cortex (gustatory cortex - GC). One such dimension, hedonic value, appears to emerge in GC via top-down modulation by the basolateral amygdala (BLA). While the importance of BLA in modulating gustatory

cortical function has been well established, the nature of this input onto GC neurons is largely unknown. Conflicting results from extracellular recordings point to either excitatory or inhibitory effects. Here, we use an anesthetized rodent preparation to directly test the hypothesis that BLA can evoke time-varying - both excitatory and inhibitory - responses in GC. The time course of BLA-evoked synaptic potentials and their influence on GC responses to inputs from the gustatory thalamus (GTh) were directly studied using intracellular recording techniques. Electrical stimulation of BLA evoked in GC neurons a post-synaptic potential (PSP) that resulted from a combination of short and long-latency components. An initial, likely monosynaptic, glutamatergic potential is followed by a multisynaptic, GABAergic hyperpolarization. This pattern differs from that observed with thalamic stimulation. To test the influence of amygdalar inputs on the processing of bottom-up signals, the effects of BLA stimulation on GTh-evoked PSP were studied. As predicted by the dynamic nature of amygdala-evoked potentials, the final effects of BLA stimulation depended upon the timing of the two stimuli. These experiments provide the first description of BLA synaptic inputs to GC and reveal that amygdalar afferents can modulate gustatory cortical network activity and its processing of sensory information via time-varying synaptic dynamics. Acknowledgements: NIDCD 1R01-DC010389

353 Parametric evaluation of the time course of PKMzeta inhibitor effectiveness

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Recently it has been suggested that infusions of a Protein Kinase M (PKM) zeta inhibitor (Zip) into gustatory cortex (GC) 3 days following training erases long term consolidated memory of conditioned taste aversion (CTA). In preparation for upcoming electrophysiological examinations, we are performing a parametric analysis of the time window over which Zip infusions into GC effectively impair CTA retrieval–that is, we are studying the effect of this PKM zeta inhibitor on consolidation and reconsolidation of CTA memories. We found that Zip injected into the Gustatory Cortex (GC) as early as 48 hours post-training erases consolidated memories. In contrast, Zip injected into the basolateral amygdala (BLA) at that time-point left learning intact, and GC injections made 44 hours post training were similarly ineffective. Moreover, the impact of Zip injected into GC 2 hours after a reminder/extinction trial was weaker than injection 2 hours before that trial, suggesting that Zip causes only a moderate disruption of CTA reconsolidation. Taken together, these experiments provide a comprehensive picture of the waxing and waning (and waxing again) processes of Long Term Potentaition (LTP) consolidation. Acknowledgements: DC 006666

354 Umami and Saltiness: do they play with the same rules in the match of tastes? – an fMRI study

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In this study, using block-design fMRI methodology, we intended to compare the cerebral processing of salt taste (NaCl) with umami taste, and also to investigate the controversy about the ipsi- or controlarality of the gustatory system. Stimuli were presented at suprathreshold concentration and delivered using a gustometer through the subject mouth. The sequence was presented in a session of 6 repetitions on/off -block per stimulus and per side; 24 healthy subjects participated. The BOLD signal (blood oxygenation level dependent) in every subject was detected by means of a 1.5 T scanner. fMRI data analysis was implemented in SPM5 ($p<0.005$ cluster level= 5). fMRI results: The main effect of the tastants was to elicit areas in the primary and secondary gustatory cortex. Different coordinates of the activated areas were found for the two tastants inside the same brain areas, suggesting a segregation of the brain areas involved with the tastants. Comparing the two stimuli we found that the positive effect of MSG on NaCl is evidently highlighted in the limbic lobe. On the contrary the positive effect of NaCl on MSG elicited activations in areas more common to taste perception. The conjunction analysis revealed common activated areas for the two tastants in the primary (SI) and secondary (SII) somatosensory cortex, premotor cortex, but also in secondary taste areas. With regard to lateralization within the gustatory system, the BOLD contrast for the MSG stimulus was significantly bigger on the right side of the brain when the stimulus was presented to the left side as compared to the right side presentation of the stimulus. Moreover the opposite contrast for MSG highlighted only few brain areas including the left orbitofrontal cortex. The contrary appeared when the stimulus was NaCl. This result suggests a contralaterality of the brain response to the MSG stimuli but an ipsilaterality for the NaCl stimuli whit a strong and general right sided lateralization of the brain for saltiness.

355 Electrical neuroimaging of gustatory perception in humans

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Questions of how in general, and where and when in particular, gustatory percepts are represented in the human brain remain largely unanswered despite decades of research. Electrical neuroimaging of gustatory perception has been hampered by difficulties with stimulus control because recording of event-related brain electrical responses (electroencephalography, EEG) requires temporally precise stimulus presentation to obtain good summation of the signal across trials. This is difficult to achieve with flowing stimuli in solution. Aim of this study was to investigate cortical response patterns of gustatory perception over time. For this, a gustometer, which met the requirements for the recording of event-related responses, was employed to present bitter, salty, sour, sweet and umami solutions to human volunteers while EEG was recorded. Taste qualities were identified equally well by panelists. Intensity and pleasantness judgements, however, varied between participants. Brain responses were analyzed with respect to their time courses, spatial distributions and neuronal generators. We present series of stable maps (microstates) for each tastant and we show overlaps between tastants and point out differential activation

patterns. Moreover, we show that these response patterns varied between participants thus bearing important implication for the grand-averaging technique commonly used in EEG research. Finally, we report the neuronal generators of each stable map, which comprised areas previously associated with the processing of taste and food-related stimuli. In particular the insular cortex and the orbitofrontal cortex are involved. The findings are discussed in the framework of current knowledge on gustatory perception in the human brain.

356 Multiple Neuronal Subpopulations emerge from the Olfactory Placode During Development

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During olfactory development a migratory mass (MM) (Valverde et al., 1992) forms in the mesenchyme as cells and axons exit the olfactory placode (OP) and begin to approach the nascent olfactory bulb (OB). Subpopulations of cells, collectively termed MM cells, emerge including ensheathing cells, odor-receptor (OR) expressing cells, and OMP+ cells from the main olfactory epithelium (OE), and GnRH+ cells from the vomeronasal (VNO) organ (Valverde et al. 1992; Conzelmann & Breer 2002; Schwanzel-Fukuda 1999; Miller et al. unpublished data). As the axons extend across the basal lamina, beginning at embryonic day (E) 10, they join and interdigitate among the MM cells. Elsewhere in the nervous system pioneer neurons may serve as guideposts for later extending axons (i.e. Zimmer et al., 2010); the cells of the MM may function similarly in olfactory nerve development. We tested the hypothesis that molecular diversity, or spatial-temporal patterning of the MM cells, contribute to the ongoing coalescence of subpopulations of OSN axons as they extend toward the developing OB. We show that by E12 the MM cells are heterogeneous, containing subpopulations distinct from those described (such as the GnRH+ and OMP+ cells) and migrating from both the main OE and the VNO. Expression patterns are complex, but occur in combinatorial phenotypes that can include expression of: MAP2, OMP, DNER, GnRH, DCX, Lectins, NQO1, and CXCR4. The diversity among the MM cells may be related to the heterogeneity of the OSN axons, each of which expresses only 1 of ~1,200 ORs and converge with like-axons. It is plausible to hypothesize that the subpopulations of OSN axons rely on specific subsets of MM cells to provide the intermediate cues that influence axon organization and trajectory as they approach the developing OB. Acknowledgements: Generously supported by NIH/NIDCD/NIA

357 Development of a Mouse Embryonic Stem Cell Model for Neurogenesis and Localization of RARa and RARg in these Cells

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Our laboratory has shown that vitamin A-deficient (VAD) postnatal rats have significantly reduced numbers of mature olfactory neurons (ORNs). VA is a precursor for retinoic acid (RA), a growth factor that positively influences embryonic development and cell differentiation. It is hypothesized that RA affects neurogenesis in postnatal mammals and the reduction in ORNs in VAD OE results from local depletion of RA. To test this hypothesis, we are developing a defined mouse embryonic stem cell (mESC) model to investigate effects of RA signaling on mESC differentiation. Addition of RA to mESCs induces neurogenesis presumably by complexing with nuclear RA receptors (RAR) that mediate gene expression. The goal of this study was to localize RARα and RARγ expression in mESCs. Two different mESC strains were grown on microscope slides. One strain (R1ES) was grown in undefinded media (supplemented with 15% FBS) and the other (Bl6 ES) was grown in chemically defined media. Fixed cells were reacted with RARα and RARγ antibodies using immunofluorescent and electron microscopy (EM) protocols. Negative controls showed no reactivity. MCF-7 cells, a positive control for RARα and RARγ, showed positive nuclear staining with both antibodies. The RARα and the RARγ antibodies positively stained the nuclei of R1ES and Bl6 mESCs. Staining was variable in intensity throughout mESC colonies, suggesting there is variable expression of the protein in different cells. Unexpectedly, incubation of mESCs with either 0.5 μM RA or 0.5 μM VA for 24h did not increase or diminish staining by the antibodies. The results further confirm the authenticity of the antibodies. Future work will be directed at monitoring RAR expression as cells differentiate into neurons and in identifying cell types in the olfactory organ and bulb of rodents. Acknowledgements: NIH/NIGMS/MBRS/SCORE S06 GM 008092

358 Genetic Manipulation of Sox2 in the Adult Olfactory Epithelium During Lesion-Induced Regeneration

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The transcription factors Sox2 and Pax6 are co-expressed in multiple cell types of the adult OE, including HBCs, ''upstream'' GBCs, and Sus cells, but not in ''downstream'' GBCs nor olfactory sensory neurons (OSNs). We used transduction by retroviral vector (RVV) to over-express them individually and together during recovery from MeBr lesion to test the hypothesis that the two factors suppress neuronal differentiation. As described last year, *Sox2-IRES-eGFP*-encoding RVV significantly suppresses OSN formation compared to empty vector (EV), though many OSN-containing clones remain; half of the GFP (+) OSNs (and only the OSNs among the cells in the clones) lack detectable Sox2 protein. Is co-expression of Pax6 and Sox2 required for complete OSN suppression and to maintain Sox2 protein in OSNs? A *Sox2-Pax6-eGFP*-encoding RVV (SEP) also produced only partial suppression of OSNs, although Sus cells were increased relative to the Sox2 RVV. As before, both Sox2 and Pax6 proteins were undetectable in many SEP-transduced OSNs. Since Sox2 over-expression suggests that it may enhance the proliferation within upstream neural progenitor cells, we eliminated *Sox2* expression by infecting $Sox2^{loxP/loxP}$ mice with a Cre-encoding RVV. Elimination of

Sox2 caused a significant reduction in clone size, probably by preventing the expansion of neural progenitor cells, but both OSNs and Sus cells are still produced, suggesting that Sox2 is not required for either cell type. In summary, *Sox2* is neither necessary nor sufficient to drive the differentiation of OSNs or Sus cells. However, Sox2 regulates progenitor cell proliferation and acting with Pax6 biases in favor of Sus cell formation. Lastly, OSNs tightly regulate their constellation of transcription factors to achieve and maintain their differentiated state. Acknowledgements: NIH grant R01 DC002167

359 The Transcription Factor p63 is Required for the Differentiation of Horizontal Basal Cells During Development

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The capacity of the adult olfactory epithelium (OE) to replace neurons lost through senescence, axotomy, or trophic factor depletion and to reconstitute itself after severe injury is incumbent on the retention of multipotent, neurocompetent stem cells. Olfactory stem cells reside amongst the basal cells of the epithelium, of which at least two types exhibit a capacity for multipotency – horizontal basal cells (HBCs) and globose basal cells (GBCs). GBCs, defined on the basis of transcription factor expression, are present early in embryonic development, repopulate the tissue throughout adult life, and contribute to tissue regeneration after severe injury. In contrast, the population of HBCs, defined by adherence to the basal lamina and by the expression of HBC-specific proteins (including cytokeratins (CK) 5 and 14), is not established until after birth. HBCs remain dormant during normal tissue maintenance, but contribute to tissue regeneration after severe injury. Here we report that p63, a member of the p53 transcription factor family, is necessary for the emergence of HBCs during the development of the OE. We show that p63 precedes both expression of CK5 and 14 and the migration toward the basal lamina, and that p63-mutant mice generate all cell types of the OE except for HBCs. Finally, we demonstrate that p63 expression anticipates HBC reappearance in the ventral OE of adult rats after methyl bromide lesion, and that HBC activation to multipotency during regeneration is accompanied by a transient loss of p63. Together these data suggest a model in which p63 acts to set aside a reserve stem cell population that can be activated by severe lesion. Moreover, loss of p63 expression appears to be a hallmark feature of such activation. Acknowledgements: R01 DC002167

360 IFT88 Regulates Olfactory Cilia Maintenance and Function

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Odorant detection begins in the cilia of olfactory sensory neurons (OSNs), where the binding of odorants to receptors initiates the canonical G-protein signaling pathway. The components of this pathway are compartmentalized in cilia, yet none are translated there. In order to reach the ciliary membrane, proteins are transported from the basal bodies into the cilia by the process of intraflagellar transport (IFT). While olfactory cilia are critical for the detection of odorants, relatively little is known about their development, maintenance and protein trafficking. IFT particles, consisting of motors and IFT complex proteins such as IFT88, are responsible for the transport of cargo into and out of cilia. Mutations in IFT88 have been shown to affect ciliogenesis in several other cell types. Here, we used the ORPK mouse, an IFT88 hypomorphic mutant, to investigate IFT in the development and maintenance of OSN cilia. Disruption of IFT88 affects olfactory cilia organization and olfactory function, but does not result in the complete loss of cilia. Although electro-olfactogram recordings show that ORPK mice are largely anosmic, scanning electron microscopy shows the OSNs from ORPK mice do contain cilia, although ORPK olfactory cilia are fewer and shorter compared to wild-type littermates. Immunohistochemistry reveals a decrease in gamma- and acetylated-tubulin staining consistent with a decrease in cilia in ORPK mice as well as a concomitant loss of the signaling proteins, ACIII, CNGA2, and Gγ13 from the ciliary layer. Staining for MOR28 shows odorant receptor protein remains localized to the dendritic knobs and remaining cilia. These data indicate that IFT88 is not required for olfactory ciliogenesis, but rather IFT88 is important for the maintenance of cilia and hence olfactory function. Acknowledgements: This work was support by National Institutes of Health Grants DC009606 (J.R.M.), DC00011 (J.C.M., P.M.J., and D.P.M.) and National Research Service Award Fellowship DC009524 (P.M.J.)

361 Fasciculation of Molecularly Defined Subsets of Axons in the Developing Olfactory Nerve Pathway

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Olfactory sensory neuron (OSN) axons exit the olfactory epithelium (OE) and grow toward the olfactory bulb (OB) where they coalesce into glomeruli. Each OSN expresses 1 of ~1,200 odor receptors (ORs) and the axons sort into 2-3 glomeruli in a reproducible manner based on OR expression. Deletion or substitution of the OR results in convergence failure, or convergence in an ectopic site. While ORs appear necessary for appropriate convergence of axons, they are likely not sufficient to fully account for the behavior of OSN axons. The OSNs expressing any 1 OR are distributed roughly in 1 of 4 zones of the OE, but within a zone are not contiguous, as their distribution appears stochastic. Recent work has implicated that pre-target axon sorting first occurs in the olfactory nerve (Bozza et al., 2009; Imai et al., 2009). We recently established that in mice OSNs expressed ORs as early as embryonic day (E) 9 and that axons first emerge from the OE, crossing the basal lamina into the undifferentiated mesenchyme, at E10. Using regional and OR specific markers, we have now asked when homotypic fasciculation first occurs and assessed the degree to which subpopulations of axons may remain segregated as they extend toward the nascent OB. Here, we show that immediately upon crossing the basal lamina axons uniformly turn sharply, at ~90 degrees toward the OB. Molecularly defined sub-populations of axons begin to segregate by E12, forty-eight hours after the OSN axons have crossed the basal lamina but prior to the axons first reaching the nascent

OB. Homophilic axon fasciculation appears to be a hierarchical process. While regional segregation occurs in the mesenchyme, ultimate convergence of OR-specific subpopulations does not occur until the axons reach the inner nerve layer of the OB. Acknowledgements: Generously supported by the NIH/NIDCD/NIA.

362 Bridging Multiple Time-Scales in the Signal Transduction of the Mouse Olfactory Receptor Neuron

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Our goal is to identify the salient or essential dynamic components of the olfactory signal transduction cascade in mouse. We use an integrative approach which takes advantage of electrophysiological data from the olfactory receptor neuron (ORN) of several mutant mouse lines. This data is used concurrently to develop physiologically-based computational models of the slow (transduction) and fast (action potential) currents in ORN. A key question for our group is, "Can a single general model be developed which predicts the ORN electrophysiology of all the observed mutant lines?" In this poster, we present application of a novel Bayesian model-fitting algorithm called Wedge to the electrophysiological data from several mutant mouse lines. We illustrate how a diversity of responses across a population of ORN can be addressed through a fully Bayesian dynamical systems approach using this Wedge algorithm. We conclude our analysis by summarizing which components of the ORN cascade appear to be most responsible for the diversity or variability observed in the current response of mouse. Acknowledgements: This work was supported by Award Number R01DC009946 (NIDCD/NIH).

363 A neural code for binary odorant mixture interactions in the nose

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As a step towards elucidating the neural mechanisms of odorant mixture interactions, we examined binary mixture interactions at the level of olfactory receptor neurons (ORN) in an intact mouse. We used transgenic mice that expressed synaptopHluorin (spH) selectively in ORNs. We measured odor-evoked neurotransmitter release, reported as an increase in spH fluorescence signal, by distinct populations of ORNs converging onto defined glomeruli in the olfactory bulb. We obtained maps of activity across the dorsal surface of the bulb in response to eugenol (EG), methyl iso-eugenol (MIEG) or their binary mixture. EG and MIEG, which have highly similar chemical structures but different smells, activated overlapping sets of receptors; however, the apparent odorant binding affinity and sensitivity varied with the different receptors. Both EG and MIEG evoked dose-response relations that showed wide dynamic range, saturation, and only weak cooperativity (Hill coefficient $\sim$ 1). Below response saturation, glomerular responses to binary mixtures showed mutual additivity, which is consistent with a model of competitive agonists binding at a single receptor site. Near response saturation, glomerular responses to the mixtures often showed a suppression effect, similar to the reduction effect seen with the highest concentration of single odorant. Little or no apparent receptor inhibition was observed in our studies. Thus, odorant interactions between structurally similar compounds might be explained by concentration-dependent adjustments in the overall signaling system at the peripheral level. It remains to be determined how these physiological interactions in the periphery give rise and are transformed into perception or behavioral responses. Acknowledgements: NIH DC004208

364 Influence of the chemical structure on odor intensity and odor character of halogenated and methylated phenols

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Objectives. A series of halogenated, methylated phenols have been reported to cause phenolic and medicinal off-flavours in food such as 2-iodo-4-methylphenol, 2-chloro-6-methylphenol and 2-bromo-4-methylphenol [1][2]. In all cases, ortho-substitution with a halogen atom resulted in high odour activity [2][3] while e.g. for 2-chloro-4-methylphenol odour activity increased significantly by insertion of a methyl group in position 4 [4]. Based on these observations the aim was a systematic variation and sensory evaluation of monohalogenated and monomethylated phenols to elucidate key structural elements for their smell. Experimental methods. The odour character of the phenols, and their threshold concentrations in air and water were evaluated by application of gas chromatography-olfactometry as well as sensory evaluation on aqueous solutions [5]. Results. Variation of the halogen (Cl, Br, I)- and methyl- substitution of the phenols resulted not only in distinctive different smells with predominantly medicinal and phenolic impressions, but also in major differences in their odor thresholds. Thereby, pronounced inter-individual sensory differences were observed between panelists, e.g. for 2-iodo-4-methylphenol. Conclusions. The odor parameters of halogenated and methylated phenols strongly depend on their respective substitution patterns, and on individual specificities. References [1] Mottram, D. S. (1998). *J.Food Sci. Technol., 33*, 19-29. [2] Strube, A., Guth, H., & Buettner, A. Water Res. 2009, 10.1016/j.watres.2009.08.026. [3] Dietrich, A. M., Mirlohi, S., DaCosta, et al. (1999). *Water Sci. Technol., 40*(6), 45-51. [4] Young, W. F., Horth, H., Crane, R., et al. (1996). *Water Res., 30*(2), 331-340. [5] Czerny, M., Christlbauer, M., Christlbauer, M., et al. (2008). *Eur. Food Res. Technol., 228*, 265-273.

365 The first quantitative model of the nasal aerodynamics in mouse

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Despite mouse being an animal model widely used in biomedical research, including those focused in the olfactory and respiratory system, there has been no published studies on its nasal aerodynamics, due to the small size. The structure and the subsequent nasal airflow features of mouse can not be assumed to be similar to that of rat, as the reported ratios of nasal surface area to nasal

volume and body weight are much higher in mouse than in rat. We have created the first anatomically accurate 3D computational model of mouse nasal cavity based on postmortem microCT scans (vivaCT 40, SCANCO USA, Inc) of an adult B6 mouse. The isotropic pixel resolution of the scans is 10.5 um. Profiles of velocity and flow distribution in the mouse nasal cavity under restful breathing and sniffing were simulated computationally adopting the quasi-steady approach and were found to be similar to those reported in rat, yet with some significant regional differences. Similar to rat, of the major nasal flow streams in mouse, only the dorsomedial (DM) stream passes through the Ethmoid (olfactory) Recess (ER) while the others flow ventrally, joined at the nasopharygeal meastus before exiting the nasal cavity. However, the DM stream in mouse did not split into medial and lateral path in the ER as found in the rat. Consequently, all the lateral and ventral ER in mouse were ventilated through the recirculation of DM stream, which may have functional implication related to the olfactory odorant transport. This phenomenon could be unique in the mouse, or that the previous rat nasal models may fail to capture the structure accurately. Future applications of the model may include: predicting the nasal odorant/toxic/aerosol uptake dosimetry and distribution pattern in mouse, and potentially extrapolating data from mouse to human. Acknowledgements: NIH DC006760 and NIH DC008187

366 Implicit modulation of preferences for odors by explicit choices in long-term memory

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Several studies have shown that preferences can be strongly modulated by cognitive processes such as decision making and choices. Remarkably, it has been demonstrated that explicit choices, traditionally considered as a reflection of preferences, can in fact create them. However, it is still unclear whether choices can influence preferences of sensory stimuli in an implicit way and if such a modulation is stable over time. This question was addressed here by asking participants to evaluate odors, to choose their preferred odors among pairs, to re-evaluate odors, and to perform an unexpected memory test concerning their choices. After one week, participants were asked to evaluate one more time the odors, and to do choices between pairs similar to those presented the previous week. Results revealed the existence of post-choice preference changes, in the sense of an overvaluation of chosen odors and a devaluation of rejected ones, even when choices were forgotten. These results suggest that chemosensory preferences can be modulated by explicit choices and that such modulation might rely on implicit mechanisms. This finding rules out any explanation of post-choice preference changes in terms of experimental demand and strongly challenges the classical cognitive dissonance reduction account of such preference changes. Moreover, preliminary results showed that this choice-induced preference modulation was still present after one week and the congruence between the choices made the first time and after one week was high. This result invites to further consider the importance of implicit processing in preference acquisition and stability across time.

367 The eyes see what the nose smells: Olfactory modulation of visual perception in binocular rivalry

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Vision is widely accepted as the dominant sense in humans and other primates, whereas olfaction is often considered a vestigial sense yielding only obscure object representations. It is well documented that vision drives olfactory perception, but the converse is hardly known. Here we introduce smells to a well-established visual phenomenon termed binocular rivalry, perceptual alternations that occur when distinctively different images are separately presented to the two eyes. We show that an odorant congruent to one of the competing images prolongs its dominance duration and shortens its suppression time in a manner that is automatic, independent of cognitive control, and partly subconscious. Our findings provide the first direct evidence that a non-visual sensory cue biases the dynamic process of binocular rivalry, thereby demonstrating olfactory modulation of visual perception – an effect that has been hitherto unsuspected. Acknowledgements: This work was supported by NIH R03DC4956 and the Knowledge Innovation Program of the Chinese Academy of Sciences (KSCX2-YW-R-250 and 09CX192019).

368 Virus-infected female mice attract male mice through pheromone up-regulation

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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 adenocarcinoma tumors. The virus is widely distributed in wild mouse populations worldwide suggesting that some factor acts to maintain the virus in the mouse population. We previously reported that inbred laboratory mice (C57BL/6 (B6 which is $H2^b$) infected with MMTV can be discriminated by scent from uninfected, genetically identical control mice long before the development of tumors. We next found that the amount of 3,4-dehydro-*exo*-brevicomin (DHB-previously identified as a pheromone produced by male mice that modulates inter-male aggression and female estrus cycling) was dramatically increased in urine of infected female mice. Subsequent studies demonstrated that, in free-choice tests, mice are attracted to the scent of MMTV-infected females, of their urine, and of DHB-spiked female urine. To evaluate the generality of these results, we have now tested females of two other mouse strains: Congenic B6-$H2^k$ mice with a different MHC haplotype than B6 mice and BALB/c, an inbred strain that differs from B6 at many genetic loci. MMTV-infected B6-$H2^k$ mice also showed up-regulation of DHB production and the urine of infected mice and DHB-spiked urine attracted B6 males. In addition, DHB-spiked BALB/c female urine attracted male mice. These

overall results indicate that female mice infected by MMTV are particularly attractive to males which may serve to enhance the transmission of MMTV to succeeding generations of mice thereby helping to explain the persistence of this virus in world-wide mouse populations.

369 Human Male Superiority in Olfactory Sensitivity to the Sperm-Attractant Odorant Bourgeonal

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Recent studies have shown that sperm chemotaxis critically involves the human olfactory receptor hOR17-4 which is activated by the aromatic aldehyde bourgeonal. Given that both natural and sexual selection may act upon the expression of receptors we hypothesized that human males are more sensitive than human females for bourgeonal. Using a 3-alternative forced-choice test procedure, olfactory detection thresholds were determined for a total of 500 subjects, 250 males and 250 females between 18 and 40 years of age. We found that male subjects detected bourgeonal at significantly lower concentrations (mean value: 13 ppb) compared to female subjects (mean value: 26 ppb) whereas no such gender difference in olfactory sensitivity was found with helional, a structural analogue of bourgeonal, and with n-pentyl acetate, an aliphatic ester, which were tested in parallel. Males and females did not differ in their frequency of specific anosmia for any of the three odorants. The frequency distributions of olfactory detection thresholds were monomodal with all three odorants in both genders. Olfactory detection thresholds did not differ significantly between pre- and postovulatory females with any of the three odorants. To the best of our knowledge this is the first study ever to find a human male superiority in olfactory sensitivity. Single nucleotide polymorphisms (SNPs) and/or copy number variations (CNVs) in genes coding for olfactory receptors may be the proximate cause for our finding whereas a gender difference in the behavioral relevance of bourgeonal may be the ultimate cause.

POSTER SESSION VII: OLFACTORY PSYCHOPHYSICS & CLINICAL STUDIES; CENTRAL OLFACTION

370 The characteristic aroma compounds in raw nonpareil almond kernel are enzymatic products

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Benzaldehyde is well recognized as the predominant aroma in bitter almond (*Prunus dulcis* var. amara), and is released from amygdalin upon enzymatic hydrolysis followed by a loss of hydrogen cyanide. Water and/or hydrolytic enzymes are added to increase the level of benzaldehyde during the processing of almond oils. Sweet almond (*Prunus dulcis* Mill. D.A. Webb) has a sweeter, nuttier aroma than the bitter variety. We observed that the sweet aroma was enhanced when water was added to the ground raw almond samples. We then compared the volatile profiles of the almond samples with and without adding water using solid phase microextraction followed by gas chromatography/mass spectrometry (GC/MS). Several alcohols were released upon addition of water and became the major volatile compounds, indicating that water may facilitate enzymatic reactions and the resulting alcohol production. Two of them, 3-methyl-3-buten-1-ol and 3-methyl-2-buten-1-ol, emitted the characteristic sweet aroma, as determined by GC/olfactometry. In order to determine whether the release of these alcohols is regulated by enzymes, we added guanidine hydrochloride to the almond samples to stop enzymatic reactions and examined their volatile profiles. The alcohols in the enzyme-denatured samples were barely detected or greatly reduced in amount. Therefore, our data suggest that 3-methyl-3-buten-1-ol and 3-methyl-2-buten-1-ol, the characteristic aroma components in raw nonpareil almond kernel, are released upon enzymatic reactions. Acknowledgements: This work was partially supported by the Almond Board of California.

371 The Relationship between Intranasal Volume and Olfactory Performance

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The volume of the nasal cavities is a crucial factor for odorant molecules to be transported to the olfactory receptors in the upper part of the nose. Whereas several studies have focused on the relationship between intranasal volume and olfactory functions in various patient populations, few studies in healthy individuals exist. To the best of our knowledge, only one study has investigated the connection between intranasal volume and olfactory functions in healthy subjects (only males). The result of this study was that the volume of a minor portion within the dorsal region of both nostrils was correlated with olfactory threshold. The aim of our study was to extend our knowledge about the relationship between intranasal volume and olfactory performance with a special focus on potential sex differences. We are currently determining bilateral intranasal volumes as well as olfactory performance scores in a larger group of healthy male (n = 40) and female subjects (n = 40). Structural high-resolution MRI scans were acquired on a 1.5T MRI scanner and the volumes of the left and right nasal cavity were delineated and measured with the help of AMIRA software. Olfactory detection threshold scores and olfactory discrimination scores of the subject's left and right nostrils as well as birhinal olfactory identification scores were acquired. Initial Bonferroni-corrected correlation analyses, with age and body mass index included as variables of no interest, based on a subset of the sample (n = 40), demonstrated no significant correlation between total nasal volume and olfactory functions. No sex differences were found. These initial results seem to indicate that total nasal volume has little to no influence on olfactory performance. The final results based on the whole sample will be presented and discussed. Acknowledgements: Supported by start-up funds from the Monell Chemical Senses Center awarded to JNL and a DAAD

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372 Olfactory Scintigraphy in Normal Volunteer by Intranasal Tl-201 Administration.

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Background –At present, there is no method to visualize the olfactory nerve damage. The aim of this study was to assess whether olfactory nerve scinitigraphy by per-nasal cavity thallium-201 (Tl) administration is feasible or not. ***Methods***- 5 normal volunteer (5 male, aged 43 ± 8) were administered 18-37 MBq of Tl on the olfactory mucosa. 30-min and 24-hr after the tracer administration, SPECT/CT was performed. CT image from SPECT/CT was fused with MRI image that obtained separately, then SPECT/MRI fused image was generated. On the SPECT/MRI coronal and sagittal fused images, ROIs were set to cover the area of Tl administered on the olfactory mucosa with the 50% threshold of maximum count, and another ROI was set on the olfactory bulb. The % uptake of the Tl of the olfactory bulb (U) was calculated as: U = 100 (counts of the olfactory bulb / counts of the olfactory mucosa). ***Results***- There were no adverse side effects on odor detection ability and nasal mucosa in all subjects. At 30-min after Tl administration, most of the Tl activity was observed at the roof of nasal cavity, however, significant activity was observed in olfactory bulb at 24-hr. Quantitatively, olfactory bulb Tl uptake at 30-min was 10.8 ± 6.8 % and it increased significantly at 24-hr (24.3 ± 11.8 %, $p<0.05$). ***Conclusion*** – Tl transport from olfactory mucosa to olfactory bulb was clearly observed in normal volunteer by per-nasal cavity administration of Tl, indicating the feasibility of olfactory nerve scintigraphy for the diagnosis of impaired olfactory nerve function due to such as trauma or infectious diseases. Acknowledgements: Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (C18591860 to TM)

373 Effects of Odor Discrimination Task Manipulation on Performance

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This study looked at five variations of the odor discrimination task using the OLFACTÔ olfactometer to determine how changes in testing protocol affect an individual's ability to discriminate between odors. The first task in the study was a traditional triangle test: three presentations of odors in each trial, two presentations were of the same odor, one was of a different odor and the participant selected the odd odor. The second odor discrimination task used the same method except the target odor or "odd" odor was named for the participant. This manipulation stemmed from the concept that the more an individual knows about an odor, the easier it would be for the participant to discriminate that odor from another. However, performance did not improve significantly. The third odor discrimination task gave two presentations of odors per trial and asked the participants to indicate if the odors were the same or different. This variation yielded the best performance of all five versions of the task and led to the fourth and fifth versions of the odor discrimination task. The fourth task asked participants to discriminate between two odors more subjectively, asking them to indicate if the odors were the same, very similar, similar, not very similar, and different. Interestingly, these results showed that some of the obviously different odor pairs were still perceived as similar. Finally, the fifth odor discrimination task used multiple presentations of only two odors that have distinctly different qualities in order to simplify the task even more, but performance did not improve significantly. This study illustrates that although discrimination of odors is often thought to be a fairly simple task, in fact, it is a complex task and there are processes involved that are not fully understood. Acknowledgements: Supported by NIDCD grant DC6369

374 Time Course of Human Perceptual Odor Disadaptation

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We have previously reported estimates for the onset time course of perceptual olfactory adaptation in humans by using a novel stimulus paradigm. That research demonstrated that prolonged exposure to an odor induces perceptual olfactory *adaptation*—temporarily decreasing the subject's sensitivity and increasing detection thresholds for a brief, simultaneous target odorant presented at various time-points after adapting stimulus onset. Results suggested that, for a self-adapting odorant, thresholds for the target odorant were increased systematically within 100 - 300 ms after stimulus onset. The current study employed the same technique to examine the time course of psychophysical olfactory *disadaptation*—the recovery of sensitivity following olfactory adaptation. Nineteen volunteers (ages 18-21; 11 females) served as subjects. To characterize adaptation's offset time course, we used a liquid-dilution olfactometer to estimate baseline two-bottle discrimination thresholds for brief (600 ms) presentations of vanilla odor. The adapting odorant concentration level was then set to twice baseline-threshold for each subject. Threshold for the 600-ms target was measured as a function of the relative delay between the *offset* of a 1500-ms adapting stimulus and the onset of the target. On average, subjects recovered significant olfactory sensitivity within 500 ms of adapting odorant termination, but detection thresholds remained above baseline to 2500 ms after adapting stimulus removal. These results, combined with previously reported data from our laboratories, suggest that this stimulus paradigm can provide a detailed characterization of the time course of perceptual odor adaptation.

375 Training the inter-nostril localization ability of olfactory chemicals

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In vision and audition, the pair of receptor organs is crucial for spatial orientation and localisation of stimuli in the 3-dimensional environment. Whether the pair of receptor organs in olfaction fulfils the same function of localizing odor sources is not yet clear. Is is widely accepted that mixed trigeminal-olfactory chemicals can be accurately localized when applied passively to one of the nostrils. Chemicals with a predominant olfactory component seem, on the contrary, hard or impossible to localize in passive stimulation procedure. The purpose of this study was to investigate whether the ability of subjects to localize an olfactory stimulus delivered passively to one of the two nostrils would improve under training. Fifty-two healthy, normosmic women aged between 18 and 30 years participated in 6 to 7 sessions. Two groups were created: 27 subjects followed an olfactory lateralisation training protocol using two chemicals known to selectively stimulate the olfactory system (hydrogen sulphide and phenylethylalcolol). The second group performed ''brain jogging''. Before and after- training subjective intensity and lateralisation ratings were recorded. Additionally, electrophysiological parameters and fMRI BOLD signal after stimulation with the two odorants were recorded at the end of the training. Comparisons between trained and non-trained subjects suggested an improvement of localisation scores in subjects specifically trained for this. Further analyses and discussion will focus on lateralisation effects.

376 Similarities and differences between sensory systems in the localsiation of unilateral nasal stimuli

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Odor stimuli presented to one nostril can only be localized if they additionally activate the trigeminal nerve's chemosensitive fibers. In this study we aimed to investigate characteristics in the localisation of unilateral trigeminal, olfactory and somatosensory nasal stimuli. We compared subjects' ability to localise monorhinally presented a) pure olfactory stimuli (phenyl ethyl alcohol), b) mixed olfactory trigeminal stimuli (eucalyptol), and c) somatosensory stimuli (air puffs). As expected, the subjects could localize the air puffs and eucalyptol, but could not localize phenyl ethyl alcohol. Interestingly, we observed a significant correlation between localization performance for eucalyptol and phenyl ethyl alcohol but not between the ability to localize somatosensory and trigeminal or olfactory stimuli. The present study provides further support for the intimate connection between the chemosensory trigeminal and olfactory systems. Acknowledgements: Fondation des Etoiles, Fondation Hopital Ste. Justine (JF) Belgian National Funds for Scientific Research (OC) CRCP, CIHR, NSERC (FL)

377 Nasal Epithelial Responses in a Murine Model of Allergic Rhinitis

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The aims of this study were to develop a murine model for allergic rhinitis (AR) induction and to analyze AR effects on the olfactory epithelium (OE) and, ultimately, on olfactory functional capabilities and effective pharmacological treatment thereof. We have previously reported (AChemS 2009) successful AR induction in Balb/C mice, shown by high ELISA serum OVA-specific IgE levels and lamina propria (LP) eosinophil infiltration, using a modification of the McCusker et al. (2002) localized asthma induction protocol. Bilateral infusions of 7.5µl/naris of sterile ovalbumin (OVA; 1% in PBS) or PBS were made M-F for 6 or 11 wks, with defined breaks in the regimen to enhance allergic response intensity. One day after the final infusion, mice were deeply anesthetized and perfused with 4% PFA following cardiac blood collection. Paraplast nasal serial sections were examined for overall histology, eosinophil distribution, alcian blue staining, and OMP and neurotubulin immunoreactivities. Histological changes in OVA-treated mice include pronounced LP eosinophil infiltration, noticeable respiratory epithelium (RE) swelling, and OE thinning and disruption with cell sloughing and Bowman's gland swelling into the OE. Significantly, LP eosinophil infiltration was confined to RE but not seen in OE. We are now using 5-bromo-2'-deoxyuridine (BrdU) and activated caspase-3 (cc-3) immunoreactivities to analyze AR effects respectively on cell proliferation and apoptosis. Pronounced BrdU labeling occurs in RE but in OE is seen only in areas that are normally thinner and/or closer to RE. Surprisingly, despite the noticeable epithelial disruption, increased cc-3 labeling is minimal, possibly suggesting non-apoptotic mechanisms of epithelial loss. Currently, TUNEL apoptotic analysis is underway to verify this result. Acknowledgements: Northwestern University FSM in-house funds

378 Influence of sinunasal diseases on olfactory function and quality of life

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Chronic rhinosinusitis is one of the most common causes of olfactory disorders and is known to have a large effect on general health and subjective well-being. Aim of this study was to investigate changes in olfactory function and quality of life after surgical treatment of sinunasal disease. A total of 788 patients with sinunasal disorders (492 men, 296 women; age range 9-81 years) were included in the study; 336 patients received sinus surgery, 358 septum surgery and 94 patients received sinus surgery involving the septum. Patient's subjective impairment and quality of life were measured before and after surgery using standardized questionnaires (Rhinosinusitis Disability Index; SF-36-Questionnaire). Olfactory function was assessed with a standardized odor identification test (''Sniffin' Sicks''). Five months (63-339 days) after surgery 361 patients were retested. Prior to surgery, 30% of the patients rated their sinunasal problems in the Rhinosinusitis Disability Index to be of a gravely impaired, 61% rated them medium and 9% minor, whereas in the SF-36 score more than 70% of the patients rated their quality of life within the norm. After surgery, symptom-severity decreased in more than 60% of the patients and also a slight improvement (p=0.012-0.08) of general quality of life was observed. An improvement of the sense of smell was found in 18%, no change

was seen in 74%, and decreased olfactory function was seen in 8% of the patients. In conclusion, surgery improved olfactory function ($p<0.001$). In the majority of the patients sinunasal disease does not seem to have a major impact on general quality of life. The SF-36-score is more affected by age and gender, meaning that older patients reported a reduced quality of life ($p<0.001$) and women rated their quality of life lower than men ($p=0.01$).

379 Olfactory neural responses of anosmics: A pilot fMRI study

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Anosmia, the complete loss of olfactory sensory perception, is a prevalent neurological disorder which has devastating impacts on nutritional health and safety. The aim of this pilot study was to assess the basic neural coding of odors within the olfactory cortex (the neural oscillations entrained to the rhythmic action of sniffing) by utilizing fMRI methods in both anosmic and healthy human subjects. Specifically, the hypothesis that anosmics possess the neural mechanisms necessary to form sniff-driven oscillations in the olfactory cortex was tested. Functional images were acquired from three healthy volunteers and one anosmic patient using a 3T MRI scanner. Each fMRI experiment consisted of two sessions, each comprised of four ''sniffing'' blocks (40 seconds each) and five baseline blocks (40 seconds each), lasting a total scan time of 320 seconds per session. Image preprocessing was performed in SPM5 including slice-timing and motion correction, spatial normalization and spatial smoothing using a Gaussian kernel (FWHM = 9 mm). Single-subject and group analyses were performed using probabilistic ICA as implemented in MELODIC v3.05 (part of FSL software suit) and revealed activity in piriform cortex and orbitofrontal cortex in both groups. These results provide important information regarding the neural substrates of olfactory information processing in anosmia. Also, our results support the hypothesis that patients suffering from olfactory loss are capable of showing activation of cerebral structures involved in olfactory processing. Future studies with larger samples will provide more and detailed information to improve anosmia therapies and thus the lives of those afflicted by anosmia and possibly other olfactory sensory disorders.

380 Investigation of detection and pain thresholds at different sites at the human nasal mucosa in healthy subjects and patients with chronic rhinosinusitis

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Background: Previous investigations in humans suggest topographical differences in the arrangement of intranasal trigeminal chemosensitivity with the highest sensitivity in the anterior part of the nasal cavity. The aim of the present study was to investigate if different sites at the human nasal mucosa react differently to unspecific electrical stimuli and if there are differences in healthy subjects and patients. *Material and Methods:* A total of 50 young, healthy volunteers (24 men, 26 women; age 22-38 years) and 10 patients with nasal polyps prior sinus surgery (6 men, 4 women; age 19 - 43 years) participated. Detection and pain threshold of trigeminal stimuli of the healthy subjects and the patients 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:* In healthy subjects a significantly higher trigeminal sensitivity was found at the anterior parts of the nose compared to the posterior part. There was a similar distribution pattern of the sensitivity for detection and pain thresholds. In patients there was a higher detection thresholds compared to healthy subjects, interestingly. *Conclusions:* The present data suggest that there are topographical differences in the arrangement of trigeminal neurons at the human nasal cavity. The highest sensitivity seems to be located in the anterior part of the nasal cavity. This finding is compatible with the idea that the trigeminal system acts as a sentinel of the human airways with regard to toxic agents. In case of the patients with chronic rhinosinusitis the sensitivity of the nasal mucosa seems to be reduced which may contribute to the sensation of a ''congested nose''.

381 Odors, Asthma and Risk Perception

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Fragrances and strong odors have been characterized as a putative trigger that may exacerbate asthma symptoms and many asthmatics readily avoid odors and fragranced products. However, the mechanism by which exposure to pure odorants can elicit an adverse reaction in asthmatic patients is still unclear and may involve both physiological and psychological processes. The aim of this study was to investigate how beliefs about an odor's relationship to asthmatic symptoms could affect the physiological and psychological responses of asthmatics. Asthmatics classified as 'moderate-persistent', according to NIH criteria, were exposed for 15 minutes to a fragrance which was described either as eliciting or alleviating asthma symptoms. During exposure, participants were asked to rate odor intensity, perceived irritation and subjective annoyance while physiological parameters such as electrocardiogram, respiratory rate, and end tidal carbon dioxide ($EtCO_2$) were recorded. Before, immediately after, and at 2 and 24 hours post-exposure, participants were required to subjectively assess their asthma symptom status using a standardized questionnaire. We also measured asthma status at each of those time points using objective parameters of bronchoconstriction (spirometry) and measures of airway inflammation (exhaled nitric oxide -eNO). Predictably, manipulations of perceived risk altered both the quality ratings of the fragrance as well as the reported levels of asthma symptoms. However, perceived risk also appeared to modulate the inflammatory airway response, suggesting that stress elicited by the instructions may affect airway physiology and impact asthma exacerbations. Acknowledgements: Supported by NIH-NIDCD grant number RO1 DC 003704 to PD.

382 "Olfaction in Burning Mouth Syndrome"

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OBJECTIVE: While Burning Mouth Syndrome (BMS) has been linked to disorders of taste, a formal assessment of olfaction has not been entertained. **BACKGROUND:** BMS is a condition of chronic neuropathic pain affecting the tongue and other intraoral structures in more then one million Americans. **METHODS:** Retrospective chart review of the most recent 22 BMS patients (7 males, 15 females) was assessed for olfactory complaints and testing. **RESULTS:** Average age was 59 years (males) and 54 years (females), the severity of burning on a scale of 1 to 10 was 7. Fifty five percent of patients (12 out of 22) had at least one olfactory complaint as follows: hyposmia - 55%, dysosmia – 23%, and phantosmia – 14%. Twenty patients (90%) who performed olfactory tests had abnormal results bilaterally. Forty one percent of these patients had 1 test abnormal, 9% of these patients – 2 tests, 27% - 3 tests and 14% - 4 tests abnormal. Of those who performed the Brief Smell Identification *Test* corrected for age and sex - 4 out of 9 (44 %) had abnormal results; Alcohol Suprathreshold Testing – 11 out of 14 (79%); Smell Threshold Test with phenyl ethyl alcohol – 8 out of 10 (80%) patients had abnormal results in both nostrils tested individually; Quick Smell Identification Test - 9 out of 19 (47%); the Pocket Smell Test – 6 out 16 (38%); the unilateral University of Pennsylvania Smell Identification Test corrected for age and sex – 5 out of 7 (71%) patients had abnormal results in both nostrils tested individually. The incidence of chemosensory complaints in the general population is approximately 5%, whereas the incidence of chemosensory complaints and dysfunction in those with BMS was far greater (55%). **CONCLUSION**: Beside taste dysfunction, BMS may manifest as a generalized chemosensory disorder.

383 Eruction Sensitive Subjective Hypogeusia

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OBJECTIVE: To illustrate the importance of retronasal olfaction for perception of taste. BACKGROUND: Physiologic synesthesia with taste/smell confusion is the chemosensory underpinning of perceived flavor which is mediated through retronasal olfaction. The importance of this mechanism in the perception of flavor is manifest in the case study. METHODS/RESULTS: 70 year-old retired teacher was nasute until one year prior to presentation when, after a severe upper respiratory infection, suddenly lost all smell, with gradual recovery of approximately 30%. Concurrent with the smell loss was a perceived decreased ability to taste all but sweet, sour, and spicy, whereas all foods tasted flavorless. With eructation, totally normal flavor would return (ie. chewing what tasted like cardboard, on eructation, would suddenly transform to garlic bread, rubber to cantaloupe, etc.). Otolaryngolgic and nasal fiberoptic endoscopic examinations and CT of the head and sinuses were normal. Chemosensory testing suggested normogeusia on quadrant testing and Accusens Taste test, but severe olfactory deficit: right and left unilateral PEA threshold testing greater than -2.0; UPSIT left nostril 16, right nostril 10; Sniffin' Stick Threshold unilaterally and dirhinously less than 1; discrimination left 3, right 5, dirhinously 6; identification left 8, right 9, dirhinously 7; odor memory test 2 at ten seconds, 3 at thirty seconds, and 1 at sixty seconds. CONCLUSIONS/RELEVANCE: The perceived normogeusia upon eructation in this otherwise subjectively hypogeusic individual demonstrated the importance of retronasal olfaction in the perception of flavor. SOURCE OF FUNDING: None

384 Identification of odor active substances in human amniotic fluid

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Objectives. Physiological evidence indicates that olfaction could already function in the human fetus [1]. Right after birth the odor of amniotic fluid (AF) is detectable to newborns and seems to attract [2] and calm them [3]. Such early attraction may derive from fetal learning/memory, and may help newborns adapt to the postnatal environment. The chemosensory basis of AF attractiveness has not been investigated yet, and this study aimed to characterize odor active compounds therein. **Methods.** The flavor profile of AF was monitored by descriptive sensory evaluation using an adult panel, while the identification of the predominant odor substances was carried out by senso-analytical techniques like gas chromatography-olfactometry and comparative dilution assays. **Results.** The prevailing AF odor impressions were described as blood- and raw meat-like. Several odor active substances were identified, amongst them some carbonyl compounds, as well as androstenone. **Conclusions.** Human AF contains a wide range of odorous substances, such as odor active steroids or ketones. These substances could have the potential ability to promote attention and hush the newborn, for example during noxious medical examination. **References**. [1] Schaal et al. 2004. Clin Perinatol 31:261-81 [2] Schaal et al. 1995. Biol Neonate 67, 397-406. [3] Varendi et al 1998. Early Hum Dev 51:47-55. Acknowledgements: Financed by the German Federal Ministry of Education and Research (BMBF) and the Bavarian Research Foundation.

385 Human Neonatal Responses to Androstenone

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Aims. Human newborns show equal attraction to the odors of amniotic fluid (AF) and mothers' colostrum [1]. As 5-α-androst-16-en-3-one (An) has been found in both fluids [2, 3], we studied it as a potential vector of this perinatal odor continuity and of neonatal attraction to the smell of breast and milk [4]. **Methods.** Two studies were run. 1) We recorded the behavior of 16 newborns (3 days) and 26 adults exposed to a saturating water solution of An and to 3 reference odorants [butyric acid, B; vanilla, V; water, W]. 2) We videotaped 26 newborns (3 days) being administered 7 stimuli: 4 dilution steps of An and 3 reference odorants [familiar milk, V, W]. Oral and facial actions to each odorant were quantified in infants, while verbal responses were recorded in adults. **Results**. In study 1, 12/16

neonates reacted with negative facial actions to An, while 10, 3 and 2 responded in such way to B, V and W, respectively. In contrast, 11/26 adults rated An as unpleasant, while 26 rated B as negative, and V and W as positive/neutral. In study 2, 24/26 newborns increased the relative duration of negative facial actions and/or decreased the duration of oro-cephalic movements when exposed to An as compared to W. **Conclusions.** A great majority of newborns detect An and, as adults who are osmic to An, respond to An in showing unpleasantness. Thus, the status of specific anosmia to An clearly differs in neonates and adults (<25% vs. > 50% being non responsive, respectively). Finally, the negative responsiveness to a compound met prenatally appears paradoxical when considering previous data on fetal odor learning. **References.** [1] Schaal B et al. (1998). Behav Neurosci, 112, 1438-49; [2] Doucet S et al., submitted; [3] Buettner A (2007). Flav Fragr J, 22, 465-73; [4] Doucet S et al. (2007). Dev Psychobiol, 49, 129–38. Acknowledgements: SD was supported by Bavarian Research Foundation and Region Burgundy. CH and AB were funded by the German Federal Ministry of Education and Research (BMBF), and BS by CNRS (EAL 549).

386 The Scent of Nurturing: Experimental Evidence supporting the Priming of Infant Nurturing Behavior by Baby Powder Fragrance

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The smell of baby powder can provoke tender feelings in parents reminding them of when their children were small. We tested whether such odors can actually elicit nurturing behavior in the perceiver. **Method:** In a laboratory experiment, 40 childless females (Mean age = 22, SD = 4.3) watched a short movie showing infants being nurtured by their parents. During this phase (Phase 1: association between odor and nurturing) they were exposed to a baby-powder like odor - either fruity or floral - of weak intensity. After an unrelated filling task they entered the second phase (test phase) of the experiment in which they were instructed to respond to the needs of a crying computerized RealCare® infant simulator doll (RealityWorks Inc., US). Participants were again exposed to a weak odor, either the same as during Phase 1, or different. Nurturing behavior, involving rocking and feeding, was automatically recorded by the doll. Grade of nurturing behavior quality was calculated by the software. **Results:** There was a significant effect of odor on nurturing behavior such that participants who had been exposed to the same odor they had experienced during the association phase showed higher quality nurturing behavior during the test phase than those who had experienced a different odor ($p < .005$). **Discussion:** The above results support that the scent of baby powder, a product that is generally used when talking care of an infant, can become an unconscious prime of nurturing. This finding can be applied to the training of nurturing behavior in e.g. teenage mothers, by supplying to the mothers similarly fragranced products that were used during training and thus improve quality of parenting. Acknowledgements: Linschoten Research Institute

387 EFFECT OF SENSORY EDUCATION ON CATEGORISATION OF UNKNOWN ODORS IN CHILDREN

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Spontaneously, unknown odors are categorised by children according to their hedonic values. This study was set up to verify whether a sensory education program, based on the pedagogical method of the ''Classes du gout'' developed by Jacques Puisais is able to switch this hedonic categorisation strategy into a more olfactory quality based ''expert'' strategy. The study was carried out in Dijon, France with school classes of children of 8 to 11 years old and with their usual teacher. It was performed using an experimental group of 87 children and a control group of 81. Children of an experimental group (N=87) participated in 12 sensory education lessons of 90 minutes each. Both the experimental and the control group (N=81) participated in two laboratory measurements: a pre-test before the education period of the experimental group and a post-test just after the education period. The children were asked to categorise 9 unknown odors in 3 groups of 3 without any other information. They also indicated their liking for the odors. Results showed that the sensory education induced a switch in the strategy of classification of unknown odours towards a less hedonic approach. The protocol was approved by a medical ethical committee and supported by the National Research Agency of France.

388 The Effect of the Stimulation of Traditional Korean Medicine Acupunctural Points on Olfactory Function

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The purpose of this study was to test the hypothesis that stimulation of specific Traditional Korean Medicine (TKM) acupuncture points will improve olfactory ability in healthy subjects. Nineteen college students (11 female, 8 male) underwent 5 minutes of gentle massage and 30 minutes of pressure stimulation using 3mm foil balls secured onto sets of control and experimental TKM acupuncture treatment points found along meridians located in the head, neck, arms, hands, legs and feet. The stimulation done first was randomly assigned for each subject with at least 48 hours between treatments. After pressure stimulation, the olfactory ability of the subject was evaluated by determining the thresholds for β-phenylethyl alcohol and ammonia and by having subjects use the Green Scale (Green *et al.*, 1996) to rate the intensity of 3 blocks of 9 odorants. Although the experimental TKM subjects rated the intensities of the nine odors higher than they did after receiving the control stimulation ($p<0.05$), the olfactory thresholds did not change. The increases in the intensity ratings raise the possibility that stimulation of the experimental TKM points enhance olfactory function by increasing nasal dilation. Males showed a greater change in the intensity ratings than the females. Since some TKM practitioners report better success when the patient is of the opposite gender, the fact that the experimenter was female may account for the observed gender difference. This observation may be due to ''complementing energies'' or it might suggest a placebo-like effect in at least a portion of the TKM response.

389 Phantosmia Treatment with Olfactory Counterstimulation- A Case Report

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Objective: Phantosmia is resistant to myriad treatments. Sensory counterstimulation in other sensory spheres has been an effective approach to sensory hallucinations. Successful treatment of a patient with phantosmia through olfactory counterstimulation is described. Methods: A case-report of a 65 Y/M with hemochromatosis. Three Yrs ago, this patient had an upper respiratory infection (URI) followed by unpleasant smell of burnt wood which morphed into a mixed gasoline onion smell. It has diurnal variations, increasing throughout the day, with intensity of 7/10, and causing eyes to water for several hours. His symptoms improve with saline irrigation, snorting salt water, xanax, flonase, distractions, holding breath or nose, clogged nose, putting head down, sleep, blowing nose, laughing, eating, humming, or talking. Nasal congestion, coughing, or breathing in and out worsen it. In an attempt to reduce the phantosmia, the patient was given odorized pens impregnated with (banana, garlic, cloves or turpentine) to sniff on onset of phantosmia. Banana and garlic had no effect, however, cloves and turpentine induced total replacement and inhibition of the phantosmia. Discussion: The potential mechanism of odor inhibition of phantosmia is through peripheral olfactory stimulation acting as a counterstimuli, causing peripheral inhibition of the phantosmia

390 Filial Catamenial Phantosmia

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Objective: To report an unusual case of filial catamenial phantosmia. **Background**: Phantosmia describes the phenomenon of odor perception when no odorant stimulus is present. **Design/methods**: Two sisters, aged 39 and 41 presented with similar recurrent catamenial smoky phantosmias in the absence of olfactory deficits. Beginning three days prior to and resolving a week after menses, all odors were perceived as a mixture of their original essence and the phantom odor. Both their medical histories were positive for migraine headaches and were negative for head trauma, upper respiratory tract illness and rhinitis. **Results**: General physical, psychiatric, neurological and head and neck examinations, nasal endoscopy, MRI and CT of the brain and sinuses were negative and chemosensory testing including phenylethyl alcohol(PEA) threshold and University of Pennsylvania Smell Identification Test were normal in the proband. The proband's symptoms resolved on initiation of L-methylfolate, Methylcobalamin and N-acetylcysteine. The sister declined treatment. **Conclusions**: Relative olfactory hypersensitivity resulting from estrogenic changes in mucosal consistency may have induced patient awareness of subthreshold odors, perceived as a phantosmia. Alternatively, relative hyposmia caused by low estrogen or a progesterone excess could explain the temporal sequence of both presentations. Estrogenic modulation of neuronal function, a shared olfactory tropic virus infection, a mass psychogenic illness, a cyclic variant of olfactory reference syndrome or an amigrainous variant of catamenial migraine could explain both the cyclical and the familial pattern of symptom production. **Relevance**: Exploration in these realms may prove beneficial in elucidating a management approach for phantosmias. **Funding Sources**: none

391 Developmental fine-tuning of olfactory discriminability

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Olfaction, generally considered well functioning at birth, is unique among human senses in its neural genesis and myelination processes. There have been few empirical studies on the developmental trajectory of human olfactory perception. Here we assessed olfactory discriminability in children aged 3-6 yrs with 16 pairs of single-compound odorants which differ in various degrees on structure, functional group, and/or smell. We observed a significant positive correlation between age and overall discriminability. Moreover, age interacts with pairs of odorants – whereas some odorant pairs were discriminated equally well by children of all ages, some were better discriminated in older children. Our findings provide insights into the fine-tuning process of human olfactory system. Acknowledgements: Knowledge Innovation Program of the Chinese Academy of Sciences Grant No. 09CX192019 & KSCX2-YW-R-250

392 Quantifying Olfactory Function in the Aging U.S. Population: A Home Test

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The aging olfactory system may contribute significantly to physical and mental health in older adults, as well as their quality of life. To characterize olfactory aging in the diverse population of the United States, we selected a representative sample of 3,005 older adults (the National Social Life and Aging Project (NSHAP)), large enough to accurately determine prevalence of olfactory dysfunction and its association with key aspects of medical conditions, mental health, sexuality, cognition, social networks, geography, and demography (e.g. socioeconomic status, ethnicity and gender). The Olfactory Function Field Exam (OFFE) is designed to be conducted in respondents' homes throughout the United States and requires a short 6-8 minute protocol to assess sensitivity to physical and social odors as well as odor identification. To this end we conducted two validation studies: 1. Validation of the olfactory sensitivity component of the OFFE: A forced choice sensitivity test for a physical odorant (n-butanol) and a social odorant (androstadienone) was administered to 30 older adults along with well-validated staircase threshold tests (Hummel, et al., 1997). We will report the test characteristics and validity of the OFFE's sensitivity component, along with frequency of dysfunction and basic demographic characteristics. 2. To determine feasibility of administration by field interviewers, we completed a pretest of both components of the OFFE (odor sensitivity and identification) embedded in the full NSHAP interview of people in their homes throughout the U.S. (n = 120). We found a 100% success rate, and will report on further refinements of the OFFE, along with frequency of dysfunction and its associations with basic demographic characteristics of this diverse population. Acknowledgements: The National Social Life, Health, and Aging Project Wave II (R37 AG030481), Gianinno Graduate Research Fund

393 Olfaction and Executive Function in the Beaver Dam Offspring Study

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Olfactory impairment is associated with the 5-year incidence of cognitive impairment in older adults but it is unknown if there is an association present in younger adults in a general population. The objective of the current study is to determine the association between olfaction and cognitive function in the Beaver Dam Offspring Study, a study of familial and birth cohort effects on aging senses. The San Diego Odor Identification Test was used to measure olfaction, and olfactory impairment was defined as correctly identifying fewer than 6 out of 8 odorants. The Trail Making Test (TMT) was used to assess cognitive (executive) function. The TMT score is the time in seconds(s) it takes to complete the tasks, drawing a line sequentially connecting randomly placed numbers (TMTA) and alternating numbers and letters (TMTB) correctly, with a longer time considered a poorer performance. There were 2837 participants, aged 21-84 years (mean age 49 years) with olfaction and TMT data; 3.8% had olfactory impairment. In preliminary analyses, adjusting for age and sex, TMTA and TMTB time were significantly longer among those with olfactory impairment versus those without impairment (TMTA = 35.9 s vs. 27.6 s; TMTB=83.7 s vs. 67.0 s; respectively, $p<0.0001$ for both). This association remained significant for both TMTA and TMTB in models adjusting for age, sex, education, ankle brachial index, history of cardiovascular disease and statin use. In this adjusted model, the mean TMTA time was 6.6 s longer and mean TMTB time was 14.3 s longer among those with olfactory impairment as compared to those without olfactory impairment (TMTA = 34.2 s vs. 27.6 s; TMTB = 81.2 s vs. 66.9 s; respectively, $p<0.0001$ for both). Olfactory impairment is associated with lower cognitive function in this middle-aged cohort. Acknowledgements: The project described was supported by R01AG021917 from the National Institute on Aging, National Eye Institute, and National Institute on Deafness and Other Communication Disorders. The content is solely the responsibility of the authors and does not necessarily reflect the official views of the National Institute on Aging or the National Institutes of Health.

394 The Effect of Aging on Human Olfactory Ability

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The hypothesis tested in this study was that even when older subjects are able to identify odorants as well as their younger counterparts, there are nevertheless subtle changes in both their olfactory acuity and memory. Eighteen subjects over the age of 70 and 18 young adults performed pair wise comparisons of 6 odorants using a dissimilarity scale. The same scale was used to evaluate the subjects' memories of these odorants. Both groups also performed butanol and phenethyl alcohol (PEA) threshold tests, as well as a 16 odor discrimination test. Multidimensional scaling analysis revealed subtle, but significant differences in both the memory and actual people spaces between the younger and the older subjects. The butanol thresholds were also significantly different, but the PEA thresholds were not. The results of the discrimination test reveal that the older subjects were significantly worse at discriminating among odors than the younger subjects. Even though all the subjects over the age of 70 were able to identify as many of the odorants as the younger subjects, the results of this study suggest the olfactory acuity of the elderly subjects was not as keen as compared to the acuity of the younger subjects. A further analysis of the pair wise comparison data suggested the perception of garlic was significantly different between the two groups. This observation, taken with the higher butanol threshold, raises the possibility of an alteration of the trigeminal nerve function in the older subjects. Further, the subtle differences in the odor memory suggest the possibility that the decline of olfactory perception in the older subjects is not simply a result of a decline in their cranial nerve functions.

395 Demographic Effects on Olfactory and Gustatory Function in Healthy Chinese

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Aim: To investigate the effect of age and gender on olfactory and gustatory function, and establish test methodology and normative values in Chinese. **Methods:** The T&T olfactometer, the Sniffin' Sticks olfactory test, olfactory event-related potentials (oERPs), trigeminal event-related potentials (tERPs), and the triple drop method for gustatory testing were used to examine chemosensation in 90 healthy adults (45 males and 45 females). **Results:** Older subjects (age 50-65 years old) had poorer olfaction compared to younger subjects (age 18-35 years old) using both T&T (young -1.71±0.41,old -0.92±0.95, ; $P<0.01$) and Sniffin' Sticks (TDI young 33.17±2.83,old 30.89±3.35 $P<0.05$) testing. Meaurements of oERPs revealed that older people (N1 471±85ms,P2 676±93ms) had longer latencies compared with younger subjects (N1 368±57ms,P2 561±74ms, $P<0.05$) of N1/P2 wave.The results of trigeminal nerve related potential examination showed that N1/P2 latencies were longer and amplitudes were lower in older people (N1 384±98ms/-5.01±4.00uv, P2 568±95ms/6.53±3.62uv) compared with younger (N1 316±31ms/-7.20±3.43uv, P2 472±66ms/8.72±3.09uv; $P<0.05$). Gustation was normal in all subjects and there was no significant difference between young and old groups. **Conclusion:** Age and gender affect olfaction as measured by standard testing methods in Chinese adults as found in other human populations. In healthy Chinese subjects, gustation was normal and did not vary with age. Our data provide preliminary normative values for future investigation of chemosensation in the Chinese population. Acknowledgements: Source of support: China Natural Scientific Foundation under project No. 30740052, Beijing Natural Scientific Foundation under project No. 5072018, Capital Medical Development Foundation under project No. 2007-1034.

396 Is there a shift in odor pleasantness with age?

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Is there a shift in odor pleasantness with age and if yes what could explain it? In this study we set out to examine this question using 2 experiments. Experiment 1 compared 50 young adults (age: 18-40) to 89 old adults (age: 60-76). Participants were asked to rate

pleasantness and intensity of 20 odorants. Results revealed that the two groups did not differ in their overall pleasantness and intensity ratings ($p>0.05$ in both cases). In Experiment 2 we hypothesized that additional factor such as edibility of the odorant source may influence odor pleasantness during aging. Thirty young adults (age: 18-40) were compared to 30 old adults (age: 60-75). Participants were asked to give their hedonic ratings of the same 20 odorants and to estimate odor intensity, familiarity, edibility as well as to give a brief verbal description about the odor. Odor threshold and identification scores were also assessed. Results revealed that the correlation between pleasantness and edibility ratings was overall significantly greater in young adults vs. old adults ($p<0.02$). Moreover, whereas odor pleasantness and edibility correlated significantly in 27 out of 30 young subjects, the same correlation was significant in only 19 out of 30 old subjects. A comparison within the group of old adults revealed that those with a non significant pleasantness/edibility correlation also scored lower in odor identification ($p<0.0007$) and used less descriptions referring to food ($p<0.05$). The two groups did not differ in odor threshold ($p>0.05$). Taken together, these results suggest that in some older people, odor hedonic perception may be disconnected from knowledge about the food significance of the stimulus. This link between edibility, olfactory pleasure and identification may be thus crucial in food perception during aging.

397 Aging does not reduce the proliferative capacity nor the distribution of progenitor cells in the VNO

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The olfactory system is unique in that it undergoes plasticity beyond a short developmental period. It is not yet clear how molecular and cellular aging occurs, nor precisely how aging affects function. The olfactory system provides an opportunity to examine the role of environmental versus biological causes of aging because it is composed of both the main and accessory olfactory systems. However, little is known about the regenerative capacity of the vomeronasal epithelium (VNE). While it is presumed that the potential for proliferation exists throughout life, little experimental evidence exists to support this idea. Previously, we established the basal rates of proliferation and apoptosis in the VNE over the lifespan of the mouse. Here, we investigate whether the regenerative capacity of the VNE remains as the animal progresses through advanced life stages. The VNE from mice of varying ages (1-24 months) were reconstructed and analyzed for BrdU distribution across the length of the VNO. While relative BrdU incorporation was found to be highest at the most rostral and caudal extent of the VNO, this distribution did not change with age. In a second experiment, mice of varying ages (1-18 months) were injected with BrdU and allowed to survive 30 days post-injection. VNE were evaluated for BrdU incorporation in conjunction with OMP labeling. Although the number of cells incorporating BrdU declined with age, the percent that were positive for OMP did not. In addition to our previous results that VNE in aged animals respond normally to a lesion challenge, these results suggest that the progenitor cell retains its proliferative capacity throughout life. Other factors influencing progenitor cells in the VNE may cause the decline in neurogenesis seen with age. Acknowledgements: J.H.B. supported by F32 DC008455.

398 Clinical usefulness of Japanese version of University of Pennsylvania Smell Identification Test (UPSIT-J) to Japanese population

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The University of Pennsylvania Smell Identification Test (UPSIT) is the most popular olfactory function test throughout the world. In Japan, however, it is not used very much because of difference of language and unfamiliarity with some odorants used in the test. Recently, a cross-culturally modified UPSIT was developed, whose words are written in Japanese and some odorants unfamiliar to Japanese population are replaced with new odorants that seem to be familiar to them. To determine if the Japanese version of the UPSIT (UPSIT-J) would be effective in the Japanese population, we administered the test to 50 normosmic Japanese subjects and 50 Japanese patients with olfactory dysfunction. They were also administered Japanese standard olfactory threshold test: T&T olfactometry, the Odor Stick Identification test for Japanese (OSIT-J) and intravenous Alinamin test to compare these test results and subjects' opinions. Most subjects reported UPSIT-J was easy, simple and interesting as well as the OSIT-J, more than the T&T olfactometry and Alinamin test. However, some odorants and distractors used in the UPSIT-J were still unfamiliar to the subjects. UPSIT-J test scores were significantly correlated with OSIT-J score, recognition threshold of T&T olfactometry and Alinamin test results. In conclusion, although a cultural bias was still detected for a few odorants, this study demonstrates that the UPSIT-J can be clinically used to assess olfactory function in Japanese population. Acknowledgements: Supported by a grant from the Mie University Medical Research Award for Young Investigators

399 The Odor Naming Power Test: Evaluating the Relationship of Odor Naming Ability and Recognition Memory Performance

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Previous research has shown a strong connection between odor naming and recognition memory. We hypothesize that this relationship is based on odor knowledge. The greater a person's odor knowledge, the more likely that it can be successfully named and remembered. To test this idea, odor recognition memory was compared to naming ability using a novel test- the odor naming power test. Study participants were asked to remember odors presented during Phase I of the experiment. During Phase II, participants were asked to indicate whether odors had been presented during Phase I or was being presented for the first time. During Phase III (the power test), participants were asked to name each odor, choosing from a list of possible odor names. Initially, participants were provided with a list of 32 odor name alternatives (including the correct name). If the correct name was chosen, the next odor was presented. If not, a list of alternatives was reduced by half

and the participant tried to name the odor again. An incorrect response lead to another halving of the alternative list until only two alternatives remained. It was assumed that the ability to choose the odor from a longer list of alternatives indicated greater odor knowledge. As predicted, odors that were correctly named when the list of alternatives was longer were more likely to be remembered. Odors identified at the 32 or 16 alternative level were correctly remembered 83% of the time while odors identified at the 8 alternative level were correctly remembered on 67% of the trials. Odors named at the 4 or 2 alternative levels were associated with only 61% correct memory responses. These results support the hypothesis that odor knowledge underlies the relationship between odor naming and recognition memory.

400 Early Neurocognitive Changes Exhibited by Those at Risk for Alzheimer's Disease

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The P300 cognitive event-related potential (ERP) was elicited with a single stimulus paradigm in an odor identification task. Participants were young (18-25 years old), middle (45-54 years old) age and older (over 65 years old), healthy adults, all free of cognitive impairment as measured by the Dementia Rating Scale. Participants were genotyped for the presence of the ApoE e4 allele, a genetic risk factor for development of Alzheimer's Disease. Fourteen common household odorants were presented via olfactometer, with a 30 second interstimulus interval between each odorant. Each odorant was presented twice per session. Immediately following presentation of the odor the participants pressed one of four buttons corresponding to four written odor options presented on a computer monitor. Olfactory event-related potentials were recorded and then averaged offline utilizing Neuroscan hardware and software. Statistical analyses consisted of ANOVA and repeated measures MANOVA. ApoE e4 positive and negative participants performed equally well in identifying the odorants via button press however, ApoE positive participants in the older group exhibited significantly longer P300 peak latencies, suggesting significantly slower cognitive processing of odor identification. These findings demonstrate that olfactory ERPs to odor identification may discriminate early neurocognitive changes in ApoE e4 positive and ApoE e4 negative individuals even at the earliest pre-clinical stages of Alzheimer's Disease. Supported by NIH Grant DC02064 to Claire Murphy. The authors would like to acknowledge the late Dr. Leon Thal and the ADRC for genotyping (P50AGO5131), Joel Kowalewski, Krystin Corby, Jessica Bartholow, Roberto Zamora, and Richard Vail for their contributions. Acknowledgements: Supported by NIH Grant DC02064 to Claire Murphy.

401 The Role of Odor Identification in Discriminating Depression from Probable Alzheimer's Disease in Older Adults

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Research suggests that odor identification tests may be a useful diagnostic tool for differentiating between depression and probable Alzheimer's Disease (AD) in older adults. Specifically, odor identification is often impaired in AD populations but relatively unimpaired in patients with depression. The objective of the present analysis was to determine the extent that group membership (i.e. depression or AD) could be predicted using the Mini-Mental State Examination (MMSE), Dementia Rating Scale (DRS), and the San Diego Odor Identification Test (SDOIT). A direct discriminant function analysis was performed for 93 individuals, 68 AD patients and 25 patients with depression, all aged 55 and older. One discriminant function was statistically significant, $\chi^2(3)=74.815$, $p<.001$, $\eta^2=0.567$, and maximally differentiated patients with AD (M=-0.686) from patients with depression (M=1.865). Although the structure coefficients showed that all predictors correlated highly with the discriminant function (DRS=0.900, MMSE=0.752, SDOIT=0.541), based on the standardized discriminant function coefficients, the MMSE (=0.257) was not a good, unique predictor of group membership while the SDOIT (=0.399) and DRS (=0.657) contributed most to differentiating between depression and AD. Overall, 90.3% of the patients (100% depressed, 86.8% AD) were correctly classified using the discriminant function, which exceeds the 60.7% (26.9% depressed, 73.1% AD) that would be classified correctly by chance. These findings suggest that the SDOIT, when used in conjunction with the DRS, may be an effective diagnostic tool for differentiating between depression and AD in clinical settings. Acknowledgements: Supported by NIH Grant #R01 AG04085 to C.M. We thank the members of the UCSD Shiley-Marcos Alzheimer's Disease Research Center (ADRC), and the support of NIH Grant #P50 AG05131 to the ADRC.

402 Olfactory dysfunction in patients with Parkinson's disease is related to gray matter atrophy in regions of the olfactory cortex

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Objectives: It is now widely accepted that early non-motor signs indicate pre-clinical stages of Parkinson's disease (PD) prior to the onset of motor symptoms. According to recent neuropathological staging concepts, impaired olfaction is assumed to indicate an early pathological process and might be associated with structural changes in primarily non-motor related brain regions. **Subjects and Methods:** Early PD patients (n=15, median Hoehn and Yahr stage 1.5), moderately advanced PD (n=12, median Hoehn and Yahr stage 2.5) and age-matched healthy controls (n=17) participated in the study. Olfactory function was assessed in all subjects birhinally using the standardized "Sniffin' Sticks" test battery (Burghart, Germany). A morphometric analysis of magnetic resonance images (voxel-based morphometry [VBM]) was used to investigate gray matter atrophy related to psychophysically measured scores of olfactory function. **Results:** In PD patients, but not in controls, cortical atrophy in olfactory-related brain regions correlated specifically with olfactory dysfunction. Positive correlations between olfactory performance and gray matter volume were observed in the right piriform cortex in early PD patients and in the right amygdala in moderately advanced patients. **Discussion:** The results provided first evidence that olfactory dysfunction in PD is related to atrophy in olfactory-eloquent regions

of the limbic and paralimbic cortex. In addition, olfactory-correlated atrophy in these brain regions is consistent with the assumption that olfactory impairment as an early symptom of PD is likely to be associated with extranigral pathology. Acknowledgements: Swiss National Science Foundation (Grant-No. 3100-068282)

403 The course of olfactory deficits in patients with Parkinson's disease - a long term study

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Objectives: Olfactory deficits are common and present in early stages in patients with Idiopathic Parkinson's disease (IPD). These deficits can be verified psychophysically or electrophysiologically using olfactory evoked potentials (OEPs). While olfactory function in IPD patients psychophysically can improve over time, OEPs were not examined in a follow up study. This was the aim of our study. **Methods:** Olfactory function in 19 (14 men, 5 women, mean age: 65 years) of initially 27 IPD patients (5 patients passed away, 3 bedridden) was re-examined psychophysically (Sniffin Sticks, TDI-Score) and electrophysiologically, 5 years after the initial visit. OEPs were recorded for two odors on each side (Phenyl-ethyl-alcohol: 40% v/v, H2S, 6ppm,). OEPs were evaluated regarding existence (yes/no) and latency if existing. Average disease duration now was 8,95 years, average Hoehn & Yahr 2,18. **Results:** Psychophysically, one patient was normosmic, 14 hyposmic (TDI > 15 <30) and 4 functionally anosmic (TDI <16) at the initial visit. Reexamination revealed 1 normosmic, 9 hyposmic and 8 functionally anosmic patients. Mean TDI score decreased by 4,2 points. OEPs were initially existent in twenty percent of the patients. Follow-up showed potentials in a comparable number of patients. **Conclusions:** Overall, mean olfactory function decreased. We confirmed previous findings regarding psychophysical follow-up results. Electrophysiological follow-up showed a pattern of fluctuation in olfactory function comparable to the psychophysical results: like a small percentage of healthy, normosmic people in IPD patients olfactory function does not seem to be mirrored by constantly detectable OEPs. Acknowledgements: This study was supported by a Grant of the Swiss National Fund 3100-068282

404 Functional and morphometric studies of the olfactory system in patients with idiopathic normal pressure hydrocephalus

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Introduction: The most common form of the hydrocephalus in elderly patients is idiopathic normal pressure hydrocephalus (iNPH). Gait disturbance, dementia and incontinence are the leading symptoms in this complex disease. It is difficult to correctly identify those patients, because other disorders are frequently accompanied by similar symptoms. Because of the hypotheses related to possible CSF absorptive pathways through the olfactory pathway, we analyzed the MRI-morphologic characteristics of the olfactory bulb (OB) in patients with iNPH and compared them to healthy controls. Comprehensive assessment of olfactory function was performed with the ''Sniffin' Sticks'' test kit. **Methods:** The prospective study comprised 20 patients with iNPH (7 women and 13 men with average age of 67). Among those 2 had secondary NPH because of aqueduct stenosis. The healthy control group comprised 7 women and 10 men with average age of 61. Cranial MRI was performed before and after operative treatment of the hydrocephalus by insertion of a ventriculo-peritoneal shunt. Functional tests and volumetric measurements of the OB were perfomed before and after surgery. However, due to decreased patients' compliance postoperative MRI could only be performed in 10 patients and olfactory function were performed in only 7 patients. **Results:** Preoperative OB in patients (n=20) with iNPH were significantly smaller that the OB in healthy controls. Postoperatively the OB has shown a growth tendency without statistical significance. Conclusion: The pathophysiological mechanism behind iNPH development is still unclear. In addition to morphologic brain alteration, we describe for the first time the volumetric change of OB. This could be a simple and convenient tool to distinguish between iNPH and other disorders with similar symptoms.

405 Pre-exposure to odour mixture modifies the perceptual quality of the components

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Associative learning between taste and smell has been demonstrated several times. It has also been suggested that an odour can acquire perceptual qualities from another odour with which it has been mixed. Such process has been referred to as odour–odour learning (Stevenson, 2001). Nevertheless there are few experimental evidences of such olfactory learning process. In the present study we investigated the impact of pre-exposure to an odour mixture on the further perception of its constituting odorants. Sixty nine subjects (Ss) participated in the experiment and were dispatched in three groups. Ss included in the first group (control) were not pre-exposed. Ss of the second group were pre-exposed, during 2 sessions spaced by a week, to a mixture of isoamyl acetate (ISA, banana-like odour) and guaiacol (GUA, smoky odour) whereas Ss of the third group were pre-exposed to the components out of mixture. ISA and GUA were chosen since their mixture was previously shown to be processed elementally by Humans. Each subject participated in a 1-hour test session and had to evaluate odour typicality of ISA and GUA among other odours. Within each group, half the Ss evaluated the banana typicality of ISA (GUA respectively) whereas the other half evaluated the smoky typicality of ISA (GUA respectively). The results showed that Ss pre-exposed to the mixture rated ISA as more typical of a smoky odour and GUA as more typical of a banana odour as compared to participants pre-exposed to single components (p=0.03, data pooled); typicality ratings of the control

group falling in-between. These results confirmed that experience can alter odour quality perception: once perceived in mixture odours may share a common outcome while odours perceived independently may conserve, even enhance, their specific perceptual qualities. Acknowledgements: Supported by grants from Burgundy Region and EU-FEDER, by IFR 92 and by French MESR.

406 Is action inherently encoded into odor?

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Several lines of research converge to suggest that pleasantness is a primary perceptual dimension of olfaction. Valence implies action: pleasant stimulus drives approach and an unpleasant stimulus drives withdrawal. Whereas this is possibly true of any sensory stimulus, visual auditory or olfactory, only in olfaction is pleasantness also the primary dimension in which the stimulus is encoded. Thus we suggest that action is inherently encoded into the stimulus. In order to test this hypothesis, four 10 minutes movies were presented, while an odorant or a sound were delivered every 12 seconds (50 repetitions of each stimulus). We used 2 pleasant and unpleasant odorants (perfume and hexanoic acid) and 2 pleasant and unpleasant sounds (guitar and chalk). Subject's EMG (foot) and EEG (C_3, C_4 and C_z electrodes) data was recorded in order to test limb motor related activity. Sniffing, heart rate and skin conductance were also measured. So far, two of the intended 30 subjects participated in the pilot experiment. In contrast to our prediction, preliminary analysis did not indicate odorant-induced preparations for action. Conclusions, however, depend on a larger number of subjects.

407 Determinants of the Pleasantness of Odor Mixtures

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Objective: To investigate if pleasantness of odor mixtures is determined by (linear) combinations of the pleasantness of the components and whether odor pleasantness can be deduced from the chemical nature of the odor. **Methods**: Thirty seven Ss participated in the experiment. Stimuli consisted of 19 *natural* (food) odors and 52 different mixtures of the 171 possible binary mixtures. The (component) odors varied in chemical complexity, consisting of between 40 and 800 different molecules each. Ss sniffed and rated the following properties of each of the 71 stimuli contained in small vials and presented in randomized sequences: pleasantness, intensity, perceived complexity and novelty. Ratings were repeated 2-4 times and were divided over two sessions on separate days. **Results**: Partial least squares analysis demonstrates that pleasantness is inversely correlated with perceived complexity and novelty. Pleasantness of odor mixtures is *not* solely determined by a linear combination of pleasantness of components as indicated by many examples where pleasantness 1)of a mixture falls below pleasantness of either of the components 2)of mixture (A,B) is different from pleasantness of mixture(A,C) even though odors B and C have similar pleasantness 3)of mixtures (A,B), A,C), (A,D) and (A,E) is similar despite very different pleasantness of B, C, D and E. Perceived complexity is *not* correlated with chemical complexity. **Conclusions:** A linear combination of pleasantness of component odors clearly cannot account for our data obtained with natural odors. Furthermore, since perceived complexity and novelty of a particular odor change with exposures to it and since pleasantness is strongly inversely correlated with these quantities, odor pleasantness cannot be deduced from the (unchanging) chemical nature of an odor.

408 Body Odors Modulate Detection Speed of Visual Emotional Stimuli

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Human body odors are able to convey an array of social and biological signals, including information about individual identity. Body odor originating from a family member is known to reduce tension and anxiety, whereas body odor originating from a stranger is thought to increase anxiety. Based on the known connection between body odor and emotion, we explored whether body odors modulate detection speed to friendly and threatening faces. Participants performed a visual search task with schematic friendly and/or threatening faces while smelling either their own (Self) or an unknown individual's (Stranger) body odor. Body odors were presented with a computer-controlled olfactometer to ensure that odor onset was time-locked to presentation of the visual stimulus. Faces were presented in 3 x 3 matrices containing either one friendly or threatening target face against a background of faces expressing the alternate emotion (Target) or only friendly or threatening faces (Same). Participants responded by indicating whether presented faces expressed the same or different emotions. Previous behavioral studies have demonstrated that the emotional content of a target face affects detection speed. Initial analyses demonstrate that participants detect friendly Target matrices significantly faster when exposed to the Self body odor relative to Stranger body odor, based on a subsample of participants. There was no significant difference in perceived pleasantness, intensity, or familiarity between body odor categories. These tentative data support the view that body odors have the capacity to modulate threat detection. The full data, which may elucidate whether this effect is mediated by signal congruency or by biological effects that reduce anxiety and threat detection, will be presented. Acknowledgements: Supported by the NIH (R03DC009869) awarded to JNL and the Swedish Research Council (VR-2008:2426) awarded to JNL and MJO.

409 Influence of Odor Hedonics, Food-relatedness and Motivational State on Human Sniffing

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Sniffing is part of an active exploratory process that, like the oculomotor system, functions to identify and locate the source of biologically salient stimuli or to gather information where the stimulus is novel. Despite increasing recognition of the role that sniffing plays in human olfaction, there are still very few data on those factors that determine the nature of the sniff – its magnitude, duration or frequency. The study reported here was aimed at increasing our

understanding of human sniffing behaviour by examining sniff magnitude and duration to odors that independently varied in both food-relatedness and pleasantness, under conditions of both hunger and satiety. In two sessions, 25 Ss sniffed odors that they had previously identified as varying in pleasantness (unpleasant; neutral; pleasant) and as food-related or not, as well as odor blanks. During one session, Ss were in a state of relative hunger, while in the second session, they had recently eaten. Sniffs were monitored using the *Sniff Magnitude Test* (Frank et al., 2006) which sampled the changes in air pressure produced by the sniff, and calculated the magnitude and time course of pressure changes. Sniffs, whether to odors or odor blanks, were significantly longer, and had higher overall and peak amplitudes when Ss were hungry. Longer sniffs and overall greater amplitude were also elicited by pleasant, than by either neutral or unpleasant, odors. Whether an odor was food-related or not had no impact on any measured sniff parameters. These data reinforce a view that sniffing in humans is, as has been shown in rats, a means of exploring the environment in response to increased motivation to consume.

410 Feeding and Ghrelin Administration Modify Sniff Behavior in Humans

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Sniffing plays a critical role in olfaction, and therefore we expected sniffing to be modulated by changes in variables that affect responses to olfactory input. The current study assessed the effects of food deprivation and ghrelin administration on sniffing behavior in humans. **Methods**: Study 1: 12 lean (4F/8M; age 25.0 ± 6.7 y [mean ± SD]; BMI 22.5 ± 2.2 kg/m^2) and 9 obese subjects (4F/5M; age 29.9 ± 7.6; BMI 34.7 ± 4.5 kg/m^2) sniffed from canisters that contained food and non-food odors after 10 h of fasting, and 30 min and 3 h after drinking 16 oz of water or Ensure on two separate days. Sniff magnitude (the sum of negative pressure generated by sniffing) was measured. Study 2: Synthetic human ghrelin was infused intravenously to 12 lean subjects (4F/8M; age 26 ± 11.4 y; BMI 24.1 ± 4.2 kg/m^2) at doses of 1, 3, and 5 μg/kg/h or saline on 4 occasions. Sniff magnitude (as in Study 1) was measured at baseline and at 45 min after the infusion of ghrelin began. **Results**: Study 1: As expected, sniff magnitude decreased with Ensure feeding in both lean ($p < 0.001$) and obese subjects ($p < 0.05$). The magnitude of this decrease was significantly smaller in the obese as compared to the lean ($p < 0.001$). At 3 h after feeding, the sniff magnitude (adjusted for fasting baseline) was significantly higher in the obese subjects when given water ($p < 0.001$) and lower when given Ensure ($p < 0.05$) as compared to the lean. Study 2: Sniff magnitude increased significantly when subjects were infused with any dose of ghrelin as compared to saline ($p < 0.001$ for all comparisons). Sniff magnitude changes were similar in food and non-food related odors. **Conclusions**: Our findings suggest that nutritional status as well as enhanced hunger signaling may modulate sniff in humans, and can in turn modulate responses to olfactory stimuli.

411 Olfactory sensitivity related to hunger state, BMI and negative mood

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The evidence that olfactory sensitivity is more acute in periods of high compared to low hunger states is rather mixed. More recently, research has demonstrated lower olfactory sensitivity in obese versus non-obese individuals which suggests olfaction might be an important marker in this condition. The study here therefore aimed to see if using a standardized method of olfactory sensitivity, whether individuals' olfactory thresholds are influenced by their state of hunger and further explore the relationship between Body Mass Index (BMI) and olfactory sensitivity. Participants (n=24) attended two separate sessions that differed only in whether they received a standard lunch prior to testing. They then completed olfactory threshold and discrimination tasks and various subjective ratings. On the first day of testing, compared to lunch deprived individuals, those tested following lunch exhibited significantly lower olfactory sensitivity. Additionally, when individuals were deprived of lunch, both BMI and negative (PANAS) mood were inversely related to olfactory sensitivity. These findings suggest that our ability to detect odours can be influenced by a number of factors including hunger state, BMI and mood.

412 Neural correlates of olfactory selective attention

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In the olfactory system, the thalamus lies downstream from cortex, and is therefore unlikely to play a gate-keeping function similar to that of other sensory modalities. Based on our recent work, we set out to clarify the roles played by piriform cortex (PC), mediodorsal thalamus (MD), and orbitofrontal cortex (OFC) in olfactory selective attention. In an event-related fMRI study, subjects were instructed to focus their attention onto a pre-determined target odor, and on each trial to indicate whether or not this target was present within an olfactory stimulus. Odorants consisted of methyl-3-nonenoate (odor A), hexanol (odor B), and cinnamaldehyde (odor C), all matched for intensity, and binary mixtures of [A+B], [B+C], and [A+C], each combined in 50:50 proportions within the vapor-phase. By comparing trials in which the delivered stimulus was identical and only the target smell differed, we were able to dissociate brain regions in which ensemble patterns of fMRI activity reflected either the attentional focus of the subject or the stimulus content of the odorant. Preliminary data from two subjects indicate that attention to a target odor modulated activity patterns in OFC, MD, and frontal PC, but not in temporal PC. For example, the activity patterns evoked by stimuli A and AB were more closely overlapping in attention-driven regions when subjects attended to the same odor (i.e. stim A/attend A and stim AB/attend A), than when subjects attended to different odors (i.e. stim A/attend A and stim AB/attend B). These findings suggest that selective attention refines perceptual codes of odorant-evoked activity across a distributed olfactory network, effectively serving to filter out non-relevant sensory information. Acknowledgements: NIH

413 Differential activation of neural networks in an odor recognition task: an event-related fMRI study

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Memory can be present, but incorrect. The present study aimed to shed light on the neural substrates activated during correct and incorrect responses in an odor recognition memory task in healthy subjects. We examined these different olfactory processes as a function of age by testing young and old subjects. Sixteen young (27 y.o.) and 22 elderly (68 y.o.) subjects were tested. Odors were presented in synchronization with the subject's inspiration according to an event-related fMRI design. Fifty odors were presented during a first run during which subjects performed a detection task, and 100 odors (50 old and 50 new) were presented during a second run, during which subjects performed a recognition task. Performance was coded with hits, misses, correct rejections (CR) and false alarms (FA). fMRI images were analysed using SPM, and ROI analysis (piriform cortex (PC), amygdala, hippocampus (Hip), parahippocampal (PH), perirhinal and entorhinal cortices). Additionally to a common neural network (of which PC), contrasts between conditions showed significantly higher activation in the left Hip and PH gyrus in Young subjects and in the right Hip in Elderly subjects. We then examined the relationhips between the structures differentially activated as a function of the four conditions by performing a canonical variate analysis. The analysis clearly separated the 4 types of responses in Young subjects and showed that areas associated with Hit were the Hip and PH and those associated with FA were the perirhinal cortex, the right PC, the middle frontal, insular, cingulate and occipital gyri and the putamen. In Elderly subjects, the patterns were less clear. Results were discussed in relation to the functional organization of the medial temporal system and the degree of subjective confidence of subjects. Acknowledgements: This study was supported by the Programme National de Recherche en Alimentation et Nutrition Humaine (PNRA) to J.P. Royet. and NIH Grant R01AG085 from the National Institute on Aging to C. Murphy.

414 Odor Coding in the Human Brain: Effect of Expectation

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There is limited evidence to suggest that cognitive context shapes odor quality perception. Freeman and colleagues have proposed that odor expectation induces spatial patterns or "templates" in the olfactory bulb, that set up subsequent perception of the input. To explore putative mechanisms of odor template matching in the human brain, we conducted a multivariate functional magnetic resonance imaging (fMRI) study to investigate whether odor-evoked ensemble activity in primary olfactory (piriform) cortex (PC) depends on which odor subjects expect to smell. Odor expectation was manipulated using semantically congruent or incongruent picture cues that preceded delivery of odor. Behavioral analysis based on 6 subjects (and 6 odorants) demonstrated a robust expectancy effect: odor detection was faster in congruent trials (*e.g.*, mint picture / mint odor) than in incongruent trials (*e.g.*, rose picture / mint odor) (P <0.01). Complementary imaging data revealed greater ensemble fMRI correlations between odors preceded by congruent visual cues, compared to the same odors preceded by incongruent cues (P's <0.01) in both posterior PC and anterior PC, but not in orbitofrontal cortex. Thus, odor-evoked patterns of spatially distributed piriform activity were more alike when an odorant's perceptual quality was expected than unexpected. Moreover, we found that the magnitude of pattern overlap in PC for congruent (vs. incongruent) odor conditions was correlated with response accuracy (r = 0.93, P <0.01) and speed (r = -0.77, trend at P = 0.07). Our findings suggest that the neural instantiation of an odor percept is as much a construct of stimulus expectation as it is of stimulus input. Acknowledgements: This work was supported by NIH/NIDCD Grants 1K08 DC07653 and 1R01 DC010014 (to J.A.G.)

415 Brain activity during lateralized olfactory stimulation and retrieval – an fMRI Study

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Objectives: The ability of humans to spatially localize intranasal applied pure olfactory stimuli is still controversial. In a functional magnetic resonance (fMRI) study, we investigated whether lateralized olfactory stimulation and identification of the side being stimulated involves different brain regions of the olfactory system. Subjects and Methods: Nineteen healthy, normosmic subjects (12 women, age range: 20 to 39 years) participated. All performed a visual orientation test (mosaic test, part of the Hamburger-Wechsler intelligence test) to assess their visuo-spatial abilities. During fMRI, two types of odorants (phenylethyl-alcohol, 40 % v/v, and H_2S, 6 ppm) were delivered either to the left or right side of the nose using an olfactometer (OM4B, Burghart, Germany). After each olfactory stimulus subjects were requested to solely identify the side of stimulation (retrieval) using a visual cue without giving any response. The functional imaging data were analysed using SPM5. Results: Compared to retrieval, olfactory stimulation induces significant higher activity in primary olfactory regions, the piriform cortex and amygdala. In contrast, higher activation of the hippocampus and parahippocampal gyrus was observed when subjects tried to localize the side of stimulation. Activity in this brain region during retrieval correlated positively with the performance of the visual orientation test. Discussion: The pattern of activation shows that retrieval of spatial olfactory information involves secondary regions of the olfactory system. Moreover, subjects with good visuo-spatial abilities rely more on visual cues to retrieve olfactory related information. This result however, does not allow any conclusion regarding correctness of the performed retrieval task.

416 Contextual modulation of odor valence coding

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It is well established that the perception of an odor varies with the context within which it is presented. This contextual modulation

holds true not only for odor identity, but also for odor valence. For example, the same odorant is perceived as more pleasant or unpleasant depending on the associated verbal label (e.g., "vomit" vs. "parmesan cheese"). Using olfactory pattern-based functional imaging and sensory psychophysical approaches, we examined the contextual modulation of valence coding in olfaction by presenting a nominally neutral odor (isobornyl acetate, IBA) in two different contexts, once with a pleasant odor (vanillin) and once with an unpleasant odor (isovaleric acid). Across 12 subjects, valence ratings for the neutral odor varied with context: IBA was rated as significantly more unpleasant ("IBA-") when it was presented with vanillin and as more pleasant ("IBA+") when presented with isovaleric acid ($p<.05$). Multivariate imaging analysis will be presented testing the hypothesis that multi-voxel pattern activity exhibits greater spatial overlap between IBA+ and IBA- in amygdala than in OFC, suggesting a context-*independent* or perceptually invariant coding of odor hedonics in this region. On the other hand, it is expected that multi-voxel correlations in the OFC will show higher correlations between odors IBA+ and vanillin (relatively pleasant odors), and between odors IBA- and isovaleric acid (relatively unpleasant odors), irrespective of odorant identity, indicative of context-*dependent* ensemble coding. These results will help clarify the contributions of amygdala and OFC to odor valence coding in the human brain, providing specific insight into how relative odor pleasantness (or preference) is represented. Acknowledgements: National Institute On Deafness And Other Communication Disorders (NIDCD)

417 Altered processing of olfactory stimuli in women with a history of childhood maltreatment: A functional MRI study

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Background: Aim of this study was to investigate how women with a history of childhood maltreatment (CM) process non-threatening and non-trauma related olfactory stimuli. This approach was motivated by the overlap of brain areas often proposed to be affected in CM patients and the projection areas of the olfactory system. In particular, this includes amygdala, orbitofrontal cortex, insula, and hippocampus. *Methods:* Twelve women with CM and 10 controls participated in the study. All of them were, or had been, patients in a psychosomatic clinic. Participants underwent a fMRI investigation during olfactory stimulation with a relatively neutral (coffee) and a pleasant (peach) odor. *Principal Findings:* Both groups showed normal activation in the olfactory projection areas. However, in the CM-group we found enhanced activation in multiple, mainly neocortical, areas, largely involved in associative networks. These areas included the precentral frontal lobe, inferior and middle frontal structures, posterior parietal lobe, occipital lobe, and the posterior cingulate cortex. *Conclusions:* The results indicate that in this group of patients, CM was associated with an altered processing of olfactory stimuli. This complements other studies on CM insofar, that we found the observed pattern of enhanced activation in associative and emotional regions even following non-traumatic cues.

418 Brain mechanisms controlling the soft palate

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Locked-in syndrome (LIS) is characterized by intact cognition with complete paralysis. LIS is the final stage of several neurodegenerative diseases, and can also follow trauma. We hypothesized that because control of the soft palate is both cranial and distributed, it may be highly spared and conserved in LIS. The position of the soft palate directs respiratory airflow through either the mouth or nose. This allowed us to develop a sniff-dependent device that enabled tasks ranging from text writing to wheelchair driving (see presentations by Sela and Plotkin). Here we set out to use fMRI (3T Siemens, TR=2s, TE=30ms, 36 slices, 4mm, 0 gap) in order ask which brain mechanisms are involved in this control, and whether volitional control (VC) of the soft palate differs from its control during speech (SC). Anterior rhinometry revealed that 7 subjects (6M) out of 10 (8M) screened were able to volitionally control the soft palate. These 7 subjects were scanned using a block-design paradigm containing a VC condition, an SC condition, and an oral-breathing baseline. VC was generated following a cue, and SC was generated by pronunciation of two meaningless words composed of consonants causing the opening and closure of the soft palate. Initial analysis ($p<0.001$) revealed that both VC and SC involved bilateral SMA, bilateral pons, bilateral cerebellum and bilateral inferior frontal gyrus. An extensive part of the parietal and temporal lobes and part of the frontal lobe were active in SC but not in VC, and the right lingual gyrus (BA 18) and bilateral cerebellum were active in VC but not SC. The significant overlap between VC and SC suggests that volitional control of the soft palate may provide an intuitive path for control of devices related to communication.

419 A Brain-Machine Interface Through the Nose: Text Writing

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Locked-in syndrome (LIS) is a condition of quadriplegia and anarthria resulting from damage to descending corticospinal tracts at the pons level, thus sparing the forebrain-based faculties of cognition and emotion. LIS patients cannot speak nor write and they depend on assistive technology and augmentative communication devices (ACD) for interaction with others. We present a novel concept of ACD based on sniffing, that can serve patients with LIS and similar conditions. Sniffs are precisely controlled sensory-motor acts that depend in part on enervation of the soft palate by at least three different cranial nerves, the 5th, 9th, and 10th. In that this enervation is cranial and distributed, we hypothesized that it may be relatively spared following injury. We present the case of a 51 year old female patient with LIS following a ventral pontine infarction. Nasal pressure was monitored with a nasal canula connected to a MEMS pressure sensor followed by a USB data acquisition system. Training software provided bio-feedback in the form of an on-screen "flame" the user had to "put out" by shifting nasal pressure as instructed by on-screen commands. Changes in nasal pressure were then used to generate a code that allowed manipulation of an on-screen courser.

After 21 days of training, the latency to volitional nasal-pressure shift decreased from ~50 to 2.3 (+/-1.14) seconds (p<.001). Following training, the patient could use the device to write text at a rate of 3 (+/-1.5) letters per minute. This allowed the patient her first meaningful post-stroke communication, and she now uses the device regularly. Because of its distributed cranial enervation, soft palate control may provide an avenue to device-control for patients with severe disability resulting from diverse pathologies.

420 Size Matters: Volumetric Relationship between the Olfactory Bulb and Olfactory Brain Areas

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It has recently been demonstrated that olfactory performance is positively correlated with size of the olfactory bulb, the first stage of neuronal processing for olfactory information. Additionally, numerous studies have shown a relationship between behavioral measures and anatomical brain structures. Based on this, we hypothesized that olfactory bulb volume would correlate with density of brain areas associated with olfactory processing. To this end, we obtained volumetric measures of the left and right olfactory bulb using the AMIRA 3D program, as well as grey and white matter density of higher olfactory brain regions, from high-resolution T1-weighted anatomical MRI scans in 98 normosmic individuals (ages 19-79). Preprocessing of the MRI data, segmentation, as well as correlations between grey and white matter density with olfactory bulb volume, were performed using the voxel-based morphometry toolbox (VBM5.1) in SPM5. Age was included as a variable of no interest in all analyses. Total olfactory bulb volume was positively correlated with grey matter density in the postcentral gyrus, the putative sensory representation area of the nose within the primary somatosensory cortex (somatosensory homunculus). In addition, total olfactory bulb volume was positively correlated with white matter density in areas just below primary olfactory cortices (piriform cortex, gyrus rectus). The results of this study imply a relationship between anatomical measures of the olfactory bulb and olfactory-related cerebral areas. This relationship might explain the link between olfactory bulb size and olfactory performance by providing evidence for increased connectivity to central areas (white matter density) and increased somatosensory processing of intranasal stimuli. Acknowledgements: Supported by startup funds from the Monell Chemical Senses Center awarded to JNL and a DAAD postdoctoral fellowship D/08/40252 awarded to JA.

421 Spontaneous Ca^{2+} Oscillations in Olfactory Bulbs of Neonatal Mice

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Pituitary Adenylate Cyclase Activating Peptide (PACAP) is a pleiotropic peptide found at high levels in the olfactory bulb (OB). The physiological effects of PACAP in the OB are unknown. We used functional confocal imaging to test the effects of 100 nM PACAP, 10 uM ATP, and 50 mM potassium on spontaneous calcium oscillations in Fluo-4 loaded slices of OB from neonatal mice. SR101 was used to distinguish astrocytes from neurons. ATP initiated calcium transients in astrocytes. When PACAP was applied, an increased number of neurons in a given area oscillated, but the overall rate of oscillation was not significantly changed. The glutamate and GABA receptor antagonists DNQX (25 uM), AP5 (50 uM), GABAzine (30 uM) and TTX (1 uM) did not affect the calcium oscillations. The PAC1 receptor antagonists (M65 and PACAP6-38) reduced the PACAP-induced increase of oscillating neurons. Furthermore, the cells that oscillated before the application of PACAP showed a reduction when blocked with PACAP antagonists. Thus, the spontaneous oscillation of the OB neurons may be motivated partly by the G-protein coupled PACAP receptors PAC1, VPAC1 or VPAC2. Acknowledgements: NIH NIDCD DC002944 and supplement to MI

422 Purinergic Receptor-Mediated Ca^{2+} Signaling in Cells of the Olfactory Bulb and the Periventricular Zone of the Lateral Telencephalic Ventricles

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Extracellular nucleotides have been shown to be involved in a myriad of important physiological processes, including the regulation of trophic effects on neurons and glial cells. Here we functionally characterized the purinergic system of the anterior telencephalon of larval *Xenopus laevis* using Ca^{2+} imaging and immunohistochemical techniques. Like in other vertebrates, the anterior part of the telencephalon of *Xenopus laevis* mainly consists of the olfactory bulb (OB), the first relay center of the olfactory system. In *Xenopus laevis* the lateral telencephalic ventricles expand deeply into the anterior telencephalon. Therefore, the periventricular zone (PVZ), known to contain proliferating neuronal stem cells, is in close contact with the OB. We found that varying purinergic receptor subtypes are expressed in cells of these brain areas, and that application of purinergic agonists initiates stereotypic $[Ca^{2+}]_i$ signals. While in cells of the OB these responses were completely abolished in absence of extracellular Ca^{2+}, in cells of the PVZ a residual response persisted. Also the order of potency of several purinergic agonists consistently differed in the two cell types. The responses of both cell types were substantially reduced by known purinergic antagonists. Taken together, our findings show that the anterior telencephalon features a complex purinergic system. Thereby our results indicate that cells of the OB exclusively express P2X receptor subtypes, while cells of the PVZ express P1, P2X as well as P2Y subtypes. Regarding the purinergic receptors expressed in the OB we could show that they are not involved in the processing of olfactory information. Possible implications of the purinergic signaling in the context of cell proliferation in the PVZ and cell turnover in the OB are discussed. Acknowledgements: Supported by grants from the Research program, Faculty of Medicine, Georg-August-University Göttingen to I.M., and the DFG CMPB (Project B1/9) to I.M. and D.S.