AChemS

Association for Chemoreception Sciences

ACHEMS XXXVII

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ABSTRACTS

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Hyatt Regency
Coconut Point
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Presentation Abstracts

#1 **GIVAUDAN LECTURE**

Nicotine addiction: molecular basis of behaviors at the complex interface between reward, food intake and taste

Marina R. Picciotto[1], Sven-Eric Jordt[2], Yann S. Mineur[1].
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Nicotinic acetylcholine receptors (nAChRs) are critical for transducing acetylcholine signals in the brain and periphery, but are highjacked by the nicotine in tobacco. Addiction to smoking is driven by multiple effects of nicotine and other constituents in tobacco, and studies in genetically-manipulated mice have been essential for identifying the molecular and cellular mechanisms underlying nicotine reinforcement. While ongoing smoking is driven by the effects of nicotine on brain areas related to reward and addiction to abused drugs, smokers report that they also use tobacco to control appetite. Distinct nAChRs and brain regions are involved in the effects of nicotinic drugs on food intake, raising the possibility that the addictive and appetite-suppressing effects of nicotine can be separated by more selective medications. Finally, other constituents in tobacco, particularly menthol and sweet tastants, likely also contribute to smoking behavior. These constituents interact with receptors involved in taste and temperature sensation, and interactions between taste, reward and appetite combine to influence ongoing tobacco smoking. These studies separate the molecular mechanisms involved in distinct behaviors that contribute to a significant public health problem, and identify potential novel targets for promoting smoking cessation. Funding Acknowledgements: DA14241, DA036151 (FDA TCORS), DA037566.
FCOI Declarations: None.

#2 **ORAL SESSION 1**

Olfactory receptor accessory proteins RTP1 and RTP2 play a crucial role in receptor gene choice, development and odor detection

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Receptor transporting proteins (RTP1 and RTP2), specifically expressed in the olfactory sensory neurons (OSNs), have been shown to greatly increase the cell surface expression of olfactory receptors (ORs) when expressed in heterologous cells. We have generated RTP1 and RTP2 double knockout mice (RTP1,2-/-) to test the function of the RTPs in vivo. RTP1,2-/- mice are viable and show no gross morphological defects. Consistent with the role of RTP1 and RTP2 in OR trafficking, cilia localization of a specific OR is lost in RTP1,2-/- mice. OMP and ACIII expression indicate fewer mature OSNs in the RTP1,2-/- olfactory epithelium which can be explained by a four fold increase in apoptosis of the olfactory epithelium in these mice. Strikingly, expression of ATF5, an indicator of the unfolded protein response and ongoing OR gene choice is vastly expanded in the olfactory epithelium of the knock out mice suggesting that these neurons may be unable to stably express a single OR. This is further reinforced by the expanded expression of LSD1, a histone modifier responsible for the desilencing and initiation of the OR transcriptional machinery. While expression of the vast majority of ORs is diminished in RTP1,2 -/- mice, some ORs are overexpressed in the mutant, suggesting a biased OR choice in the absence of RTPs. We hypothesize that mutant OSNs that choose to express most ORs undergo OR gene switching, while a minor fraction of OSNs that choose to express ORs that are transported to the surface in the absence or RTPs, stabilize OR expression by down regulating ATF5 and LSD1. Acknowledgements: NIH R01: DC005782 and DC012095.
FCOI Declarations: None.

#3 **ORAL SESSION 1**

Active Sampling Motor Centers project to Primary Olfactory Networks Resulting in State Dependent Modulation of Olfactory Function

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Active odor sampling behaviors shape spatiotemporal input patterns and thus also shape processing and perception. However, these behaviors also result in rhythmic reafferent input to primary olfactory networks that must be distinguished from true olfactory input. Like most sensory domains, primary olfactory centers receive input from motor pathways driving active sampling, yet little is known about how the mechanisms that resolve the stimulus temporal structure in primary olfactory networks, integrates input from motor systems to optimize olfactory processing during odor guided behavior. We sought to address this using the moth *Manduca sexta*. Using anatomical techniques characterize two histamine (HA) immunoreactive cells that link flight motor centers with the antennal lobe (AL), where a small population of local and output cells express the HA_B receptors. This circuit is the only source of AL HA and is only developed in adults. Electroantennograms indicated that antennae weakly track odor presented as pulse trains, designed to simulate the physical effects of wing beating. Single cell and multiunit recordings reveal that populations of AL neurons clarify this weakly periodic input resulting in enhanced periodic odor representations as measured by AL power spectral and population analyses. Pharmacological receptor blockade reveals that these enhanced representations are locally mediated by

GABAA and modulated by HA input. These findings are further supported by psychophysical results demonstrating lowered detection and discrimination thresholds to rhythmical stimuli that is also GABA and HA dependent. Overall these results suggest that this simple and elegant motor-to-sensory circuit provides a corollary discharge that modulates AL function to enhance odor representations during odor-guided flight. Acknowledgements: NIH DC009417 to KCD.
FCOI Declarations: None.

#4 **ORAL SESSION 1**

Anxiety-dependent modulation of olfactory fear conditioning: a multidimensional approach

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Recent findings emphasize the impact of anxiety on early stages of stimulus perception, a link that is exclusively based on audio-visual perception. However, due to their direct access to limbic areas, odors represent unique triggers for strong emotional reactions. By means of a multidimensional approach, we examine the impact of anxiety on perception, physiological arousal, and neural activation patterns in an odor-based fear conditioning paradigm. Twenty-one healthy participants were divided in two subgroups on the basis of low (LAV) or high (HAV) trait anxiety vulnerability. Event-related perceptual ratings of odor intensity, skin conductance responses (SCR), and fMRI were co-registered within participants over a 20-min period in which odor-threat associations were repeatedly induced. Perceptual odor intensity increased post-conditioning for both groups, suggesting learning-dependent sensory evaluation processing. Overall, SCR was heightened for the HAV as compared to the LAV group and resulted in significant post-conditioning differential learning (CS+ vs. CS-). Anxiety vulnerability selectively impacted neural processing in amygdala, insula, and cingulate cortex. Multivariate pattern analyses of fMRI activity revealed learning-dependent effects on odor representations within piriform cortex and amygdala post-conditioning. Over time, piriform patterns correlated with perceptual odor intensity, whereas amygdala patterns correlated with learning-dependent SCR amplitude. A stronger association between amygdala patterns and SCR was evident for HAV participants. These results demonstrate that anxiety vulnerability differentially and dynamically modulates perceptual, physiological, and neural responses to emotionally salient perceptual stimuli. Acknowledgements: Supported by the Knut and Alice Wallenberg foundation (KAW 2012.0141) awarded to JNL. MNC is funded by a National Research Service Award from the NIH. FCOI Declarations: None.

#5 **ORAL SESSION 1**

Smell training induces functional plasticity in patients with long-term smell loss

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Olfactory training has recently been proven to offer a very promising therapeutic option for patients with olfactory dysfunction. However, less is known about the neuronal basis of this training program and its impact on functional networks. Therefore, we aimed to investigate the neuroplasticity of chemosensory perception in patients with smell loss induced by an olfactory training. Functional MRI (fMRI) experiments included three different types of chemosensory stimuli: CO2, menthol, and cinnamaldehyde. Ten anosmic patients (7f, 3m) and 14 healthy controls (7f, 7m) underwent the same testing sessions. Following 12-weeks of olfactory training seven patients (4f, 3m) were tested again using the same fMRI protocol. Functional imaging data was analyzed using independent component analysis and functional connectivity analysis. The results of this study revealed that anosmic patients and healthy controls initially use the same three networks to process chemosensory input: the olfactory, the somatosensory, and the integrative network. Even though those networks did not differ in their spatial extent, alterations in their functional connectivity were determined. Assessment of olfactory performance revealed an increase in odor threshold. Furthermore, training-induced modifications of functional connections in anosmic patients were found in particular in the olfactory network. These findings indicate that olfactory training program may reorganize functional networks while the spatial distribution of neural activity is not altered. Thus functional plasticity in olfaction-related neuronal networks may be the key mechanism underlying the success of olfactory training. Acknowledgements: FWF (P23205-B09). FCOI Declarations: None.

#6 ORAL SESSION 1

Neural circuitry underlying expected food odor value in humans

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Nervous systems must encode information about both the specific identity and general value of expected outcomes to guide appropriate behavioral responses. While general value signals have been well-studied, relatively little attention has been paid to the processing of specific reward identity, and the neural mechanisms underlying this critical biological function remain poorly understood. Here we conducted a functional magnetic resonance imaging (fMRI) experiment on 15 hungry human participants, wherein abstract visual symbols served as conditioned stimuli (CS) and appetizing food odors served as unconditioned stimuli (US). By independently manipulating the value and identity of the US, we were able to test the hypothesis that the human brain employs distinct neural substrates to encode general and identity-specific reward value. Using multivoxel searchlight pattern analysis techniques, we discovered predictive value representations of identity-specific reward in central/lateral orbitofrontal cortex (OFC), and identity-general value representations in ventromedial prefrontal cortex (vmPFC). Functional connectivity analyses revealed parallel pathways supporting these two types of value processes, such that OFC was functionally coupled with piriform cortex, and vmPFC was coupled with amygdala. These results challenge widely accepted models of OFC as a general value coding region, and lend credence to more recent findings from non-human organisms demonstrating that OFC encodes a predictive model of environmental and task parameters. Moreover, our connectivity findings highlight a potential organizational principle, such that identity-based sensory features are extracted from sensory-relevant cortical areas, and abstract value is accessed from general affective processing regions. Acknowledgements: NIDCD 1F31DC013500 (J.D.H), NIDCD R01DC010014 (J.A.G), Swiss National Science Foundation PP00P1_128574, PP00P1_150739, and CRSII3_141965 (P.N.T).
FCOI Declarations: None.

#7 ORAL SESSION 1

Busting a myth: humans are not generally less sensitive to odors than nonhuman mammals

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Humans are traditionally considered to have a poorly developed sense of smell that is clearly inferior to that of nonhuman mammals. This view, however, is mainly based on an interpretation of neuroanatomical and recent genetic findings, and not on physiological or behavioral evidence. To assess whether human subjects are indeed generally less sensitive to odors than nonhuman mammals I compiled a complete list of all published olfactory detection threshold values in nonhuman mammals and compared them to human olfactory detection thresholds. More specifically, I compared the lowest mean threshold values reported in human subjects to the lowest individual threshold values reported in a given mammal species. The total number of nonhuman mammal species for which olfactory detection thresholds using operant conditioning procedures have been published is 17, the total number of odorants tested with these species is 138, and the highest number of odorants tested with a given mammal species is 81. I found that human subjects have lower olfactory detection thresholds, that is, a higher sensitivity with the majority of odorants tested so far compared to most of the nonhuman mammal species tested so far. This includes species traditionally considered to have a highly developed sense of smell such as mice, hedgehogs, shrews, pigs and rabbits. Humans outperform rats with 31 of the 41 odorants tested with both species. Humans even outperform the dog, often considered as the undisputed super-nose of the animal kingdom, with 5 of the 15 odorants tested with both species. Based on these comparisons, and contrary to traditional textbook wisdom, humans are not generally inferior in their olfactory sensitivity compared to nonhuman mammals.
Acknowledgements: Supported by Institutional Funds.
FCOI Declarations: None.

#8 ORAL SESSION 2

Alterations in the Fatty Acid Signaling Pathway Affect Dietary Fat Intake

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There is a strong correlation between consumption of a high fat (and calorically dense) diet to an increase in body fat and correspondingly to the development of obesity. Recent research has elucidated the pathway for fatty acid transduction in chemosensory cells and it appears similar to that described for other tastants, including sweet, bitter and umami involving GPCRs, PLCß, IP_3 and TrpM5 (Liu et al., 2011; Shah et al., 2012) . In the current study, we are investigating the role that alterations in the fatty acid signaling pathway have on dietary fat intake. Specifically, we are studying the effects of TrpM5 deletion ($TrpM5^{-/-}$) in mice compared to wild-type mice ($TrpM5^{+/+}$) while on a high-fat diet (60% fat diet for 46 days). To test the fat specificity of the diet-induced changes, separate cohorts of KO and WT mice were fed a high sucrose diet that followed the same parameters. KO male mice took in significantly less calories than their WT counterparts and subsequently gained significantly less body weight while on the high fat diet. Similar, though less dramatic, effects were seen in mice lacking the IP_3 receptor (IP_3R3) or the fatty acid transport protein, CD36, which are implicated in the fatty acid pathway. Interestingly, differences between KO and WT mice were limited to male mice; female

TrpM5$^{-/-}$ mice showed similar caloric intake to WT on a high fat diet. In response to a high sucrose diet, there was no significant difference between KO and WT mice in both the male and female groups when assessing caloric intake and body weight. Our data are consistent with the interpretation that alterations in fatty acid signaling, pre- and/or post-ingestively, lead to specific changes in the intake of dietary fat in male mice and that this effect is gender specific. Acknowledgements: NIH DC013194.
FCOI Declarations: None.

#9 **ORAL SESSION 2**

The Effects of Base Temperature and Taste Context on Perception of Thermal Sweetness

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Heating the tongue tip from a cool temperature (< 30°C) can induce the perception of sweetness (Thermal Taste). Thermal sweetness (TS) had previously been produced only by heating a thermode touched to the tongue tip. The first objective of this study was to determine if TS can be experienced when heating is produced by water, i.e., whether pure H_2O can taste sweet. The second objective was to determine whether TS would be impaired by experiencing actual taste solutions on alternate trials. Twenty-one Ss (11M, 10F) served in 2 testing sessions that began with a TS pre-test block of 3 trials in which dH2O was flowed over the tongue tip as temperature was increased to 37° from 12°, 20°, or 30°C at the rate of 1.0°C/s. When water temperature reached 37°C the Ss were prompted to rate the intensity of sweetness, saltiness, sourness, and bitterness on the gLMS. A series of 15 test trials followed in which base temperature was varied from 12° to 30°C in 5 steps for solutions of pure dH_2O, 0.18M sucrose, and 0.18M NaCl presented in pseudorandom order. The same 3 trials of dH_2O alone given at the beginning of the session were presented at the end of the session as post-test measurements of TS. In the pre-test, 9 of the 21 Ss reported "weak" or stronger TS for the 12° and/or 20°C base temperature conditions. No taste quality other than sweetness was consistently reported. However, the incidence and intensity of TS rapidly decreased during the 15-trial test block and was insignificant at post-test. This finding supports the hypothesis that TS arises from a selective but weak activation of sucrose-best gustatory neurons by heating, and further implies that the encoding of combined thermal and gustatory stimulation is disambiguated if chemical taste stimuli are experienced within the same thermal context. Acknowledgements: NIH grant DC05002.
FCOI Declarations: None.

#10 **ORAL SESSION 2**

The Taste Bud Connectome: First Results From Scanning Blockface EM

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A major question in the field is whether the nerve fibers innervating taste buds show specificity in terms of forming functional contacts with specific subsets of taste cells, or whether nerve fibers are promiscuous in their innervation of taste cells. Of the 3 morphological classes of taste cells, Type II cells transduce either sweet, umami or bitter stimuli, while Type III cells transduce sour. The transducing elements for salty are unclear, and Type I cells serve a glial-like function. If nerve fibers selectively innervate different functional types of taste cells, then nerve fibers innervating Type III cells (sour) should not innervate Type II cells (sweet, umami or bitter) and vice versa. We tested this proposition by reconstructions of serial sections from serial blockface scanning electron microscopy (sbfSEM) sections through circumvallate taste buds of mice. By following individual nerve fibers throughout the taste bud, we can identify all points of specialized contact with any type of taste cell. Specialized contacts with Type III cells have the form of classical synapses with presynaptic vesicles. Specialized contacts with Type II cells exhibit no presynaptic vesicles, but rather display atypical mitochondria closely apposed to the presynaptic membrane. We have analyzed in detail the patterns of connectivity of 12 nerve fibers. Each nerve fiber either forms contacts with Type II cells or with Type III cells, never with both. Therefore our initial results support the hypothesis of connectional specificity at least at the level of cell type as is suggested by the labeled-line hypothesis of taste coding. Acknowledgements: NIH, NIDCD R21DC013186 NIH NIDCD P30DC004657.
FCOI Declarations: None.

#11 **ORAL SESSION 2**

Bidirectional plasticity at basolateral amygdala synapses in primary gustatory cortex

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The basolateral amygdala (BLA) is an important limbic structure that sends a large projection to the primary gustatory cortex (GC). Experimental evidence suggests that different forms of taste learning involve modifications in the connection between BLA and GC. Electrophysiological studies in anesthetized and alert animals have demonstrated that the connectivity between BLA and GC is increased following taste learning, suggesting

that this projection undergoes some form of plasticity. The forms and mechanisms for BLA-GC plasticity, the patterns of activity that may induce plasticity, and the GC neurons involved in BLA-GC plasticity are currently unknown. Here we use whole-cell recordings in GC slices combined with optogenetic stimulation of BLA terminal fields to investigate the capacity for plasticity of the BLA-GC synapse. We began by focusing on the connection from BLA onto GC pyramidal neurons and found that long term potentiation (LTP) and depression (LTD) rely on distinct patterns of BLA activity. Repetitive phasic activation (5ms) of BLA-GC inputs at 20 Hz paired with post-synaptic depolarization led to LTD (% change: -24.2 ± 3.1, n = 10/14). Tonic activation of BLA-GC synapses using ramping light stimuli paired with post-synaptic depolarization induced LTP (% change: 72.0 ± 12.7, n = 13/19). Our results indicate that the amygdalar input onto GC neurons is bidirectionally plastic and that the sign of plasticity depends on the regime of BLA activity. Acknowledgements: Supported by NIDCD grant RO1-DC013770 to A.M. and A.F. FCOI Declarations: None.

#12 **ORAL SESSION 2**

Complex taste responses of neurons in gustatory cortex as revealed by 2-photon imaging

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Previous studies of neurons in gustatory cortex of rodents suggest that their responses are complex and multimodal. In mice, taste neurons appear to map into discrete clusters based on a highly tuned response to one of the primary taste qualities although other studies suggest this may not be true. It is not clear in any of these studies, however, how taste responses may vary according to layer and depth within cortex. We conducted in vivo 2-photon (2P) imaging in C57BL/6J mice, combining neuronal labeling via an AAV-GCaMP6 virus with the ability to image clusters of labeled cells at different depths from the cortical surface. Typical labeled clusters comprised an area of approximately 500^2 μm across the cortical plane spanning several cortical layers. Basic taste stimuli (NaCl, sucrose, and quinine) were delivered to the oral cavity of mice and changes in calcium fluorescence were measured from multiple cells simultaneously. Overall, we find taste responses from a majority (>80%) of labeled cells within each cluster of varying magnitudes and temporal structure. Moreover, the majority of taste-activated neurons within a cluster displayed responses to more than one stimulus with no apparent spatial organization. In several cases, we verified cluster location within gustatory cortex via anterograde tracing from gustatory thalamus (VPMpc). Current work is underway examining the selectivity of individual cells according to cortical depth and cell type. Acknowledgements: R01 DC000353. FCOI Declarations: None.

#13 **ORAL SESSION 2**

Epigenetics of the human TAS2R38 gene

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We investigated whether there are epigenetic modifications to the bitter receptor gene *TAS2R38* and whether these modifications have functional significance. To do this, we obtained genomic DNA from saliva and taste tissue of 18 human subjects. In addition, *TAS2R38* mRNA was quantified from taste tissue and its abundance expressed relative to a housekeeping gene. To identify the methylation patterns within the *TAS2R38* gene body, genomic DNA was treated with bisulfite, amplified by the polymerase chain reaction, and amplicons sequenced using next generation methods. The degree of methylation at each base pair location was compared with *TAS2R38* mRNA abundance. The methylation of one particular base (chr7:141672605, GRCh37) was inversely related to *TAS2R38* mRNA expression in taste tissue (r^2=-0.35); the opposite relationship was observed in saliva (r^2=+0.27). This differentially methylated base is one base pair downstream (5') of a functional single nucleotide polymorphism (rs10246939; I296V). Our previous work has demonstrated that mRNA abundance is related to ratings of bitterness intensity for a ligand of the T2R38 receptor. Thus, epigenetic modifications to genomic DNA may in part determine individual differences in bitter taste perception. Funding Acknowledgment: NIH DC011287; FCOI Declaration: None.

#13.5 **INDUSTRY WORKSHOP**

Application of Chemosensory Science to Industry Needs: Sugar Replacement, Salt Reduction and Aromatherapy

Christopher T. Simons, Department of Food Science and Technology, The Ohio State University, Columbus, OH, United States

Evolving health and wellness needs are driving innovation within the consumer packaged goods industry with specific interest in sugar replacement, salt reduction and aromatherapy. The opportunity to convene experts from industry and academia makes the AChemS Annual Meeting the perfect forum to apply knowledge and findings from the chemosensory community to a broader discussion of these issues. As such, this year's Workshop will entail three sequential facilitated roundtable discussions (approximately 40-min each) in which panels of experts from industry and academia have been assembled to discuss the needs, issues and potential solutions relevant to each of these areas and entertain larger questions from the audience. Participants include:

Sugar Replacement: John Hayes (Penn State University, Facilitator), Grant DuBois (Sweetness Technologies, LLC), Rick Mattes (Purdue University), Steve Munger (University of Florida) and Jay Slack (Givaudan Flavors Corp).

Salt Reduction: Chris Simons (The Ohio State University, Facilitator), Steve Gravina (PepsiCo), Jane Leland (Kraft Foods), Stuart McCaughey (IUSM-Muncie at Ball State University) and Paul Wise (Monell Chemical Senses Institute).

Aromatherapy: Rachel Herz (Brown University, Facilitator), Pam Dalton (Monell Chemical Senses Institute), Bryan Raudenbush (Wheeling Jesuit University), Monique Smeets (Unilever) and Stephen Warrenburg (International Flavors and Fragrances). Funding Acknowledgements: None. FCOI Declarations: None.

#14 CLINICAL LECTURE

The importance of the chemical senses during early life

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Many chronic illnesses that plague modern society derive in large part from poor food habits, which often are established in childhood. As guidelines shift toward children's dietary patterns, chemosensory research is examining how to account for poor choices and the difficulty in changing from bad to good food habits. At least two factors conspire to predispose some children toward obesogenic diets: (a) inborn, evolutionarily driven flavor preferences and (b) lack of exposure to flavors of healthful foods early in life. Basic chemosensory research has revealed that children naturally prefer higher levels of sweet and salty and reject lower levels of bitter than do adults. Thus, their basic biology does not predispose them to favor recommended low-sugar, low-sodium, vegetable-rich diets and makes them especially vulnerable to our current food environment high in salt and refined sugars. Nor does it predispose them to readily accept liquid medicines: "taste" is often cited as a primary issue for noncompliance. However, research also shows that sensory experiences, beginning early in life, can shape preferences toward healthful foods. We need more chemosensory research on how to change dietary habits (lowering sugar and salt and increasing vegetable intake) early in life, enhance medication compliance by making medicines taste better, and develop evidence-based practices aimed at infant feeding difficulties, which constitute a medically and economically important complication for some neonatal diseases. Knowledge gleaned from research and clinical practice, which takes into account the changing sensory world of the child, could help promote healthy eating and adherence to medication regimens, which may have a significant impact on many pediatric illnesses and chronic diseases associated with taste.FUNDING ACKNOWLEDGMENTS: Research was supported by NIH Grants DC01128, HD37119 and HD072307. FCOI Declarations: None.

#15 SYMPOSIUM: NON CALORIC SWEETENERS AND THEIR NOT-SO-SWEET METABOLIC EFFECTS

Non caloric sweeteners and their not-so-sweet metabolic effects

M. Yanina Pepino
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Nonnutritive sweeteners (NNS) have existed since the end of the 19th century, when saccharin was serendipitously discovered. Until recently, the general belief was that NNS could promote diet healthfulness, by delivering a pleasant sweet taste without calories or glycemic effects. However, recent data suggest that NNS are not inert but have metabolic effects. The goal of this symposium is to present a comprehensive and integrative understanding on the potential effects of NNS on metabolic health. This symposium will integrate approaches that are both cross-disciplinary (from behavioral neuroscience, to metabolism, to sensory perception) and multiple model systems (from rodents to people). By providing a critical review of the literature linking use of NNS with metabolic disturbances, and closely examining findings from animal models and clinical studies on the effects of NNS on metabolism and taste perception, we hope this symposium will shed new light, motivate new research, and increase awareness on potential effects of these commonly used food additives. Acknowledgements: Non applicable. FCOI Declarations: None.

#16 SYMPOSIUM: NON CALORIC SWEETENERS AND THEIR NOT-SO-SWEET METABOLIC EFFECTS

Not so sweet revenge: Unanticipated consequences of high-intensity sweeteners

Susan E. Swithers
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One solution that has been proposed to combat the ongoing obesity epidemic has been to replace caloric sugars with artificial sweeteners that provide sweet tastes without providing the associated calories. While such an idea seems to be common sense, scientific data supporting artificial sweeteners as beneficial for weight loss are weak. Further, more recent epidemiological data from long-term studies in a variety of human cohorts have indicated that daily consumption of artificial sweeteners may exacerbate metabolic disturbances like type 2 diabetes, metabolic syndrome and stroke. One explanation for such a counterintuitive result is that consuming sweet tastes without typical post-ingestive outcomes could interfere with basic learning processes that normally operate to regulate energy balance. Using data from an animal model, work from our lab has explored how interfering with predictive relations between tastes and calories may contribute to negative health outcomes. The results suggest that obesity and its attendant co-morbidities are unlikely to be helped by consuming "diet" foods manufactured with sugar substitutes. Acknowledgements: Supported by Purdue University and NIDDK R01 076078. FCOI Declarations: None.

#17 SYMPOSIUM: NON CALORIC SWEETENERS AND THEIR NOT-SO-SWEET METABOLIC EFFECTS

Separate brain systems mediate the hedonic and metabolic actions of sugar

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Unlike artificial sweeteners, sugar exerts its potent reinforcing actions via both gustatory and physiological pathways. I will discuss evidence in favor of the notion that separate brain systems mediate the hedonic vs. metabolic effects of sugar on ingestive behavior. Specifically, the role of the basal ganglia in sugar reinforcement will be explored, with emphasis on the idea that while artificial sweeteners activate the ventral striato-pallidal system, a distributed pattern of activation involving both the ventral and dorsal systems arises during sugar ingestion. I will discuss the implications of these findings for the hypothesis that sugars and artificial sweeteners differently shape our behavioral repertoire. Funding Acknowledgements: NIH DC011287.
FCOI Declarations: None.

#18 SYMPOSIUM: NON CALORIC SWEETENERS AND THEIR NOT-SO-SWEET METABOLIC EFFECTS

Sucralose Consumption Decreases Sweet Taste Sensitivity

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Accumulating evidence links artificial sweetener (AFS) consumption to body weight and obesity-related co-morbidities. However, the physiological mechanism by which AFSs influence feeding and weight gain remains unknown. One possible explanation is that repeated high-affinity binding of AFSs to sweet taste receptors (TRs) leads to down-regulation of TR expression, ultimately resulting in decreased signaling from sensory afferents and diminished taste intensity perception. To determine whether repeated exposure to AFSs decreases perceived intensity of sweet tastants in humans, participants (N = 32) were randomly assigned to a sucrose- or Splenda®-exposure condition. Sweet taste intensity perception was tested during 6 consecutive behavioral sessions in which subjects rated the perceived intensity, sweetness and sourness of 4 test solutions (sucrose, sucralose, citric acid, sucralose/citric acid mixture). After completing perceptual ratings, participants consumed a 355mL beverage sweetened with either Splenda® (2g) or iso-intense sucrose (16g). The perceived sweetness of the sweetened stimuli decreased significantly across the 6 days in the Splenda®- but not the sucrose-exposed group. These changes were perceptually meaningful, with ratings of the mixture decreasing 3-fold from "moderate" to "weak". Sourness ratings remained stable for both groups. These findings demonstrate that repeated exposure to Splenda® decreases sweet taste sensitivity. We speculate that this results through peripheral actions on sweet TRs. Acknowledgements: NIDCD Grant R01 DC006706, Kavli Graduate Fellowship in Neuroscience.
FCOI Declarations: None.

#19 SYMPOSIUM: NON CALORIC SWEETENERS AND THEIR NOT-SO-SWEET METABOLIC EFFECTS

Metabolic effects of sucralose in subjects with obesity

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It is generally believed that non-nutritive sweeteners (NNS) promote diet healthfulness, by delivering a pleasant sweet taste without calories or glycemic effects. However, data from several epidemiological studies have found that consumption of NNS, mainly in diet sodas, is associated with an increased risk to develop type 2 diabetes, and metabolic syndrome. In this presentation, I will briefly review recent work on potential mechanisms underlying this paradoxical association, and present findings from our laboratory in which we evaluated the acute effects of sucralose ingestion on the metabolic response to an oral glucose load in subjects with obesity. I will discuss and contrast our findings, that sucralose is metabolically active in people with obesity, with findings from other studies that also aimed to examine the effects of NNS on glucose homeostasis in people. Finally, I will present data on a positive association between individual sensitivity to detect sucralose taste and effects of sucralose on glycemic responses, which suggest the study of taste perception can provide novel insights into chemical sensing mechanisms in the gut that regulate metabolic function. Acknowledgements: This project was supported in part by the National Institutes of Health (NIH) Clinical and Translational Sciences Award UL1 TR000448, sub award KL2 TR00045 (to MYP), and by NIH grants P30 DK05634 (NIH DK 56351) (Nutrition Obesity Research Center) (to S. Klein (PI)
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#20 SYMPOSIUM: SIGNAL TRANSFORMATION AND ROUTING IN THE OLFACTORY SYSTEM

Signal Transformation and Routing in the Olfactory System

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The olfactory system is key to chemical communication throughout the animal kingdom. However, coding of the environmental chemical 'space' by different signal transformation and routing mechanisms in the olfactory system is, to some extent, still poorly understood. In different model systems, recent studies have elucidated some unexpected principles of odor signal transformation in the brain. Using a

variety of experimental approaches, the research presented in this symposium will both highlight some of these exciting novel findings and provide new insight from unpublished research on-going in the speakers' laboratories. The symposium will focus on rodent models, but also include studies in Drosophila, illustrating both in and ex vivo approaches from different experimental angles. Thus, the symposium aims to promote a lively debate on several novel aspects of olfactory neurobiology.
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FCOI Declarations: None.

#21 SYMPOSIUM: SIGNAL TRANSFORMATION AND ROUTING IN THE OLFACTORY SYSTEM

trans-Tango: Trans-synaptic Mapping and Manipulation of Neural Circuits

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Most behavioral responses to odors require experience and learning before an odor stimulus acquires its value for the organism. However, some odors, including pheromones and environmental chemicals with special importance for the organism's survival, elicit innate and stereotyped behavioral responses. These innate behaviors are governed by genetically programmed, hard-wired neural circuits. Due to lack of adequate tools for trans-synaptic labeling of neurons within a circuit, little is known about the circuit level of the brain, including hard-wired circuits. We have combined molecular biology and genetics to develop a new technique for circuit mapping and manipulation in fruit flies. At the core of our system is a synthetic signaling pathway that is introduced into all neurons. Selective activation of this pathway within a particular circuit is used to trace projections within this circuit or to alter its function. To this end, we genetically modify pre-synaptic neurons, for which a genetic marker is known, to express in their synapses the ligand that activates the signaling pathway in their post-synaptic partners. Since our system is modular, its use can be readily expanded to multiple neural circuits in the fly. Furthermore, it can be easily adapted to experiments in which the properties of specific circuits are modified and the functional consequences are analyzed. We are using this system to trace circuits that mediate olfactory-governed innate aversion and attraction in flies. We are also currently using this proof of concept in flies to establish an equivalent technique for labeling circuits in mice.
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#22 SYMPOSIUM: SIGNAL TRANSFORMATION AND ROUTING IN THE OLFACTORY SYSTEM

Neural Identity and Odor Coding in Piriform Cortex

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The piriform cortex is a simple, three-layered cortical structure, which receives direct sensory inputs from the olfactory bulb and is thought to play key roles in odor perception and olfactory-driven behaviors. We have used molecular genetic and in vivo imaging approaches in mice to determine the organization and function of piriform neural circuits. We have performed micro-dissection and RNA deep sequencing to identify gene expression patterns that define the neural subtype and layer-specific identities of piriform neurons. Anterograde and retrograde neural tracing experiments reveal that genetic identity and layer-specific positioning of piriform neurons specify their connectivity with distinct piriform target areas. Moreover, we have used *in vivo* two-photon imaging to characterize odor-evoked neural activity in piriform cortex. We found that odors activate sparse, dispersed patterns of piriform neurons, which exhibit considerable trial-to-trial variability. We have quantified the similarities of piriform odor responses across multiple different odorants and odorant concentrations, and we have found that despite this variability, odor-evoked response patterns can correctly be classified according to stimulus quality. Interestingly, and in contrast to the olfactory bulb, odor-evoked activity in piriform does not scale with odorant concentration, and correct classification of stimulus quality could be achieved across a wide concentration range. We will discuss preliminary results that provide insights into the neural circuit mechanisms underlying the transformation of scaled olfactory bulb inputs into concentration-invariant odor representations in piriform cortex.
Acknowledgements: Fondation pour la Recherche Médicale (FRM), Marie Curie International Reintegration Grant, PSL* (Paris Science and Letters) Research grant.
FCOI Declarations: None.

#23 SYMPOSIUM: SIGNAL TRANSFORMATION AND ROUTING IN THE OLFACTORY SYSTEM

Entrained Oscillatory Discharge in an Accessory Olfactory Bulb Microcircuit

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In the accessory olfactory bulb (AOB), mitral cells (MCs), the main AOB projection neurons, receive sensory input from peripheral vomeronasal neurons and relay this information to the vomeronasal amygdala and the hypothalamus. A subpopulation of MCs exhibits slow oscillatory discharge that persists upon pharmacological inhibition of fast synaptic transmission. Here, we identify an excitatory circuit within the AOB network that entrains oscillatory activity in a second MC subpopulation. Using a battery of physiological techniques in acute AOB tissue slices, we

investigate the physiological mechanisms underlying entrained oscillatory discharge. Entrained MCs display periodically increased excitatory synaptic input that correlates with rhythmic discharge patterns. Block of fast glutamatergic synaptic transmission reveals that (a) neural entrainment depends on an intact glutamatergic network, and (b) excitation reinforces burst intensity and increases precision in intrinsically oscillating MCs. Ongoing experiments aim to unravel the network mechanisms underlying MC entrainment and the role of slow rhythmic burst activity in AOB sensory information processing. Acknowledgements: This work was supported by the Deutsche Forschungsgemeinschaft SPP 1392: "Integrative Analysis of Olfaction" and the Volkswagen Foundation (83533). FCOI Declarations: None.

#24 SYMPOSIUM: SIGNAL TRANSFORMATION AND ROUTING IN THE OLFACTORY SYSTEM

Inhibition and Olfaction

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Inhibitory circuits are a hallmark of computation in the brain. In the olfactory system, we have recently shown that inhibition in the mouse olfactory bulb (OB) is involved in odour fine discrimination behaviour. Optogenetic silencing experiments in anaesthetized and passive awake mice have suggested that distinct interneuron circuits fulfil specific roles in the OB, namely that superficial interneurons shape slow temporal features while deep interneurons, most notably including granule cells (GCs), orchestrate activity across the OB on faster timescales. Surprisingly, silencing GCs in the anaesthetized preparation had essentially no impact on baseline activity of projection neurons both in anaesthetized and awake animals. Moreover, GC themselves were virtually silent (largely less than 1 Hz baseline firing) in both anaesthetized and passive awake mice. Here I will discuss these results and compare the contribution of inhibitory circuits in anaesthetized, passive awake and behaving mice using cell-specific optogenetic silencing, imaging and whole-cell recordings in head-fixed preparations. Acknowledgements: MRC MC_UP_1202/5 BIF. FCOI Declarations: None.

#25 SYMPOSIUM: ADAPTIVE EVOLUTION OF INSECT OLFACTORY SYSTEMS

Adaptive Evolution of Insect Olfactory Systems

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The ability to respond to chemical signals from the environment is essential for the survival and procreation of most organisms. Olfactory behavior represents an important substrate for adaptive evolution, as evident from the rapid evolution and diversification of chemoreceptor families. Evolution acts on genetic variation within a population. Thus, an assessment of the genetic underpinnings that give rise to natural variation in olfactory perception is critical for understanding the relationship between evolution of olfactory systems and environmental adaptation. Insect systems are ideal for formulating fundamental concepts of evolutionary adaptation of olfactory behavior, because they are highly amenable to integrative interdisciplinary strategies that combine ecological studies, genetic analyses, behavioral assays, and neuroanatomical approaches. This symposium will showcase studies to explore natural variation, evolution and adaptation in insect olfactory systems. Speakers will present genetics of variation and adaptation of Drosophila to host plants, the ecological significance of discrete olfactory pathways in flies, adaptive evolution of gustatory responses in cockroaches, and genetic studies on moths that give insights into the coevolution of diversification of pheromone blends and concomitant adaptations of partners responding to these blends, including the identification of genes that mediate such adaptation. Acknowledgements: Research in the laboratory of the symposium chair is supported by NIH grant R01-GM059469. Participation in the symposium by Dr. Katsumata is made possible through support from the W. M. Keck Center for Behavioral Biology at NC State University.
FCOI Declarations: None.

#26 SYMPOSIUM: ADAPTIVE EVOLUTION OF INSECT OLFACTORY SYSTEMS

The paradox of evolutionary diversification in sexual signaling

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Most night-flying moth species locate mates through production of, and response to, a very precise blend of volatile chemical compounds. Within a population, females with atypical blends are less attractive to males than females with the common blend. Similarly, rare males that respond to atypical blends are at a disadvantage in finding mates. This type of sexual communication system is expected to be evolutionarily constrained by stabilizing selection. Therefore, it is difficult to account for the great diversification of pheromone blends used by over 100,000 moth species. Genetic, physiological, and ecological studies will be discussed that give insights into the coevolution of diversification of female pheromone blends and concomitant adaptations of male partners responding to these blends, including the identification of genes that mediate such adaptation. Funding Acknowledgement: NSF DEB-1025217; FCOI Declaration: None

#27 SYMPOSIUM: ADAPTIVE EVOLUTION OF INSECT OLFACTORY SYSTEMS

Linking Genotype to Phenotype: Olfactory Behavior in Drosophila mojavensis

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Chemoreception is a principle sensory modality by which many organisms gain information from, and survive in, their environment. Variation in responses to the chemical environment, such as the olfactory preferences of insects for their host plants, may result in differential reproductive fitness, and shifts in preference may lead to divergence in host-adaptive traits, and ultimately result in reproductive isolation among populations. Here we examine differences in the genetic and neuronal underpinnings of the olfactory system between populations of the cactophilic fly, Drosophila mojavensis. This fly feeds and breeds on cacti in arid regions of Baja and the deserts of Mexico and the southwestern U.S.A. Four geographically distinct populations exploit four different cactus species that emit specific combinations of volatiles that serve as primary cues for host plant identification. Data show divergence between the populations in olfactory electrophysiology, behavioral preferences to cactus volatiles, and in the chemosensory transcriptome. These results suggest that the peripheral nervous system has changed in response to different ecological/chemical environments and that these changes contribute to population divergence. Acknowledgements: NIH GM080592 University of Cincinnati.
FCOI Declarations: None.

#28 SYMPOSIUM: ADAPTIVE EVOLUTION OF INSECT OLFACTORY SYSTEMS

A bitter-sweet adaptive change in cockroach taste

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In response to the anthropogenic assault of toxic baits, populations of the German cockroach have rapidly evolved a novel adaptive behavior a behavioral aversion of glucose, a phagostimulant component of baits that lets cockroaches avoid the bait. To understand the mechanisms of glucose aversion, we compared the electrophysiological responses of gustatory receptor neurons (GRNs) of the mouthparts to glucose, fructose and caffeine between wild-type and glucose-averse cockroaches. In both strains, the phagostimulant fructose stimulated a sugar-GRN, whereas caffeine, a bitter deterrent compound, stimulated a bitter-GRN. Glucose, like fructose, also simulated the sugar-GRN in wild-type cockroaches, but in glucose-averse cockroaches it stimulated both sugar- and bitter-GRNs. The results suggested that an acquisition of sensitivity for glucose in bitter-GRNs is responsible for glucose-aversion behavior. Moreover, we hypothesized that the native glucose-GRs, which should be expressed only on sugar-GRNs, are also misexpressed on the bitter-GRN in glucose-averse cockroaches, and carried out chemical structure-GRN activity experiments with glucose and three related compounds. The results indicated that the glucose-GRs of the bitter-GRN in glucose-averse cockroaches recognize glucose molecules differently from the native glucose-GRs of the sugar-GRN. We suggest that in glucose-averse cockroaches the expression of a broadly tuned receptor or multiple narrowly tuned receptors may contribute to the broad acceptance of glucose and related compounds by the bitter-GRN, driving aversive behavior. Acknowledgements: NSF (IOS-1052238) and HUD (NCHHU0001-11) awards to C.S. and by the Blanton J. Whitmire Endowment at NCSU.
FCOI Declarations: None.

#29 SYMPOSIUM: ADAPTIVE EVOLUTION OF INSECT OLFACTORY SYSTEMS

Drosophila Olfactory Neuroecology

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In the past 15 years, the fundamental molecular and neuronal logic of peripheral olfactory coding in the vinegar fly, *Drosophila melanogaster* has been largely deciphered. The next major challenge is now to identify how the olfactory system fits the ecological needs of the organism. I will here outline recent work from our lab aiming at unraveling the ecological significance of discrete olfactory pathways in the fly. Acknowledgements: The presented work is supported by the Crafoord Foundation and the Swedish Research Council.
FCOI Declarations: None.

#30 SYMPOSIUM: COGNITIVE INFLUENCES ON SMELL AND TASTE: MECHANISMS IN MICE AND MEN

Cognitive Influences on Smell and Taste: Mechanisms in Mice and Men

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The influence of cognitive, "top-down" factors on chemosensation has been appreciated since the nineteenth century, but the neurophysiological mechanisms of these effects are only beginning to be understood. This symposium will present recent developments in both taste and smell research exploring how the neural response to a stimulus and the perception of that stimulus can be influenced by the organism's expectations and emotional state (e.g. anxiety, surprise, etc). These talks will begin to integrate our current understanding of these phenomena across experimental approaches from human

psychophysics to fMRI to rodent neurophysiology. Because cognitive factors may be particularly important in taste and smell (in comparison to vision or audition), this session will discuss them as a shared challenge for all chemosensory researchers. John McGann will briefly introduce the problem of cognitive influences on chemosensation, including traditional psychological perspectives (e.g. attention, learning of associations among stimuli) and modern theoretical frameworks (e.g. Bayesian probabilistic networks, mutual information). This leads to the main question of the symposium: How does the brain actually implement these cognitive effects on chemosensation? This question will be addressed in four talks: 1) Dana Small on how expectations can influence taste perception and gustatory cortical activity in human subjects, 2) Wen Li on how emotion, anxiety, and threat perception can influence olfactory perception and brain physiology in human subjects, 3) Anan Moran on changes in the neural representations of tastants in the rat gustatory cortex as the behavioral significance of the tastant changes, and 4) John McGann on how expectation can influence neurophysiological responses to odors in the mouse olfactory bulb. Acknowledgements: Support indicated for each talk individually.

#31 SYMPOSIUM: COGNITIVE INFLUENCES ON SMELL AND TASTE: MECHANISMS IN MICE AND MEN

Acute and prolonged top-down modulation of taste

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Perceptual experiences result when physiochemical stimuli are transduced into nerve impulses and then elaborated and integrated in the central nervous system to inform ongoing behavior. Central processing is then sculpted by a number of "top-down" mechanisms to optimize behavior. In prior work we have shown that the canonical attention network upregulates insular response during directed attention to taste and breaches of taste expectation presumably to support orientation, identification, and learning about salient stimuli. More recently, we have uncovered evidence that this same network supports more prolonged benefits to taste sensitivity. Specifically, the rated intensity of taste stimuli significantly increase following ~15 minutes of performance of a difficult flavor, but not extra-oral, discrimination task requiring sustained directed attention. This effect is replicated across four days, but the change in sensitivity is transient, with intensity ratings equivalent to the day 1 baseline at the start of each day. We also found that the effect is equal for all the taste stimuli, supporting a central, rather than a peripheral mechanism. A subsequent fMRI study confirmed this hypothesis showing enhanced insular responses to taste following the discrimination task (e.g. post – pre response). Connectivity analyses then identified a pathway of information flow from the canonical attention network (intraparietal sulcus and frontal eye fields) to the amygdala and finally insular cortex. This suggests that, in addition to acute modulation, the canonical attention network may produce prolonged effects on gustatory cortex via an amygdala-mediated mechanism. Acknowledgements: Supported by NIH 2R01DC6706. FCOI Declarations: None.

#32 SYMPOSIUM: COGNITIVE INFLUENCES ON SMELL AND TASTE: MECHANISMS IN MICE AND MEN

Emotional influences on olfaction— Anxiety-state-dependent olfactory processing and neural circuitry adaptation

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Phylogenetically the most ancient sense, olfaction ties closely with primitive needs related to homeostasis and emotion, and changes in these internal states can greatly impact olfactory processing and experience. However, mechanisms underlying such state-dependent olfactory processes remain unclear. Perturbing the internal state with anxiety induction in human subjects, we interrogated emotion-state-dependent olfactory processing in a functional magnetic resonance imaging (fMRI) study. Following anxiety induction, initially neutral odors became unpleasant and took longer to detect, accompanied by augmented response to these odors in the olfactory (anterior piriform and orbitofrontal) cortices and emotion-relevant pregenual anterior cingulate cortex. In parallel, the olfactory sensory relay adapted with increased anxiety, incorporating amygdala as an integral step via strengthened (afferent or efferent) connections between amygdala and all levels of the olfactory cortical hierarchy. This anxiety-state-dependent neural circuitry thus enabled infusion of limbic affective information throughout the olfactory sensory progression, driving affectively charged olfactory perception. These findings could constitute an olfactory etiology model of emotional disorders, as exaggerated emotion-olfaction interaction in negative mood states turns innocuous odors aversive, fueling anxiety and depression with rising ambient sensory stress. Moreover, this circuitry adaptation raises the intriguing possibility of an additional mechanism of olfactory perceptual learning via aversive conditioning as (almost invariably) induced anxious/negative states enhance amygdala discharges to the olfactory cortex, promoting its plastic changes and consequent perceptual improvement. Acknowledgements: This work is supported by R01MH093413 (W.L.).
FCOI Declarations: None.

#33 SYMPOSIUM: COGNITIVE INFLUENCES ON SMELL AND TASTE: MECHANISMS IN MICE AND MEN

Gustatory cortex neuronal ensemble response dynamics during learning and extinction

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Gustatory cortical (GC) neurons encode information relevant to behavior: information that should change as that behavior

changes with learning, for example when a palatable taste becomes aversive and elicits rejection behavior rather than consumption. The observed relation between the mutual changes in the neural responses and behavior, although theorized to be of a causation nature, might be misleading, and cannot be validated in simple learning studies which may simply show neuronal response changes with experience. Neural activity that truly tracks behavior (as opposed to simply changing with experience) will not only change with learning but also change back when that learning is extinguished. To probe for this pattern we recorded the activity of ensembles of GC single neurons as rats that normally consumed sucrose avidly were trained first to reject it (i.e., conditioned taste aversion [CTA] learning) and then to enjoy it again (i.e., extinction), all within 49 hours. We have found that single GC neuron altered their responses through both the learning and extinction phases, but consistent with the suggestion that extinction is a new type of learning, their responses did not return to naïve responses following extinction. In contrast, using hidden Markov models (HMM) to probe for ensemble dynamic activity we have found that these dynamics actually followed the behavior pattern, changing with learning and reverting with extinction. Specifically, we have found that both the speed of ensemble-level taste processing and the relationships among ensemble responses to the different stimuli tracked behavioral relevance. These data suggest that population coding is linked to behavior with a fidelity that single-neuron coding is not. Acknowledgements: The Swartz Foundation, NIH. FCOI Declarations: None.

#34 SYMPOSIUM: COGNITIVE INFLUENCES ON SMELL AND TASTE: MECHANISMS IN MICE AND MEN

Surprise and Expectation Modulate Early Olfactory Processing in Mice

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Olfactory signaling in the brain is shaped not only by the external odor stimulus but also by the organism's prior knowledge of the sensory world. This knowledge can include the relative *frequency* that odors are encountered in the environment, the *significance* of olfactory stimuli, and the *relationships between odors and other sensory stimuli*. By using this knowledge, the olfactory system can potentially adapt to different behavioral or sensory circumstances by incorporating *expectations* into its analysis of ongoing sensory input. This talk will present neurophysiological evidence that each of these types of information is incorporated into olfactory processing as early as the olfactory sensory neurons (OSNs) themselves. Several labs have now demonstrated that odor exposure induces odor-specific plasticity in the behavior of OSNs as the system adapts to the changing natural statistics of the olfactory environment. We have also shown that the OSNs become selectively hyper-responsive to odorants that predict an impending footshock after fear learning. This talk will attempt to synthesize these findings with new data showing that establishing and violating expectations about stimulus sequence can strongly impact the early olfactory system's response to odors. Using optical neurophysiological techniques in gene-targeted mice, we have found that certain expectation violations induce a burst of activity in GABAergic interneurons that suppresses the synaptic output of OSNs via $GABA_B$ receptor-mediated presynaptic inhibition. This incorporation of stimulus contingency information into peripheral sensory processing is unexpected and may help to relate sensory processing to higher-level cognitive functions like attention and memory retrieval. Acknowledgements: This work was supported by grants from NIDCD and NIMH. FCOI Declarations: None.

#35 PRESIDENTIAL SYMPOSIUM

Metabolic State Shifts Sensory Systems

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Given the rising incidence and complications attributed to obesity and metabolic disorders, the purpose of the 2015 presidential symposium is to highlight the impact of obesity on the physiological processes of brain and sensory function. Denis Burdakov (National Institute for Medical Research and King's College London) will address how the brain estimates and regulates energy balance. He will describe his electrophysiological and behavioral work on integration of nutrient information in the brain using widely-projecting hypothalamic circuits as a model. Sensory physiology experts will then present their findings of how excess energy perturbs the sensory modalities in terms of function or structure. Tim Kern (Case Western Reserve) will address how hyperglycemia causes diabetic retinopathy and his current research strategies designed to prevent this disease, Robin Dando (Cornell University) will discuss inflammation and cell cycle disruption of taste cells following diet-induced obesity, Nicolas Thiebaud (The Florida State University) will present data demonstrating sustained problems attributed to diet-induced obesity on olfactory structure and function, and Claire Murphy (San Diego State University) will present data on humans that demonstrate changed fMRI activity correlated to BMI in response to odor stimulation.

#36 PRESIDENTIAL SYMPOSIUM

Sweet talk in the brain: hypothalamic glucose sensing influences reward neurocircuitry

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Neurons whose activity is regulated by glucose are widespread throughout the brain. Glucose-excited (GE) neurons increase while glucose-inhibited (GI) neurons decrease their action potential frequency as extracellular brain glucose levels increase.

We hypothesize that these neurons evolved to sense and respond to severe energy deficit (e.g., fasting) that threatens the brains glucose supply. We have shown that their response to falling glucose levels is enhanced during energy deficit. While this would be beneficial during times of famine, it would also reinforce the drive for compensatory feeding after voluntary weight loss. Consistent with this hypothesis, we find that activation of lateral hypothalamic orexin neurons in low glucose enhances excitatory drive onto downstream reward neurocircuitry. This lecture will discuss the mechanisms by which glucose sensing neurons sense changes in extracellular glucose and explore the roles of these specialized glucose sensors in glucose and energy homeostasis. Particular emphasis will be paid to the potential of lateral hypothalamic orexin glucose-inhibited neurons to induce persistent changes in glutamate signaling onto ventral tegmental dopamine neurons under conditions of glucose and energy deficit. These data support the hypothesis that altered glucose sensing in orexin neurons contributes to weight regain after dieting as well as binge-eating disorders by enhancing the reward value of food. Supported in part by an AHA Grant in Aid (14GRNT20380639), JDRF 2-SRA-2014-269 and NIDDK RO1081538.

#37 **PRESIDENTIAL SYMPOSIUM**

Oxidative Stress, Inflammation and a Sensory Neural Cell: Diabetic Retinopathy

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Diabetic retinopathy is a major cause of vision loss in adults, and includes both a local vascular and neural disease. Using selective pharmacologic inhibitors or genetically modified animals, an increasing number of therapeutic approaches have been identified in animals that significantly inhibit the development of the diabetes-induced degeneration of retinal capillaries. A common feature of these approaches is that they inhibit production of oxidative stress or inflammatory mediators. Inhibition of the oxidative stress, which comes from both NADPH oxidase and mitochondria, inhibits the local induction of inflammatory molecules, suggesting that the oxidative stress is upstream of the induction of inflammatory molecules. Leukocytes play a critical role in oxidative stress, inflammation and development of vascular pathology, because deletion or blocking the interaction of leukocytes with endothelial adhesion molecules, or deleting proinflammatory signaling selectively within the leukocytes inhibits the diabetes-induced induction of oxidative stress, inflammatory molecules and degeneration of retinal capillaries. Recent evidence demonstrates that the diabetes-induced oxidative stress that develops in the retina is generated largely in a specialized sensory neuron, the retinal photoreceptors, and deletion of retinal photoreceptors or slowing of the visual cycle inhibits both the oxidative stress and the local inflammation in the retina. These hypotheses offer novel targets at which the microvascular disease might be inhibited, but therapies that inhibit the vascular disease do not necessarily correct the diabetes-induced dysfunction of retinal neurons (vision). Acknowledgements: NIH (EY00300, EY022938), VA Merit.
FCOI Declarations: None.

#38 **PRESIDENTIAL SYMPOSIUM**

Inflammatory factors trigger apoptosis in taste cells, resulting in fewer taste buds in obese mice.

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Obesity is a powerful inflammatory state, governed primarily by the foods we eat. Despite our knowledge of nutrition being higher than at any point in history, we still select our foods primarily by their hedonic properties, we eat the foods that taste good. For many years, human psychophysical studies have reported lower sensitivity to tastes in the obese. There has however, been some disagreement over cause and effect, whether those born with a weak sense of taste are more likely to become obese, or becoming obesity weakens a healthy sense of taste. Recently, my lab has examined the molecular effects of obesity on the taste bud. Mice fed a regimen high in dietary fats (HFD) for only a few weeks rapidly become obese, and interestingly have significantly fewer taste buds (assayed via immunohistochemical cell-counting) than littermates fed standard lab chow. These mice also express lower levels of mRNA for receptors (via qRT-PCR) sensitive to the basic tastes of sweet, sour, umami, bitter and salty. In a state of obesity, many inflammatory markers are elevated, leading to a number of well-documented pathological consequences. Several such inflammatory markers, particularly the adipokine Tumor Necrosis Factor alpha (TNF□), are preferentially elevated in the taste buds of obese mice. TNF□ plays many biological roles, but classically triggers apoptosis, through the extrinsic (TNF receptor) pathway. HFD-fed mice exhibit prominent apoptosis (measured with TUNEL staining) in taste buds and taste stem cells, with vastly elevated markers for the extrinsic apoptosis pathway. These results would allude to the presence of an obesogenic feedback loop within the taste bud, with consumption of more tasteful, calorie-dense foods resulting in a lower ability to taste such stimuli, thus driving higher consumption. Acknowledgements: Cornell CALS startup funds.
FCOI Declarations: None.

#39 PRESIDENTIAL SYMPOSIUM

Hyperlipidemic Diet Disrupts Olfactory Structure and Function

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Energy homeostasis is achieved through a coordinated regulation between the peripheral organs and the brain. To determine the effect of chronic energy imbalance and reveal any structural or functional changes associated with obesity, we challenged mice to long-term high fat diets to induce obesity: (1) in an obesity-prone (C57BL/6J) and obesity-resistant (Kv1.3(-/-)) line of mice, and compared this with (2) late-onset, genetic-induced obesity in MC4R(-/-) mice in which diabetes secondarily precipitates after disruption of the hypothalamic axis. We quantified loss of olfactory sensory neurons and their axonal projections after exposure to a fatty diet, with an associated reduction in electro-olfactogram amplitude. Loss of olfactory neurons and associated circuitry was linked to changes in neuronal proliferation and normal apoptotic cycles. By placing obesity-prone mice on high-fat diets upon weaning and at middle age we were able to stimulate mice with odors and assess a reduction in immediate early gene activation following sustained consumption of fatty diets. Using a computer-controlled, liquid-based olfactometer, mice maintained on fatty diets learned reward-reinforced behaviors more slowly, had deficits in reversal learning demonstrating behavioral inflexibility, and exhibited reduced olfactory discrimination. When obese mice were removed from their high-fat diet to regain normal body weight and fasting glucose, olfactory dysfunctions and concomitant anatomical losses were retained. We conclude that chronic energy imbalance therefore presents long-lasting structural and functional changes in the operation of the sensory system designed to encode external and internal chemical information and leads to altered olfactory- and reward-driven behaviors. Acknowledgements: This work was supported by the National Institutes of Health (NIH) grants R01DC003387 and R01DC013080 from the NIDCD and the Council for Creativity and Research (CRC) from FSU. FCOI Declarations: None.

#40 PRESIDENTIAL SYMPOSIUM

FMRI of chemosensory response: relationship to BMI and metabolic status

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Obesity has reached epidemic proportions, motivating research into the underlying mechanisms driving obesity and its consequences. Olfaction is a powerful motivator for food consumption. Research in animal models suggests that metabolic status and hormones involved in satiety may play critical roles in modulating olfactory sensitivity. In rodents olfactory sensitivity is increased during the hunger state and decreased in satiety. Rodents fed a high fat diet show electrophysiological responses that indicate decreased sensitivity to odor. In humans, event-related potentials indicate slowed brain response as BMI increases in older adults. Neuroimaging of the human brain demonstrates differences in response to taste in gustatory and reward areas during hunger and satiety. Here we consider the response to odor in brain regions of interest for olfaction, reward and memory in participants tested under the conditions of hunger and satiety. We used functional MRI to scan at 3T in a GE excite scanner while participants provided modified gLMS ratings of the pleasantness of citral, a food related odor presented orally, under conditions that mimic natural flavor perception. Overall, response to odor was greater in the hunger than in the sated state. Responses in reward related brain areas decreased with increasing BMI, indicative of a blunted reward response, which was especially prominent in the hunger condition. Results support modulation of central response to odor in humans by metabolic state. Acknowledgements: Supported by NIH grant #AG004085-26 from the National Institute on Aging to CM. We gratefully acknowledge the assistance of the members of the Lifespan Human Sense Laboratory. We thank Dr. Thomas Liu and the UCSD Center for fMRI. FCOI Declarations: None.

#41 POLAK PLATFORM PRESENTATIONS

Alterations in Brain-derived Leptin-homolog Unpaired 1 Lead to Obesity Phenotypes in Drosophila through Regulation of Food Odor Value Signaling

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Motivated feeding results from the interplay of homeostatic and hedonic drives and dysregulation of either may contribute adversely to conditions of overweight and obesity. Recent work demonstrates domeless receptors in Drosophila can be activated by human Leptin, a typically adipose-derived "satiety hormone" with a long-established role in weight regulation. Interestingly, knockdown of endogenous domeless-ligand upd2 in the fat body

of flies leads to smaller body size and has no effect on feeding, the opposite behavior of leptin-deficient mammals. While we replicate the lower weight and unchanged food intake in upd2-manipulated flies, we further show manipulations to another endogenous ligand for the domeless receptor, brain-based unpaired 1 (upd1), recapitulate mammalian obesity phenotypes in flies. Flies with reductions in upd1 restricted to neural tissue show increased weights, increased food intake, and increased attraction to food odors. We additionally report domeless receptors likely mediate observed phenotypes. We show behavior-relevant domeless receptors are located on neurons expressing Drosophila Neuropeptide F (dNPF), the Neuropeptide Y (NPY) homolog, in the central brain with targeted receptor knockdown specifically to these cells replicating increased weight, attraction and intake phenotypes. We speculate upd1 acts as the homeostatic regulator of our previously reported dNPF food odor value signal, up- or down-regulating this hedonic signal as a function of satiety state. Our findings suggest Leptin-NPY and upd1-dNPF represent functionally homologous circuits across diverse species and imply in mammals adipose- and less understood brain-derived Leptin may play different roles in feeding and weight regulation.
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FCOI Declarations: None.

#42 POLAK PLATFORM PRESENTATIONS

Serotonergic Modulation of Sensory Processing in the Rodent Olfactory Bulb

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Serotonergic inputs from the raphe nuclei innervate all layers of the olfactory bulb (OB), yet the effects of serotonergic modulation on OB circuits remain unclear. Here, we examine how raphe projections modulate OB inter- and output neuron activity in vivo. First, we tested whether raphe stimulation affected juxtaglomerular interneuron activity by imaging calcium transients from periglomerular (PG) or short axon (SA) cells expressing GCaMP. For each cell type, brief (1-4 s) electrical stimulation of dorsal or median raphe nuclei caused a substantial (> 5-fold) increase in the amplitude of inhalation-evoked transients during inhalation of clean air or low odorant concentrations, indicating that serotonin enhances the responsiveness of PG and SA cells to weak sensory inputs. We next expressed the genetically-encoded reporter of glutamate transmission iGluSnFR in PG or SA cells and observed a similar enhancement of inhalation-evoked glutamate transients in each cell type, suggesting that raphe inputs increase sensory-driven excitation onto PG and SA cells. In contrast, raphe stimulation did not alter inhalation-evoked GCaMP transients in mitral/tufted (MT) cells but instead evoked a tonic increase in fluorescence lasting several seconds. Consistent with this, optogenetically activating serotonergic projections from raphe during extracellular recording of putative MT cells led to a tonic increase in MT cell firing rate without altering inhalation-driven transients. Together, these results demonstrate a differential effect of serotonergic modulation on interneurons and output neurons in the OB and point to glutamatergic juxtaglomerular neurons – such as external tufted cells - as a key target of serotonergic modulation from the raphe.
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FCOI Declarations: None.

#43 POLAK PLATFORM PRESENTATIONS

Pharmacologic and genetic disruption of Smoothened reveals dependence of taste organs on Hedgehog signaling

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Hedgehog Pathway Inhibitors (HPIs) are used to treat cancers that derive from deregulated Hedgehog (HH) signaling through targeting of Smoothened (SMO), a key HH pathway component. However, HPI treatment results in severe taste disturbances. To understand HPI effects on taste sensation, we analyzed fungiform papillae and taste buds in adult mice treated with the HPI LDE225. Strikingly, we had shown that 16 days of LDE225 gavage caused disruptions in taste organs and taste sensation, while 28 days of treatment eliminated normal taste organs and taste responses. To dissect timing effects, we gavaged mice with LDE225 (20mg/kg) or Vehicle for 5, 10, 16, 28 or 36 days. Papillae, taste buds and taste cell types were quantified. There was a duration-dependent effect of LDE225 treatment with a continuous decline to complete absence of normal fungiform papilla morphology and a concomitant increase in distorted papillae (27% to 100% from 5-36 days). Complete taste bud loss in these aberrant papillae increased (7% to 92% from 5-36 days). Importantly, we observed recovery from these effects within 14-21 days after discontinued HPI treatment. Further, conditional genetic disruption of SMO in the lingual epithelium resulted in similar alterations in taste organ morphology. Remaining taste bud cells in HPI-treated or SMO mutant mice expressed the taste cell marker K8 and there was retained innervation to papillae and apical epithelium seen with neurofilament and P2X3 antibodies. All taste bud cell types were reduced progressively over treatment days. These data identify a requirement for HH signaling in maintaining papilla and taste bud integrity, and strongly support the concept that taste disturbances in LDE225-treated patients reflect a direct response to HH pathway inhibition in human taste organs.
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#44 POLAK PLATFORM PRESENTATIONS

The Taste System Modulates Smell Perception via Neural Interactions at the Level of Primary Sensory Cortex

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Taste and smell are intimately connected, as evidenced by the multisensory nature of our sense of flavor. Psychophysical studies on odor intensity perception in humans, as well as behavioral work on odor preference and aversion learning in rodents have suggested that taste influences smell. By combining multi-site electrophysiology and optogenetics in behaving rats, we demonstrate that the gustatory system modulates the olfactory system via interactions at the level of primary sensory cortex. We recently identified neurons in the piriform cortex (PC) of rats that receive taste-selective input, and form functional interactions with neurons in primary gustatory cortex (GC): optogenetic inhibition of GC (GCx) during taste processing eliminates taste-selective responses from PC. Moreover, optogenetic inhibition of "spontaneous" GC activity (i.e., neural activity in the absence of taste stimuli) modulates odor-selective responses in PC. GCx significantly altered neural activity in 23% of all odor-selective responses. Increases and decreases in firing rate were observed equally likely, effectively changing the neural ensemble activated by a given odorant. Next, we probed the possible effect of altered odor representation during GCx on odor perception in a unimodal olfactory learning task. Rats were conditioned to prefer a novel, previously neutral odor by associating that odor with a reward. GCx during subsequent preference testing revealed that rats were unable to express this learnt preference, suggesting that GCx changes the way an odor is perceived. Thus, we demonstrate an unexpected consequence of intrinsic functional connectivity between the taste and smell systems. That is, "unimodal" odor perception is dependent on the taste system, even in the absence of taste stimuli.
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#45 POLAK PLATFORM PRESENTATIONS

Predicting human odor perception from olfactory receptor activation

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Correlating olfactory receptor (OR) activation patterns with human odor perception is a challenge due to the combinatorial nature of the olfactory code and our limited knowledge of odor ligands for these receptors. By examining cases in which loss-of-function of a single OR has a significant role in odor perception, we can begin to determine the conditions in which in vitro OR function predicts human behavior. To this end, we asked 321 human subjects to rate the intensity and valence of 68 odors and then sequenced each subject's OR subgenome. Despite the large number of intact human olfactory receptors, variation in a single olfactory receptor was significantly associated with changes in perception for 20 of the 68 tested odors (29%) ($p < 0.05$, with FDR correction). We then set out to identify the causal receptor underlying each association using an in vitro assay. Although this and other assays have identified ligands for only 49 of the over 400 human ORs (~12%), we were able to identify causal receptors for 9 of our top 10 associations. For these ORs, human behavior, in particular perceived intensity, correlates with in vitro receptor function. For example, human subjects with genetic variants of OR411 that reduce response to 2-ethylfenchol in vitro rated the intensity of the odor to be lower ($F(3,325) = 13.08$, $p < 0.001$) in comparison to subjects with a functional allele. However, using in vitro receptor function to predict human behavior is less clear. In vitro OR function strongly predicts perceived odor intensity for high affinity odor ligands, but poorly for low-affinity ligands. These results provide a potential approach for identifying behaviorally relevant OR/odor interactions and predicting odor perception using a heterologous assay. Acknowledgements: R03-DC11373, R01-DC1339, T32-DC000014. FCOI Declarations: None.

#46 POLAK PLATFORM PRESENTATIONS

Direct evidence for BBSome-associated intraflagellar transport reveals distinct properties of native mammalian olfactory sensory cilia

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Cilia dysfunction underlies a class of human diseases with variable penetrance in different organ systems. Across eukaryotes, intraflagellar transport (IFT) facilitates construction of olfactory sensory cilia and ciliary cargo trafficking, thereby enabling olfaction, but our understanding of mammalian IFT is insufficient. Here we perform live analysis of cilia ultrastructure, composition and cargo transport dynamics in native mammalian olfactory sensory neurons using Total Internal Reflection Fluorescence microscopy (TIRFm). Proximal and distal axonemes of these neurons show no bias towards IFT kinesin-2 choice, and Kif17 homodimer is dispensable for distal segment IFT. We identify anosmia-associated Bardet–Biedl syndrome proteins (BBSome) as bona fide constituents of IFT in olfactory sensory neurons, and show that they exist in 1:1 stoichiometry with IFT particles. Conversely, subpopulations of peripheral membrane proteins, as well as transmembrane olfactory signalling pathway components, are capable of IFT but with significantly less frequency and/or duration. Our results yield a model for IFT and cargo trafficking in native mammalian cilia

and may explain the penetrance of specific ciliopathy phenotypes in olfactory neurons. Acknowledgements: R01DC009606 to J.R.M. T32DC00011 to C.L.W. FCOI Declarations: None.

#47 SYMPOSIUM: AVIAN SPECIES AS A MODEL FOR TASTE DETECTION: MOLECULAR RECOGNITION, DIET CHOICE AND EVOLUTION

Avian species as a model for taste detection: molecular recognition, diet choice and evolution

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It has been argued that birds have a lower taste acuity compared to mammals due to their low taste bud numbers. In addition, chicken seem have fewer taste receptor genes: the sweet taste receptor is missing and their bitter taste receptor repertoire is very small, consisting of only three members. However, knowledge emerging from genomes sequencing suggests that birds have a well-developed taste system which differs significantly among different species. Behavioral and genetic evidence show that birds have an accurate capacity to detect different taste modalities. In fact, the bitter taste reception in chicken is a minimalistic model for studying bitter taste, which is much more complex in human (25 receptors) and mice (30 receptors). Furthermore, birds present a unique opportunity to study taste evolution in different ecological niches.
The symposium features recent contributions from several researchers. In particular, Dr. Roura will present work on identifying genes (beyond T1R and T2R) putatively involved in nutrient sensing and estimating oral volumes and ratio taste bud/oral volume in chicken. Dr. Behrens will present results on chicken bitter taste receptors repertoire, in comparison with bitter taste receptors in other species. Masha Niv will present studies on molecular recognition of bitter tastants, focusing on issues of selectivity and promiscuity of both ligands and receptors. Finally, Maude Baldwin will present the study on the repurposing of an amino acid taste receptor to respond to sugars and other sweeteners in the hummingbird. The symposium is aimed at audience interested in evolution, molecular recognition, taste programming and farm animal nutrition.

#48 SYMPOSIUM: AVIAN SPECIES AS A MODEL FOR TASTE DETECTION: MOLECULAR RECOGNITION, DIET CHOICE AND EVOLUTION

Oral Nutrient Sensing in the Chicken: A Look Beyond T1R/T2Rs

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Nutritional chemosensing has gained momentum thanks to the discovery of a broad array of nutrient sensors, including taste receptors (Tas1r and Tas2r), most of them known to be expressed in the oral cavity in mammals. Compared to mammals taste in birds has been regarded to be less developed because they seem to have a low number of taste buds, lack the Tas1r2 and have only few bitter taste receptors. The aim of our research is to evaluate the nutrient sensing system in birds using the domestic chicken as a model. We have measured the size of the oral cavity with the hypothesis that the number of taste buds is proportioned to the oral volume with a similar ratio in birds and mammals. Thirty-three oral cavity plaster moulds were constructed from chicken heads. The oral volumes were calculated using a regression equation based on known standards of wet plaster volumes to dried weight (Volume=0.8628×plaster weight+0.09224; $P< 0.001$, $R2=0.9959$). We found that the average oral volume of a chicken was 2.26±0.11ml concluding that the ratio of number of taste buds to the oral volume is similar to humans. We then identified 11 nutrient sensing genes present in the chicken genome including the 2 Tas1r (Tas1r1 and Tas1r3), 3 Tas2r (Tas2r1, Tas2r2, Tas2r7), 2 fatty acid sensors (FFAR2 and 3) and 4 amino acid sensors (GPR92, GPRC6A, GRM1 and GRM4) to study the expression by real-time qPCR in three different oral tissues: upper palate, base of the tongue and lower palate. We found all the target genes expressed in the three tissues but to a higher extent in the upper palate ($P < 0.0001$). We conclude that chickens have a well-developed nutrient sensory system. Our results set the scene for future studies in chickens related to taste and nutrient appetites.
Acknowledgements: The research has been partially covered by the University of Queensland post-doctoral fellowship scheme. FCOI Declarations: None.

#49 SYMPOSIUM: AVIAN SPECIES AS A MODEL FOR TASTE DETECTION: MOLECULAR RECOGNITION, DIET CHOICE AND EVOLUTION

Recognition Profiles of Avian Bitter Taste Receptors

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The detection and avoidance of potentially harmful bitter food compounds is important for the survival of animals. Recognition of bitter substances is facilitated by bitter taste receptors located in the oral cavity. The number of functional bitter taste receptor genes deviates considerably among animal species ranging from very few to more than 50. Although it has been hypothesized that birds may possess inferior tasting abilities due to low salivation, a lack of mastication and a low number of taste buds in their oral cavity, all bird species investigated so far possess small to mid-sized Tas2r gene repertoires. This raised the question of whether the sizes of avian Tas2r gene repertoires correlate with the relative importance of the bitter tasting abilities of bird species, or whether such predictions are not possible.
In order to determine the recognition profiles of avian Tas2rs we cloned bitter taste receptor cDNAs of two phasianid (*G. gallus* and *M. galloparvo*) and one passerine (*T. guttata*) bird species and performed functional heterologous expression studies. After

screening of the receptors with 46 mostly natural bitter compounds, the responding receptors were further analyzed with the activating bitter substances to determine more detailed response characteristics. We found that all of the chicken and turkey receptors exhibit broad tuning properties suggesting that small Tas2r gene repertoires do not necessarily indicate inferior bitter compound detection. An expansion of the Tas2r gene number in zebra finch, however, seems to allow the development of more specialized bitter taste receptors. Our results show that the investigated bird species are well equipped for the detection of potentially harmful bitter substances, a finding that agrees well with behavioral data obtained for chicken. Acknowledgements: Supported in part by Deutsche Forschungsgemeinschaft (Ko1046/7-1 to S.I.K.). FCOI Declarations: None.

#50 SYMPOSIUM: AVIAN SPECIES AS A MODEL FOR TASTE DETECTION: MOLECULAR RECOGNITION, DIET CHOICE AND EVOLUTION

The evolution of sweet taste perception in hummingbirds

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Sensory systems are powerful systems for understanding molecular mechanisms underlying phenotypic adaptation: they define an animal's capacity for perception and can evolve to detect different stimuli when species diverge and encounter new selective pressures. In addition, chemosensory receptors represent a direct link between genotype and phenotype and yield insight into basic aspects of the evolutionary process. In mammals, sweet taste perception is mediated by a G protein-coupled receptor complex; however, the gene encoding one subunit of the mammalian sweet receptor (T1R2) has not been detected in any bird genome, suggesting loss in the avian common ancestor. Nevertheless, many nectar-feeding birds, such as hummingbirds, lorikeets, and honeyeaters display high behavioral affinity for sugars found in nectar. To understand the molecular basis of sugar sensing in hummingbirds, we cloned members of the T1R taste receptor gene family from oral tissue of hummingbirds, swifts, and chickens. Receptor expression studies revealed that the ancestral umami receptor (T1R1-T1R3 heterodimer) was re-purposed in hummingbirds, but not in swifts, their closest relatives, to function as a carbohydrate receptor. Behavioral choice tests and high-speed videography in wild and captive hummingbird populations indicated sweet taste preferences that correlated with *in vitro* functional studies. This change in taste receptor function may have been one of many key adaptations enabling hummingbirds to detect and utilize nectar, facilitating the radiation of hummingbird species. Acknowledgements: NSF DDIG, Sigma Xi, Society for Integrative and Comparative Biology, Harvard University student research grants, Japan Society for Promotion of Science, Fulbright Commission, Science Foundation Ireland Research Frontiers Program, NIH. FCOI Declarations: None.

#51 SYMPOSIUM: AVIAN SPECIES AS A MODEL FOR TASTE DETECTION: MOLECULAR RECOGNITION, DIET CHOICE AND EVOLUTION

Taste and Promiscuity – Structural Determinants in G-protein Coupled Chemosensory Receptors

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Chemosensory receptors belong to the large superfamily of G-protein-coupled receptors (GPCRs). Bitter compounds are numerous and chemically diverse[1]. These compounds are recognized by one or more GPCRs from the T2Rs subfamily, which, in turn, may recognize either few or numerous ligands[2]. Iterative homology modeling, docking, and site-directed mutagenesis suggest that common strategies enabling T2Rs to recognize diverse ligands include ligand-specific engagement of different sets of partially overlapping positions within the same binding pocket, and employment of different types of interactions by the same residues[3]. Analysis of experimental GPCR structures highlights hydrophobicity and binding site exposure as general features predictive of the levels of promiscuity of GPCRs[4]. A ligand promiscuity predictor was developed for identifying bitter ligands that activate multiple T2Rs. The molecular determinants responsible for activity and selectivity of bitter compounds for chicken bitter receptors were investigated by ligand-based and structure-based methods. Identification of novel T2R-selective and T2R-promiscuous bitter ligands for chicken and for human is currently underway. 1. Wiener, A.; Shudler, M.; Levit, A.; Niv, M. Y., BitterDB: a database of bitter compounds. Nucleic Acids Res 2012, 40 2. Di Pizio, A.; Niv, M. Y., Computational studies of smell and taste receptors. Isr J of Chem, special issue 2014. 3. Born, S.; Levit, A.; Niv, M. Y.; Meyerhof, W.; Behrens, M., The human bitter taste receptor TAS2R10 is tailored to accommodate numerous diverse ligands. J Neurosci 2013, 33 (1), 201-13. 4. Levit, A.; Beuming, T.; Krilov, G.; Sherman, W.; Niv, M.Y., Predicting GPCR promiscuity using binding site features. J Chem Inf Model 2014, 54 (1), 184-94. Acknowledgements: DFG, ISF, Chief Scientist of Agriculture, COST action GLISTEN. FCOI Declarations: None.

#52 SYMPOSIUM: FEEDBACK AND MODULATION IN CHEMICAL SENSES

Feedback and modulation in chemical senses

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As is true in all sensory systems, chemosensory perception reflects not only the external stimulus, but also the internal state and past experiences of the perceiver. This means that perception

of stable stimulus input may be highly variable as the perceiver's state (e.g., hunger/satiety, fearful/secure, etc.) and experience with the stimulus (e.g., novel/familiar, expected/unexpected) changes. That is, the same basic sensory circuit may produce different outputs depending on internal state and past experience. These changes in sensory coding and circuit function appear to derive from changes in both neuromodulatory tone and from feedback from higher order, non-sensory circuits. While these processes occur in all sensory systems, they may be particularly relevant in the chemical senses which monitor stimuli relevant to nutrition, reproduction, kin recognition and predator avoidance. This symposium will present new data from both the olfactory (Kay, Mandairon, Sadrian) and gustatory (Fontanini) systems exploring how this internal modulation occurs. The talks will include diverse research techniques primarily in awake animals (e.g., single-unit recordings, local field potential recordings, pharmacological and optogenetic manipulations, novel behavioral assays) which examine the role of neuromodulatory systems as well as inputs to primary sensory regions providing feedback information regarding expectation, memory and hedonics. TEST Acknowledgements: N/A.
FCOI Declarations: None.

#53 SYMPOSIUM: FEEDBACK AND MODULATION IN CHEMICAL SENSES

Top-down control on adult-born neurons during olfactory learning

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Discrimination learning through experience serves as the basis for adaptive behavior thanks to structural and functional changes in the brain. In the olfactory system, two main forms of experience, perceptual and associative learning have been show to modulate discrimination and neural activity in the olfactory bulb. This cortical structure is the first central relay of olfactory processing and the target of adult neurogenesis. The olfactory bulb is in fact capable of generating new neurons that can integrate into its complex circuitry. Both associative and perceptual learning have been shown to increase neurogenesis but while perceptual learning requires neurogenesis as soon as its acquisition, associative learning needs it to maintain memory suggesting different role of adult-born neurons in learning processes and thus different mechanisms underlying their integration into the bulbar network. It is well known that centrifugal inputs to the olfactory bulb and more specifically noradrenergic and cholinergic systems contribute to learning processes. We will discuss how these neuromodulatory systems may differentially interact with neurogenesis to allow perceptual and associative learning processes. Acknowledgements: CNRS and Lyon 1 University. FCOI Declarations: None.

#54 SYMPOSIUM: FEEDBACK AND MODULATION IN CHEMICAL SENSES

Gamma and beta oscillations describe early and late cognitive processing during odor discrimination

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Over the past decade different studies have shown that either gamma (~70 Hz) or beta oscillations (~20 Hz) of the olfactory bulb (OB) local field potential dominate in odor processing. These oscillations are not just different frequencies but appear to rely on different circuits. Gamma oscillations increase in amplitude when central input to the OB is silenced; beta oscillations are ablated in this condition. Gamma oscillations are enhanced during fine odor discrimination, while beta oscillations are not. Within our lab a 2-alternative choice (TAC) task and a go/no-go (GNG) task have shown enhanced gamma and beta oscillations, respectively. We tested the task requirements of these two oscillations by training separate groups of rats on each task in parallel. We found that both tasks produce both types of oscillations, but gamma and beta represent different parts of the discrimination process. Gamma oscillations dominate the first 2-3 sniffs of the odor, while beta oscillations correlate with the end of odor sampling and preparation for a learned response. Beta oscillation power also depends on the odors being sampled. During fine odor discrimination gamma oscillations are enhanced and beta oscillations are increased before and decreased during odor sampling. These results show that the early olfactory system can occupy several different functional states within an odor sampling bout and across tasks and odor sets. Computational modeling indicates that changing the state of OB granule cells can transition the system from gamma to beta modes. These states are likely determined by the cognitive context of the behavioral task, which is associated with dynamics in neuromodulatory tone and centrifugal input to the OB. Acknowledgements: NIDCD R01-DC014367 Institute for Mind and Biology Seed Grant. FCOI Declarations: None.

#55 SYMPOSIUM: FEEDBACK AND MODULATION IN CHEMICAL SENSES

Limbic System Modulation of Olfactory Cortex

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The rodent piriform cortex (PCX) is a paleocortical structure known to support olfactory perception toward learned behavior. While the anterior PCX is used in associative odor object information decoding, the posterior PCX receives more descending input fibers from brain structures such as the amygdala that are thought to provide a qualitative relevance to raw odor percepts. Here we investigate the influence of top-down

influence of specific brain regions on spontaneous and odor-induced activity in the posterior PCX at the single unit level. Using optogenetic techniques, we artificially stimulated descending fibers in the posterior PCX that were virally transduced from one of two interconnected brain regions. Specifically, the lateral and basolateral amygdala (LA/BLA) and the lateral entorhinal cortex (LEC) were independently targeted to express Channelrhodopsin in pyramidal neurons that also express CaMKII. Photostimulation at 473nm and 1mW near infected axon terminals in the posterior piriform was sufficient to drive temporally coincident responses of unit activity and local field potential, as recorded in anaesthetized animals injected at any one of the two target regions. Odor-paired photostimulation of descending fibers at the posterior PCX modulated local single unit response patterns compared to odor only. Photo-induced effects on unit odor responses ranged from suppressive to stimulatory, which often varied depending on the combinatorial timing of odor and light stimulation. These results demonstrate the importance of top-down inputs to piriform cortex in odor coding, and highlight that cortical odor processing takes place in a rich milieu of sensory, emotional and contextual information. Acknowledgements: DC03906 AA023181.
FCOI Declarations: None.

#56 SYMPOSIUM: FEEDBACK AND MODULATION IN CHEMICAL SENSES

Processing of anticipatory and chemosensory signals in the gustatory system: where's the top and where's the bottom?

Alfredo Fontanini
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The gustatory cortex (GC) of alert animals does much more than process the physiochemical properties of taste. Besides flexibly representing the chemosensory information, GC neurons also encode hedonic value and expectation of taste. Over the past years, our group has focused on understanding how GC represents anticipatory information. I will present recordings from the GC of alert rodents showing that neurons respond to cues that anticipate the general availability of taste and cues that predict specific tastants. Cue responses develop with learning and can be triggered by stimuli belonging to multiple sensory modalities. I will present evidence of the involvement of anticipatory activity in modulating taste coding and feeding behaviors. In addition, I will discuss the role of thalamic and amygdalar inputs in mediating responses to anticipatory cues. Initial evidence suggested a functional segregation of these inputs, with the gustatory thalamus (VPMpc) carrying "bottom-up" sensory information and the basolateral amygdala providing "top-down" anticipatory signals. However, recent recordings from the VPMpc of alert rodents demonstrate that the thalamus can encode taste-anticipatory cues in a way that predicts the animal's behavior. These results challenge the idea of a clear separation between bottom-up and top-down channels of information. The presence of anticipatory signals in the VPMpc emphasizes how the integration of sensory and contextual signals is not just a cortical process, but can occur at any level of the gustatory system. On this bases, I will argue that current conceptualizations of the gustatory system as a pure labeled-line analyzer of chemical information need to be replaced by more complex models emphasizing context-dependency and integration. Acknowledgements: This work was supported by National Institute of Deafness and other Communication Disorders Grants R01-DC010389, R01-DC012543 and R01-DC013770. FCOI Declarations: None.

#57 SYMPOSIUM: CGMP SIGNALING IN THE OLFACTORY SYSTEM: IMPLICATIONS FOR CELLULAR AND BEHAVIORAL RESPONSES TO SENSORY STIMULI

cGMP signaling in the olfactory system: implications for cellular and behavioral responses to sensory stimuli

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There is growing recognition that the cellular heterogeneity of the peripheral olfactory system is a major contributor to the complexity of olfactory function. Olfactory neurons can differ in their stimulus selectivity, transduction mechanisms, CNS targets and behavioral roles. Strikingly, several neuronal subtypes in the olfactory system rely, at least in part, on cGMP (as opposed to the canonical OSN messenger cAMP) as a second messenger. This symposium will explore several cellular subpopulations in the olfactory periphery where cGMP signaling has been implicated, with a focus on their mechanisms for sensory transduction, their central targets, and their contributions to behavior. In his introduction, Steven Munger will provide a short history and important context for cGMP signaling in olfaction. Next, Elissa Hallem will present her ongoing studies on CO_2-sensing by nematode OSNs that utilize cGMP. Trese Leinders-Zufall will then discuss work from her and her colleagues dissecting the role of the Grueneberg ganglion and other olfactory subsystems in mediating aversive behaviors in the mouse. Joerg Fleischer will speak about studies in the Grueneberg ganglion from his group that dissect the mechanisms of chemo- and thermosensation in this still obscure sensory organ. Finally, Peter Mombaerts will discuss his recent work on a novel subpopulation of mouse MOE neurons that express both Trpc2 and cyclic nucleotide-gated channels, some of which express a soluble guanylyl cyclase. Together, the speakers will provide a new understanding of the importance of heterogeneous signaling mechanisms in the olfactory system across multiple species and will suggest implications for olfactory functions. Acknowledgements: Olfactory studies in Steven Munger's laboratory have been supported by grants from the NIDCD and by TEDCO of Maryland.
FCOI Declarations: None.

#58 SYMPOSIUM: CGMP SIGNALING IN THE OLFACTORY SYSTEM: IMPLICATIONS FOR CELLULAR AND BEHAVIORAL RESPONSES TO SENSORY STIMULI

Mechanisms of Carbon Dioxide Sensing in Nematodes

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Carbon dioxide (CO_2) is a critical sensory cue for many animals that can signal the presence of food, mates, predators, or hosts. We are investigating the neural basis of CO_2 sensing in the free-living nematode C. *elegans*, entomopathogenic nematodes (EPNs), and mammalian-parasitic nematodes. We have found that C. *elegans* adults are repelled by CO_2, while dauer larvae are attracted to CO_2. CO_2 is detected by the BAG neurons using the receptor guanylate cyclase GCY-9 and the cGMP-gated cation channel TAX-2/TAX-4. A network of interneurons mediates CO_2 response, and behavioral sensitivity is modulated by opposing interneurons. Like C. *elegans* dauers, EPNs are attracted to CO_2. Moreover, CO_2 is an essential host cue for EPNs: attraction to insect odor is greatly decreased or eliminated when CO_2 is chemically removed. CO_2 attraction by EPNs requires BAG neurons, indicating that the neural basis of CO_2 response is at least partly conserved across free-living and parasitic species. The passively ingested livestock parasite *Haemonchus contortus* is also attracted to CO_2, a behavior that may increase the likelihood of passive ingestion. By contrast, skin-penetrating nematodes, including the human threadworm *Strongyloides stercoralis*, are repelled by CO_2. However, skin-penetrating infective larvae require CO_2 for development inside the host, indicating that CO_2 is a critical developmental cue for these parasites. The BAG neurons of S. *stercoralis* are activated by CO_2 and express a *gcy-9* homolog. We are now testing the hypothesis that cGMP signaling by BAG neurons is required for skin-penetrating worms to infect hosts. Our results will provide insight into the role of cGMP signaling in mediating sensory behaviors, and may enable the development of new strategies for combating harmful nematode infections. Acknowledgements: Supported in part by a McKnight Scholar Award, a Rita Allen Foundation Scholar Award, and a Searle Scholars Award to E.A.H. FCOI Declarations: None.

#59 SYMPOSIUM: CGMP SIGNALING IN THE OLFACTORY SYSTEM: IMPLICATIONS FOR CELLULAR AND BEHAVIORAL RESPONSES TO SENSORY STIMULI

Dissecting innate predator odor aversion: Circuit logic and genetic substrates

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Detection of predators elicits stereotyped fear and innate evading responses on prey species. Preys frequently coexist with a large number of predator species, each emitting numerous distinctive chemical cues. The mouse olfactory system is composed of diversified subsystems that are distinguished by the signaling mechanisms they employ to transduce sensory stimuli and their axonal projections to specific regions of the olfactory forebrain. This organization enables the recognition of a broad range of chemicals, some of which activate developmentally programmed neural circuits eliciting avoidance. How olfactory signals are processed to exhibit innate avoidance remains largely unclear. We observe that mice display robust avoidance to specific odors released by predators. Different olfactory subsystems – the vomeronasal organ (VNO), the trace-amine-associated receptor (TAAR) system, and the Grüneberg ganglion (GG) – are independently dedicated to the detection and avoidance of specific predator chemosignals. Mutant mice deficient for essential subsystem-specific signal transduction components fail to display innate avoidance responses to some predator odors while avoidance to odors detected by other subsystems remains unaltered. We use Ca2+ imaging and neural activation markers to map the primary neurons governing avoidance to predators. Predator odors are broadly detected by distributed populations of sensory neurons in the nose that innervate multiple glomeruli in the olfactory bulb, organized as independently initiated sensory circuits. We have begun to investigate the central brain areas that participate in the processing and integration of these olfactory signals to determine a circuit logic of innate kairomone avoidance. Acknowledgements: Supported by grants from the Deutsche Forschungsgemeinschaft to P.C. (CH 920/2-1), F.Z. (SFB 894) and T.L.-Z. (SFB 894), the Volkswagen Foundation (to T.L.-Z.), and a HOMFORexzellent gant to to P.C. and the National Institute of Health (NIDCD) to S.D.M. FCOI Declarations: None.

#60 SYMPOSIUM: CGMP SIGNALING IN THE OLFACTORY SYSTEM: IMPLICATIONS FOR CELLULAR AND BEHAVIORAL RESPONSES TO SENSORY STIMULI

Relevance of cGMP Signaling in Sensory Neurons of the Grueneberg Ganglion

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The Grueneberg ganglion - a cluster of neurons in the anterior nasal region – is considered as an olfactory subsystem that is activated by distinct odorants, in particular given pyrazine analogues. The odorant-responsive neurons are characterized by the expression of the guanylyl cyclase subtype GC-G and the cyclic nucleotide-gated ion channel CNGA3. The results of experiments with knockout mice disclosed that GC-G and CNGA3 are important for odor-evoked responses, suggesting a unique chemo-electrical transduction mechanism in the Grueneberg ganglion. The GC-G-/CNGA3-positive Grueneberg ganglion neurons are also activated by cool temperatures.

Accordingly, the same subpopulation of neurons in the Grueneberg ganglion responds to both coolness and odorants. In search for temperature-responsive signaling proteins, it was found that the Grueneberg ganglion lacks the canonical cold receptor, the TRPM8 channel. Instead, the thermosensitive ion channel TREK-1 is expressed. However, it turned out that even in the absence of TREK-1, Grueneberg ganglion neurons were still activated by cool temperatures, leading to the notion that other coolness-responding molecular elements must exist. In this context, comprehensive biochemical analyses led to the unexpected finding that the guanylyl cyclase subtype GC-G is a very unusual enzyme which is directly activated by cool temperatures. In addition, the observation that Grueneberg ganglion neurons from GC-G-deficient mice showed a largely reduced responsiveness to coolness supports the concept that the guanylyl cyclase subtype GC-G may serve as a major thermosensor in neurons of the Grueneberg ganglion. Acknowledgements: This work was supported by the Deutsche Forschungsgemeinschaft. FCOI Declarations: None.

#61 SYMPOSIUM: CGMP SIGNALING IN THE OLFACTORY SYSTEM: IMPLICATIONS FOR CELLULAR AND BEHAVIORAL RESPONSES TO SENSORY STIMULI

Trpc2-expressing sensory neurons in the mouse main olfactory epithelium of type B express the soluble guanylate cyclase Gucy1b2

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Chemoreception in the mouse olfactory system occurs primarily at two chemosensory epithelia in the nasal cavity: the main olfactory epithelium (MOE) and the vomeronasal epithelium (VNE). The canonical chemosensory neurons in the MOE, the olfactory sensory neurons (OSNs), express the odorant receptor (OR) gene repertoire, and depend on Adcy3 and Cnga2 for chemosensory signal transduction. The canonical chemosensory neurons in the VNE, the vomeronasal sensory neurons (VSNs), express two unrelated vomeronasal receptor (VR) gene repertoires, and involve Trpc2 for chemosensory signal transduction. Recently we reported the discovery of two types of neurons in the mouse MOE that express Trcp2 in addition to Cnga2. These cell types can be distinguished at the single-cell level by expression of Adcy3: positive, type A; negative, type B. Some type A cells express OR genes. Among MOE cells, type B cells are unique in their expression of the soluble guanylate cyclase Gucy1b2. We came across Gucy1b2 in an explorative approach based on Long Serial Analysis of Gene Expression (LongSAGE) that we applied to single red-fluorescent cells isolated from whole olfactory mucosa and vomeronasal organ of mice of a novel Trcp2-IRES-taumCherry gene-targeted strain. The generation of polyclonal antibodies against Gucy1b2 and a novel Gucy1b2-IRES- tauGFP gene-targeted strain enabled us to visualize coalescence of axons of type B cells into glomeruli in the main olfactory bulb. Our molecular and anatomical analyses define Gucy1b2 as a marker for type B cells within the MOE. Acknowledgements: Max Planck Society. FCOI Declarations: None.

#62 SYMPOSIUM: TRANSMITTING CHEMICAL WARNINGS IN ANIMALS AND MEN: THE ROLE OF CHEMOSIGNALING IN SOCIAL COMMUNICATION

Transmitting chemical warnings in animals and men – The role of chemosignaling in social communication

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Chemical signals constitute an important medium for communication across a range of species, including humans, as recent evidence suggests. The type of information that can be communicated via chemical signals varies. For instance, mice have been shown to warn their conspecifics by sending alarm and disease signals. In the case of humans, there is growing evidence that chemosignals contain communicable information about the sender's emotional state, disease status, and specific characteristics such as gender or age, among other things. This symposium consists of four speakers whose work demonstrates that chemosignals of emotion and disease status constitute an important facet of social communication, not just in animals but also in humans. Lisa Stowers (Scripps Research Institute) will present data exploring the link between chemical communication and emotion in mice, and the opportunity for using molecular genetics for understanding the underlying mechanisms. Gün Semin (Utrecht University, Koç University, Institutio Superior de Psicologia Aplicada) will review some work on how communication of emotion between human senders and receivers guided by olfactory signals within a framework of human social communication previously believed to rely only auditory and visual modalities. Johan Lundström (Monell Chemical Senses Center, Karolinska Institute) will present evidence demonstrating the human ability to communicate disease highlighting potential underlying neural mechanisms. Wen Li's group (Florida State University) will close by drawing a comparison between how the human brain is involved in the processing of chemical versus physical (visual) social signals of threat. Acknowledgements: R01MH093413. FCOI Declarations: None.

#63 SYMPOSIUM: TRANSMITTING CHEMICAL WARNINGS IN ANIMALS AND MEN: THE ROLE OF CHEMOSIGNALING IN SOCIAL COMMUNICATION

Leveraging pheromones to study emotional behavior in the mouse

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While it is understood that mouse pheromones regulate social behavior, it is less well appreciated that these behaviors are tightly coupled with emotion. Emotional processing is one of the three major functions of the human brain (along with sensory- and cognitive-processing). 'Emotion' describes an ancient evolutionary motivating system that drives action essential for life. Inappropriate, exuberant or diminished display of emotion can be devastating. Even smart, logical and decisive individuals can

suddenly become irrational, foolish, and reckless when emotion is strongly engaged. How can it be that almost nothing is known about the information processing and underlying mechanisms that generate emotion in any species? We are utilizing synthetic, emotion-generating pheromones provides an experimentally simple means to identify and study currently unknown subsets of relevant neurons that underlie the generation of emotion. I will describe our recent progress to use pheromones to study emotion in the mouse. Acknowledgements: NIDCD, Ellision Medical Foundation. FCOI Declarations: None.

#64 SYMPOSIUM: TRANSMITTING CHEMICAL WARNINGS IN ANIMALS AND MEN: THE ROLE OF CHEMOSIGNALING IN SOCIAL COMMUNICATION

The multimodal nature of human communication

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Communication and its diverse facets have been the subject of considerable research in psychology. Often the multiple research strands on communication have not connected and have developed as separate lines of inquiry. These diverse strands have examined the role of 'language' as a representational medium for communicating thought and emotion. Other research has focused on visual signals, such as body postures or facial expressions, and signals from other modalities, such as sound. In the interim, there is a growing literature showing that human sweat carries information and therefore functions as a signal. Indeed, most of the research we have conducted (e.g., de Groot, Semin, & Smeets, 2014a, 2014b; de Groot, et al., 2014; de Groot, Smeets, Kaldewaij, Duijndam, & Semin, 2012) was driven by a general framework on embodied social communication (e.g., Semin, 2000, 2007; Semin & de Groot, 2013; Semin & Smith, 2013) examining the social signal function of human sweat. This research has shown that human sweat produced under specific emotional states (e.g., fear, happiness) induces a simulacrum of the same emotional state in a recipient of this sweat. This research has underlined the significance of the role played by human chemosignals produced under emotion-inducing conditions in the communication of emotions (e.g., emotion contagion). In this presentation, a sketch for the multimodal communication of emotions is presented as well as an overview of the work on human chemosignaling of emotions. The presentation also raises a question inspired by Darwin's (1872/1998) notion that emotions induce beneficial action for the organism rather than for conspecifics and the implications of this notion for the communicative function of emotion chemosignaling. Acknowledgements: The research reported in this paper was supported in part by the Dutch Science Foundation and in part by Unilever. FCOI Declarations: None.

#65 SYMPOSIUM: TRANSMITTING CHEMICAL WARNINGS IN ANIMALS AND MEN: THE ROLE OF CHEMOSIGNALING IN SOCIAL COMMUNICATION

Chemical and physical warning signals: Common and distinct effects

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Effective signaling of salient biological events to conspecifics confers critical ecological value to an organism. Emotional expressions provide powerful cues in such communication. Beyond physical emotion transmission (through facial and vocal expressions), accruing evidence demonstrates chemical emotion transmission via olfactory cues (e.g., sweat and body odor). However, it remains unclear how olfactory social signals are analyzed and whether they play unique roles in social signaling. Therefore, we compared behavioral and neural responses to visual and olfactory social warning signals (disgust faces and disgust sweat), relative to their neutral counterparts, in two fMRI experiments. Importantly, these responses were contrasted with those elicited by direct disgust-evoking stimuli (disgusting scenes and disgusting odors, relative to neutral scenes/odors) to isolate specific processing of social signals. We observed that social warning signals (disgust faces/sweat) facilitated healthy-unhealthy food judgment relative to disgust-evoking stimuli whereas both social disgust signals and disgust-evoking stimuli exerted similar effects on social affective judgment, negatively shifting pleasantness rating of novel, anthropomorphic objects. Neurally, both visual and olfactory social disgust signals evoked greater response in the insula. Nevertheless, olfactory social disgust was associated with specific response enhancement in the olfactory orbitofrontal cortex in both tasks. Therefore, to the extent that social signals of disgust transmitted via visual and olfactory channels lead to similar behavioral influences, they elicit both common and unique brain responses, informing the perceiver of the threat of disgust tinted with specific sensory characteristics. Acknowledgements: This work is supported by R01MH093413 (W.L.). FCOI Declarations: None.

#66 SYMPOSIUM: TRANSMITTING CHEMICAL WARNINGS IN ANIMALS AND MEN: THE ROLE OF CHEMOSIGNALING IN SOCIAL COMMUNICATION

Chemical Warning Signals in Humans

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Behavioral and neuroimaging studies have demonstrated that visual signals that throughout evolution has been associated with threats enjoy automated and prioritized processing. However,

seen over human history, the two deadliest experiences have been interactions with unknown (strangers) and infected (sickness) individuals. Based on this, we hypothesized an ability to detect these threats also via chemical signals. In this talk, an overview of findings from our recent project on chemosensory threat signals will be presented. In respect of exposure to a stranger's body odor: we recently demonstrated that smelling the body odor from a stranger activates the cerebral network processing threat-related stimuli. Moreover, the presence of body odors originating from a stranger, but not from oneself, renders the visual processing of a neutral face more similar to that of an angry face as well as increase general detection performance. These results suggest that the body odor of a stranger acts as a threat signal that biases the visual processing of social stimuli towards over-generalizing inputs as potential threats. In respect of exposure to a sick individual's body odor: using an experimental model where we render individuals sick by endotoxin injections, we recently demonstrated that humans are able to detect sickness based on chemical signals alone. In subsequent experiments, this was demonstrated to be a dose-dependent effect where larger endotoxin dose rendered a better detection. Taken together, our findings demonstrate that, much like other animals, humans are able to extract chemical information warning us about the presence of unknown and sick individuals in our presence and that this information affect our perceptual processing. Acknowledgements: The Knut and Alice Wallenberg Foundation (KAW 2012.0141) and Swedish Research Council (421-2014-13) to JNL as well as the Swedish Research Council (421-2012-1125) and the Bank of Sweden Tercentenary Foundation (P12-1017) to MJO.
FCOI Declarations: None.

#1 POSTER SESSION I

The transcription factor Phox2b identifies taste from non-taste neurons in the geniculate ganglion

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The basic characteristics of gustatory neurons remain unknown, partly due to the absence of a specific marker. Gustatory neurons cannot be distinguished from somatosensory neurons in the geniculate ganglion without nerve labeling, which is not possible during development and alters gene expression. An intrinsic genetic label for taste neurons would provide a significant advantage. We hypothesized that Phox2b would identify taste from non-taste neurons in the geniculate ganglion. Phox2b is a homeodomain transcription factor essential for the development of placodal-derived gustatory ganglia. To determine if Phox2b specifically identifies taste neurons, we characterized mice in which *Phox2b*-Cre mediated gene recombination labeled neurons with tdtomato. Nerve labels revealed that all taste neurons projecting through the chorda tympani and greater superficial petrosal nerves expressed Phox2b during development, while somatosensory neurons innervating the ear did not. We found robust tdtomato-positive innervation within all taste buds; however, additional regions of the tongue were also innervated. Half of the fungiform papillae had labeled innervation only in taste buds, while the other half also had addition innervation to the epithelium. Chorda tympani nerve transection eliminated all of the labeled innervation to taste buds, but the additional innervation in the fungiform papillae remained. Using these mice, we developed a whole-mount staining preparation which permits 3-dimensional analysis and quantification of innervation within whole taste buds. We conclude that *Phox2b*-Cre mice provide a useful tool for identifying taste from non-taste neurons in the geniculate ganglion, analyzing innervation in whole mount taste buds, and removing genes of interest from gustatory neurons.
Acknowledgements: Supported by NIH grant DC007176.
FCOI Disclosure: None.

#2 POSTER SESSION I

Determining the role of BASP1 in peripheral taste development and maintenance

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The taste receptor cells located in the oral cavity are the primary cells that detect chemicals in potential food items and determine consumption. Currently, our understanding of the development and maintenance of peripheral taste system is still quite limited. Recently we demonstrated that the transcription factor Wilms' tumor protein 1 (WT1) plays an important role in the development of the peripheral taste system. One of the primary interacting partners of WT1 is Brain Acid Soluble Protein (BASP1). When BASP1 is bound to WT1, they associate at the target gene promoters and repress transcription. Therefore we are trying to determine if BASP1 is involved in the development and/or maintenance of the peripheral taste system by regulating the activity of WT1 on its target genes. Our preliminary studies show that BASP1 is expressed in both the developing and adult peripheral taste system. ChIP analyses indicate that BASP1 is bound to the promoter of the known WT1 target genes Lef1 and Ptch1 in adult taste cells. Interestingly, our observations also show that BASP1 is not expressed in the developing CV papillae until the first postnatal week, though it is present in the developing gustatory nerve beginning at E12.5. Since BASP1 is a known corepressor protein, it may aid WT1 in regulating its target genes during the development and maintenance of the peripheral taste system.
Acknowledgements: This work is supported by NSF1256950 and NIH DC006358 to KM.
FCOI Disclosure: None.

#3 POSTER SESSION I

Pannexin 1 knockout mice release ATP and respond normally to all taste qualities

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ATP is required for all taste qualities to communicate with afferent nerve fibers. It is released from Type II taste cells in a non-vesicular manner and activates purinergic P2X2 and P2X3 receptors expressed on gustatory afferent fibers and adjacent taste cells. CALHM1 (Taruno et al., 2013) has been proposed to underlie the release of ATP from Type II taste cells since knockout mice show a significant decrease in their taste responses to sweet, bitter and umami stimuli. However, taste responses are not completely abolished implying that other channels may be involved in ATP release including Pannexin 1 (Panx1) (Huang et al., 2007; Dando and Roper 2009; Murata et al., 2010), Connexin 43, and Connexin 30. In order to clarify the role of Panx1 in taste buds, we used global Pannexin 1 knockout ($Panx1^{tm1b(KOMP)Wtsi}$) mice in a series of experiments. RT-PCR results confirm that Panx1 is not expressed in taste buds and other ATP-releasing channels, including CALHM1, Connexin 43, Connexin 30 are not up-regulated in the knockout. To determine whether Panx1 participates in ATP release from taste cells, a peeled epithelium containing circumvallate taste buds was collected and the amount of ATP released was assessed using a luceferin/luciferase assay upon apical taste stimulation. Results show that taste tissue from Panx1 KO mice release ATP in a similar fashion as the control mice, as also shown previously (Romanov et al., 2012). Furthermore, integrated recordings from the chorda tympani

nerve and behavioral studies did not indicate a difference in the response to various taste stimuli between Panx1 KO and control animals. These results confirm that Panx1 is not necessary for taste evoked release of ATP or for neural and behavioral responses to taste stimuli. Acknowledgements: Funded by NIH grants R01 DC012555 to SCK and P30 DC04657 to D. Restrepo. FCOI Disclosure: None.

#4 **POSTER SESSION I**

Genetic dissection of amine sensitivity in mice

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A fundamental question in olfaction is how individual olfactory receptors contribute to odor perception. The Trace Amine-Associated Receptors (TAARs) are a small set of evolutionarily conserved main olfactory receptors that respond preferentially to amines and that contribute significantly to amine perception. We are using a combination of gene targeting, electrophysiology, in vivo imaging, and behavior to dissect the contribution of individual TAARs to amine sensitivity. Odorant detection thresholds were measured in mice lacking specific TAAR genes using a go—no go behavioral assay. Genetic deletion of all olfactory TAARs causes a 10-fold decrease in sensitivity to isopentylamine and a 50-fold decrease in sensitivity to phenylethylamine. This indicates that TAARs play a significant role in determining behavioral sensitivity to amines. Our electrophysiological and in vivo imaging experiments indicate that the TAARs are broadly tuned to amines. Phenylethylamine preferentially activates TAAR4, and isopentylamine activates both TAAR4 and TAAR3, with TAAR3 being slightly more sensitive. Genetic deletion of TAAR4 by itself elicits a 10-fold decrease in sensitivity to phenylethylamine, indicating that TAAR4 is the most sensitive receptor for this odorant. Behavioral threshold for isopenylamine is not affected by TAAR4 deletion, indicating that isopentylamine sensitivity may be set by TAAR3, or may be set by either TAAR3 or TAAR4. Our results indicate that single olfactory receptors can contribute significantly to odor detection, and that the TAARs are most likely the most sensitive amine receptors. More generally, our approach allows us to characterize for the first time in mammals how chemical detection at the molecular level relates to olfactory performance at the behavioral level. Acknowledgements: NIH/NIDCD F32DC012004(AD), DFG CI 222/1-1(AC), NIH/NIDCD R01DC009640(TB), R01DC013576(TB),. FCOI Disclosure: None.

#5 **POSTER SESSION I**

Genome-scale analysis of olfactory system heterogeneity

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It has long been known that peripheral inputs to the olfactory bulb are labeled, modular, and topographically organized. By contrast, we have comparatively little understanding of whether the bulb's intrinsic circuits show similar modularity. At one extreme, the bulb may consist of only a single canonical circuit providing uniform physiological 'readout' of segregated, parallel inputs. Alternatively, the bulb may be segregated into parallel channels that are physiologically and genetically distinct, allowing for input-specific readout. To investigate these and intermediate possibilities, we have clustered spatially-registered genome-scale expression data from the Allen Brain Atlas (ABA; Lein et al, 2007), using non-negative matrix factorization (NMF). Applying NMF to ABA voxels corresponding to the accessory olfactory bulb (AOB), we observed a robust dichotomous clustering that recapitulated the well known division of the AOB into anterior and posterior divisions. Specifically, we found this division to be driven by genes [leading rank ordered genes for posterior and anterior: Pcbp3 and Fam108b, respectively] and gene categories corresponding to axonal guidance and dopaminergic synaptic transmission functional classes. That is, clustering expression data was sufficient to reveal genetically delineated, spatially contiguous regions of the bulb. In ongoing work, we are using this approach in an exploratory context to identify genetically defined subregions of the bulb that can then be validated using targeted physiological recordings. References: Lein et al (2007). Genome-wide atlas of gene expression in the adult mouse brain. Nature 445: 168-76. Oh et al (2014). A mesoscale connectome of the mouse brain. Nature 508: 207-14. Acknowledgements: Maine INBRE (NIH) P20GM103423 NIH R01 GM076990. FCOI Disclosure: None.

#6 **POSTER SESSION I**

Single olfactory sensory neuron transcriptome analysis

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The main olfactory system detects a wide range of odorants by olfactory sensory neurons (OSNs) expressing the repertoire of 1100 ORs encoded in the mouse genome. A single OSN is thought to express only one type of ORs on one chromosome, making the OSNs as one of the most heterogeneous cell types. These monogenic and monoallelic features of OSNs were supported by various methods including single-cell RT-PCR, fluorescent marker gene knock-in and RNA in situ hybridization, but have not been vigorously verified. Here we applied single-cell

RNA-Seq to ask whether single OSNs exclusively express one OR. We obtained 5-86 million reads per cell (n=26), and average 52.4% of reads uniquely mapped to the genome. Realizing technical challenges of single cell RNA-Seq, we performed a series of control analyses. First, by using the OSNs from hybrid mice (n=22), we observed the monoallelic expression of the X chromosomal genes, verifying that the sequenced materials were derived from single cells. Second, reverse-transcribed cDNAs (n=7) of single cells were split into three tubes and subjected to amplification and sequenced independently as replicates to demonstrate that the biases introduced by amplification only affected the low-expressed genes. Third, the average gene expression of single OSNs was well correlated to the published mature OSNs RNA-Seq data. We adopted recently published full-length OR annotation to improve the quantification of OR expression. Among the 26 sequenced OSNs, we found single OR expression in 20 cells (77%) whereas secondary OR expression were detected in 6 cells (23%). These secondary ORs were much less abundant (< 5%) than the primary ORs. This finding suggests that "one receptor-one neuron rule" is proved in the majority of mature OSNs, but may not be strictly true in all OSNs. Acknowledgements: DC010857. FCOI Disclosure: None.

#7 **POSTER SESSION I**

Molecular profiling of activated olfactory neurons using phosphorylated ribosome immunoprecipitation and RNA-Seq identifies odorant receptors responding to odors in vivo

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The mammalian olfactory system uses a large family of odorant receptors to detect and discriminate amongst a myriad of volatile odor molecules. Understanding odor coding requires comprehensive mapping between odorant receptors and corresponding odors. Here we present a high-throughput *in vivo* method to identify repertoires of odorant receptors responding to odorants, using phosphorylated ribosome immunoprecipitation of mRNA from olfactory epithelium of odor-stimulated mice followed by RNA-Seq. This approach screens the endogenously expressed odorant receptors against an odor in one set of experiments, using awake and freely behaving mice. In combination with validations in a heterologous system, we identify sets of odorant receptors for two odorants, acetophenone and 2,5-dihydro-2,4,5-trimethylthiazoline (TMT), encompassing a total of 73 odorant receptor-odor pairs. We also identified shared amino acid residues specific to the acetophenone or TMT receptors, and developed a model to predict receptor activation. This study provides a means to understand the combinatorial coding of odors *in vivo*. Acknowledgements: This work is supported by grants from the NIH. FCOI Disclosure: None.

#8 **POSTER SESSION I**

Expression of Phospholipase C Isozyme Gene Transcripts in Mouse Olfactory Sensory Neurons and Supporting Cells

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Phospholipase C (PLC) isozymes play important roles in cellular responses to diverse stimuli and modulators. Thirteen distinct PLC isozymes have been identified in mammals, including PLC-β (1-4), γ (1, 2), δ (1, 3, 4), ε, ζ and η (1, 2). These isozymes are distributed in a tissue- and organ-specific manner, exhibiting many unique functions, which are regulated by diverse mechanisms. We previously showed that direct activation of PLC leads to an increase in intracellular Ca^{2+} in mouse olfactory sensory neurons (OSNs) using Ca^{2+} imaging. We also showed that eleven different PLC isozyme gene transcripts are expressed at various levels in the mouse main olfactory epithelium (MOE), using reverse transcriptase polymerase chain reaction (RT-PCR) and real-time qPCR with primers specific for each isozyme (Szebenyi et al., 2014). Here, we determined cell-type specific expression of PLC isoforms in the MOE using RNA *in situ* hybridization (RISH) with riboprobes specific for PLC-β (2, 3,4), γ (1, 2) and η2. We found that PLC-β (2, 3), γ (1, 2) gene transcripts are localized in the OSN layer, whereas PLC β4 is diffusely expressed in multiple cell types in the MOE. We also found positive RISH signal for PLCη2 expression in supporting cells. These results confirm the expression of multiple PLC isozymes in the MOE, suggesting diverse roles of these isozymes in a cell type-specific context. Acknowledgements: This work was supported by research grants NIH/NIDCD 009269 and 012831 to W. Lin and Kuwait University Scholarship to A. Al-Matrouk. FCOI Disclosure: None.

#9 **POSTER SESSION I**

Three-dimensional Synaptic Analyses of Mitral and External Tufted Cell Dendrites in Rat Olfactory Bulb Glomeruli

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Recent physiological studies in the olfactory bulb have provided surprising evidence that olfactory sensory neurons (OSNs) can signal to output mitral cells (MCs) through two parallel paths, a direct OSN-to-MC path and a multi-step path that is partly mediated by external tufted cells (ETs) (OSN-to-ET-to-MC) and appears to be more dominant under a number of experimental conditions. To examine the morphological basis for these signaling mechanisms, we performed ultrastructural studies of the apical dendrites of MCs and ETs in young rats (P9-14). MCs and ETs (n = 2 for each) in bulb slices were filled with biocytin and the slices then fixed and incubated with an avidin-biotin complex and 3,3'-diaminobenzidine (DAB) to form an electron dense substrate within labeled cells. Three-dimensional analyses of DAB-labeled dendrites imaged on an electron

microscope revealed that MCs have a significantly lower density of presumed OSN-synapses than ETs (MCs: 0.3 + 0.06 syn/µm; ETs: 0.54 + 0.09 syn/µm, p< 0.05), although the densities of inhibitory dendrodendritic synapses were similar. In longer dendritic segments (≥ 6 µm), OSN synapses were clustered at the distal ends in MCs, but not ETs. Analysis of unlabeled dendrites within glomeruli indicated that some dendrites displayed both OSN synapses and gap junctions. In the gap junction-rich dendrites, which most likely reflected MCs, OSN synapses were preferentially distributed in distal regions, but gap junctions were evenly distributed. Together, these data suggest that multiple mechanisms could favor a multi-step OSN-to-ET-to-MC signaling over direct OSN-to-MC signaling, including a higher density of OSN synapses on ETs versus MCs, a distal location for OSN synapses on MC dendrites, and proximally-located gap junctions that could act to shunt direct OSN signals. Acknowledgements: NIH R01 DC006640.
FCOI Disclosure: None.

#10 **POSTER SESSION I**

Analytical Processing of Odorant Structure Critically Contributes to Human Olfactory Perception

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Matching human odorant receptors to ligands and odor percepts has seen limited success thus far. Olfactory perception is generally viewed as a configural process strongly driven by experience. As such, small changes in chemical features are believed to have little impact on the overall perception of odor objects. To verify if this is the case, we took advantage of olfactory adaptation and functionally manipulated participants' sensitivities to certain chemical features. We found that two perceptually distinct odorants sharing part of their compositional features (eg. functional group) became perceptually significantly more similar after participants were adapted to a third odorant with their non-shared compositional features. Moreover, the effect was independent of general olfactory habituation. Our results hence argue that analytical processing of odorant structure is indispensible to the representation of odor quality. Acknowledgements: This work was supported by the National Basic Research Program of China (2011CB711000) and the National Natural Science Foundation of China (31422023).
FCOI Disclosure: None.

#11 **POSTER SESSION I**

Odorant-activated Transmitter Release in Olfactory Glomeruli

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In the periphery, olfactory receptors (ORs) are expressed in cilia of olfactory sensory neurons (OSNs) where they mediate detection and transduction of odorous ligands in the external environment. However, OR mRNA and protein expression are also localized centrally in the olfactory bulb, in convergent projections of OSN terminals of glomeruli (Ressler et al, 1994; Barnea et al, 2004; Strotmann et al, 2004). These axonal/presynaptic receptors are thought to participate in axon sorting or guidance during formation of glomerular OR topographic maps. Recent evidence indicates central ORs can couple to canonical olfactory transduction components (adenylyl cyclase, CNG channels), and modulate cAMP and Ca^{2+} in OSN growth cones and terminals in glomeruli (Maritan et al, 2009). Since presynaptic CNG channels can elevate intracellular Ca^{2+} and boost transmitter release from OSN terminals (Murphy & Isaacson, 2003), we reasoned that central ORs should also be able to directly enhance transmission at this first synapse of the olfactory system. To test this, we prepared bulb slices from heterozygous OMP-spH mice and applied optical imaging to detect OSN transmitter release (Bozza et al, 2004). When slices were perfused with different odorants (e.g. eugenol, menthone; 200 µM) we observed sparse, odorant-specific patterns of increased glomerular fluorescence. This is consistent with odorants activating specific central ORs to trigger selective transmitter release from OSN terminals. Our findings suggest possible novel central pathways for modulating odor coding and processing via unknown endogenous OR ligands. Our experimental approach may offer alternative means to study local odor maps in the bulb. Acknowledgements: Supported by a grant from Japan Tobacco, Inc. FCOI Disclosure: This work was supported by a grant from Japan Tobacco, Inc.

#12 **POSTER SESSION I**

Molecular Nearest Neighbors Determine Mouse Olfactory Generalization to Overlapping Odorant Mixtures

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When an animal learns to associate a stimulus (CS+) with reward, this learning may generalize to other, novel stimuli. Typically, the greater the physical similarity between the CS+ and the novel stimulus, the greater the generalization, and the greater the presumed perceptual similarity. However, perceptual relatedness among olfactory stimuli is hard to determine, as the physiochemical similarity does not always predict similar behavioral responses. Natural odors are typically mixtures of multiple components, which pose a further challenge when estimating their perceptual similarities. Similarity can be achieved either by strictly changing the number of overlapping

molecular components (OVERLAP) between stimuli, or by manipulating the overall physicochemical similarity of the components across mixtures (SIMILARITY), or both. We asked how OVERLAP and SIMILARITY contribute to olfactory generalization by training mice to associate a binary reference mixture (CS+) with reward, and then testing response probability to other, perceptually similar binary test mixtures. In 4 experiments, we parametrically varied OVERLAP and SIMILARITY for the test mixtures relative to the reference, and found that OVERLAP is the dominant factor, with SIMILARITY of secondary importance. These results were robust across individuals and strains of mice. We found a good fit between the data and a model in which, for each component 'A' of the CS+, only the similarity of the "nearest" component 'A*' of a test mixture was useful for predicting behavioral generalization, with nearness determined by physicochemical distance. In this model, OVERLAP is a special case of nearness that has outsized influence over generalization. Acknowledgements: Arizona Alzheimer's Foundation. FCOI Disclosure: None.

#13 **POSTER SESSION I**

Mosaic representation of odors in the output layer of the mouse olfactory bulb

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Characterizing the neural representation of chemical space is a formidable challenge in olfaction research. Unlike many other sensory systems, low-dimensional metrics for characterizing odors have remained elusive and it is unclear what features of chemical stimuli are represented by neurons. Here, we have endeavored to relate neural activity in the early olfactory system of mice to the physico-chemical properties of odorants. We imaged odor-evoked responses in identified tufted and mitral cells in awake mice using multiphoton microscopy. Although both mitral and tufted cells responded with diverse amplitudes and dynamics, mitral cells responses were on average sparser and less sensitive to changes in concentration of odorants compared to tufted cells. We characterized odorant features using a comprehensive set of 1,666 physico-chemical properties. Similarity of physico-chemical features of odor pairs was a poor predictor of similarity of the corresponding neuronal representation by mitral or tufted cells. Dimension reduction revealed that ~22 dimensions could explain more than 90% of the variance in neural responses across the population, but fewer dimensions (~12) were necessary if neural activity was projected on to the space of physico-chemical properties. This suggests that factors other than the physico-chemical properties we considered, including non-sensory signals, are required to fully explain the neural responses. We found only limited and variable dependence of mitral/tufted cell position on odorant characteristics. Our data indicate that novel descriptors are needed to link chemical space to neuronal representations and that odor information leaves the bulb in a mosaic pattern, with substantial local diversity. Acknowledgements: na. FCOI Disclosure: None.

#14 **POSTER SESSION I**

The Role of Piriform Associational Connections in Odor Identity and Category Coding

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The ability to identify and categorize odors is critical for making sense out of the complexity of olfactory space, and involves recognizing different odor qualities and retrieving related knowledge and associations. Studies in animals and humans have shown that odor identity and category information is encoded in the form of distributed ensemble patterns in piriform cortex, which receives afferent inputs from the periphery, as well as associational inputs from higher order brain areas. To examine the role of piriform associational connections in odor identification and categorization, we combined functional magnetic resonance imaging (fMRI) techniques with pharmacological manipulation in human subjects in this study. In line with previous animal work, we used a GABA(B) receptor agonist, baclofen, to selectively attenuate associational connections, while leaving the afferent inputs in piriform cortex unaffected. By performing a placebo-controlled, double-blind drug experiment, we found that baclofen weakened odor identity pattern representations in anterior piriform cortex (APC), and weakened odor category pattern representations in the olfactory portion of orbitofrontal cortex (OFC) and posterior hippocampus. These findings suggest that associational connections of human piriform cortex are necessary for constructing neural representations of odor identities and categories, and highlight additional limbic and paralimbic brain areas that help to support these processes. Acknowledgements: NIH institutional training grant T32 AG020506 (to X.B.) Fyssen Foundation grant (to L.R.) NIH grant R01 DC010014 (to J.A.G.). FCOI Disclosure: None.

#15 **POSTER SESSION I**

Perceptual Antagonism in Odor Mixtures: Independence from Odor Quality

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A material in an odor mixture will smell less intense than the material smelled alone at the same vapor-phase concentration. Such perceptual antagonism provides a time-honored route to

diminish an unwelcome odor. One can blend it away without change in its physical presence. The rules of such antagonism reveal themselves even in the elementary case of the binary mixture. We formulated binary mixtures of a body malodor simulant, hexanoic acid, and each of 7 fragrance materials that appear in personal hygiene products. (e.g., lilial, linalol, geranyl acetate). Subsets of subjects from a panel of 17 sought to detect hexanoic acid against a background of just air and equi-intense backgrounds of the fragrances. A protocol from signal detection theory governed presentation of well-controlled stimuli delivered from our Vapor Delivery Device 8. The outcome, evident via psychometric functions for hexanoic acid, displayed no meaningful differences in the antagonistic effects of the fragrance materials. They antagonized the perception of hexanoic acid equally. The uniformity of effect also held upon reversal of malodor and fragrance material. It also held in the dichorhinic case where malodor and antagonist "met" only in the CNS. As found previously, dichorhinic presentation diminished antagonism by about half. Historical results on the perceived intensity of binary mixtures, often noisy because of poor stimulus control and use of subjective scaling techniques, has nevertheless indicated that binary mixtures follow a uniform rule of combination. The outcome goes hand-in-glove with that found here. Such convergence etches an optimistic path toward understanding perception of the complex odor stimulus. Acknowledgements: Acknowledgement: Support from NIDCD [R01] 05602 to WSC. FCOI Disclosure: None.

#16 **POSTER SESSION I**

Drawing the Borders of Olfactory Space

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A common refrain in the olfactory literature is that humans can detect 10,000 different odorants, however both the source and quality of this estimate is unclear. Here we set out to answer this question quantitatively. We developed a model that can distinguish odorous from odorless molecules based on their physicochemical properties with 94% accuracy (AUC = 0.96) in cross-validation. To further test this model, we asked 15 participants to distinguish test molecules from blank jars using five 3-alternative forced choice tests for each compound. In this external validation, our model could distinguish between odorous and odorless molecules with 72% accuracy (AUC = 0.82). Next, we applied the model to the Chemical Universe Database, a collection of 166 billion molecules that are both chemically stable and synthetically feasible with up to 17 atoms of carbon, hydrogen, nitrogen, oxygen, sulfur, or halogens. Since existing catalogs of odorous molecules rarely contain compounds with more than 21 heavy atoms (Reymond, 2014), we then extrapolated the result to 21 heavy atoms. We estimate that there are approximately 2.7 trillion molecules with 21 or fewer heavy atoms. We predict that over 27 billion of these 2.7 trillion molecules will have an odor. Our finding defines the borders of olfactory space, and enables rational sampling of all volatile compounds. Such a set can be applied to build desirable odor screening panels that will facilitate research in the field of olfaction. Acknowledgements: Grants R01 DC013339. FCOI Disclosure: None.

#17 **POSTER SESSION I**

Predicting olfactory abilities from olfactory structure

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Olfaction is vital for survival, helping animals detect and discriminate between predator and prey, food sources, and mates. An animal's sensory system (including olfaction) evolves to suit the demands of its particular ecological niche (described by territory size, habitat, diet etc.). Although studies have elucidated the function of various parts of the olfactory system, understanding how they contribute to the animal's olfactory abilities, and how these abilities relate to its ecology remains an open question. To accommodate the varied demands of different ecologies, evolution must make sensory systems scalable: as peripheral organs become bigger, downstream regions become bigger too, improving computational power and performance. An essential requirement for scalable systems is that sizes of components should vary according to a fixed relation across species. We obtained a quantitative description of two components of the olfactory system (bulb and olfactory cortex) in three species of rodents and one carnivore. We found that the number of neurons in the olfactory cortex scale with the number of glomeruli per olfactory receptor gene (and brain size in rodents): evidence of scalable design. We propose that increasing glomeruli per gene increases sensitivity, matched by an increase in piriform cortex neurons (and odor code resolution), which aids discrimination. Acknowledgements: NSF EAGER Award : 2014-2016. FCOI Disclosure: None.

#18 **POSTER SESSION I**

Olfactory Scene Analysis

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Studies often focus on odor concentration as a potential directional cue for odor source location in olfaction. However, turbulent environments create unsteady, highly discontinuous concentration gradients, raising question as to the reliability of concentration as a directional cue. Other sensory modalities such as vision and audition rely on time for such spatial perception or 'scene analysis'. Time has received less attention in the context of spatial perception in olfaction, although the intermittency

inherent in odor plumes, i.e., the time interval between odor encounters, is known to affect behavioral response to odor plumes. Recent modeling work on a novel population of bursting primary olfactory receptor neurons (bORNs) in the olfactory organ of the spiny lobster showed the capability of bORNs to peripherally encode intermittency. We therefore tested the hypothesis that bORNs play a role in olfactory search. To verify that intermittency can serve as a potential directional cue, we applied recurrence theory to quantify the nonlinear dynamics of realistic odor plumes using the recurrence time index (T2). We show that bORNs can estimate T2 in real time, and that T2 in turn provides a discriminative directional cue. We then developed a real time olfactory search model (an animat) in a synthetic environment to compare the quality of bilateral sensing of intermittency vs the instantaneous concentration. We show that search based on intermittency is significantly more efficient than that based on the instantaneous concentration. Our study suggests that in olfaction, as in vision and audition, time provides a key variable for what can be called 'olfactory scene analysis'. Acknowledgements: Supported by award R21 DC011859 from the NIDCD. FCOI Disclosure: None.

#19 **POSTER SESSION I**

Stereo olfaction sharpens sense of smell

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Bilateral sensory information facilitates the localization of visual and auditory objects in space but its function in olfaction in humans remains to be clarified. Here we address this issue using triangular-forced-choice odor discrimination tasks, comparing detection accuracy in the binaral (different smell to each nostril) and mononaral (same smell to each nostril) conditions. We focus on the effect of an undetectable smell, finding that it remains undetectable in the mononaral condition, but becomes detectable in the binaral condition. Our result demonstrates that, in parallel to vision and audition, binaral olfaction enables stereo sensing and enhances object detection in humans. Our finding sheds new light on the workings of bilaterally distributed sensory processing. In addition, it uncovers a mechanism with which the human nose functions better than we think it does, thereby posing a challenge to the conventional wisdom that human olfaction is a feebly developed evolutionary vestige. Acknowledgements: Baylor College of Medicine internal funding. FCOI Disclosure: None.

#20 **POSTER SESSION I**

The Perception of Odorant Mixtures: Limits on Potency Ratios

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When two odorants are mixed at equal potency the perceived intensity of the mixture is less than the intensity of either when sniffed alone. This mixture suppression can be as high as 40% when the odorants have different odor qualities, i.e. they do not cross-adapt each other (Kurtz, 2009). When 60 different odors were mixed at equal potency they had a faint non-descript odor, "olfactory white" smell (Weiss, 2012). Furthermore, the smell of a random 30-member subset of the mixture of 60 had the same faint white smell, indicating a limit to the number compounds able to form an odor image. Using sniff olfactometry (SO) to study binary mixtures of three compounds methional, methanethiol, and 3,5-dimethylpyrazine (the dominant odors in potato chip head space), we observed that each compound can be perceived in a binary mixture when their potency ratios are between 1:1 and 20:1 or 1:20 (elemental perception). But there is no elemental or configural response at ratios above 30:1 or below 1:30. The implication of these and similar results for odor image formation will be discussed. Acknowledgements: contracts. FCOI Disclosure: None.

#21 **POSTER SESSION I**

Comparison of odor spaces by two approaches: verbal profiling and perceptual similarity rating

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Many studies have investigated odor perceptual spaces by multivariate analysis which was applied to the verbal profile or similarity rating. Since odor category or descriptors used in each study were inconsistent, it is difficult to directly compare the dimensions of odor space. This study investigated odor dimensions by rating methods (verbal profiling vs. similarity rating) and odor categories (daily odors, flavored tea odors, and jasmine tea odors: each eight). Odors became more similar to each other from the categories of daily to jasmine tea odors. The profiling group (n = 30) rated the applicability of 24 adjectives to each odor. The similarity rating group (n = 31) judged the similarities of possible pairs of odors. Principle component analysis was carried out on the profiling data. The 1st component, "pleasantness", was identical in all categories. The 2nd component, "roundness", was commonly shown in both the daily and the flavored tea odors. The 3rd component was different among categories. To compare the odor arrangements between rating methods, multidimensional scaling analyses were carried out. In the profiling data, the 1st dimension was related to pleasantness in the daily and flavored tea odors.

However, in the similarity data, it was related to "edibility" in the daily odors, and "fruitiness" in the flavored tea odors. For the jasmine tea odors, odor arrangements were so different between methods that we could not interpret. While the verbal profiling yielded the pleasantness as primary dimension common to odor categories, the similarity rating yielded the odor spaces represent the principal characteristics of each category. Thus, the perception of odor quality mediated by the provided descriptors may cause the congruence of odor spaces except for odors which have identical quality. Acknowledgements: University of Tsukuba. FCOI Disclosure: None.

#22 **POSTER SESSION I**

Individual Differences in Taste and Odor Sensitivity

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Taste sensitivity has been considered the primary chemosensory factor in studies of chemical senses/ingestive behavior. Our recent findings, however, suggest that retronasal odor perception is equally important in food preference and selection, and further presence of a taste can modulate responsiveness to a retronasally perceived odor. In this study, we measured individual differences in sensitivities to food odors in the presence and absence of a congruent taste. We hypothesized that when measured separately among healthy individuals, variations in sensitivity to retronasal odors are greater than those of tastes, but that these variations are effectively reduced by the presence of a congruent, nutritive taste. Ss were asked to sample 2 tastants (sucrose, NaCl), 4 food odorants (vanilla, strawberry, chicken, soy sauce), and the congruent taste-odor mixtures, and rate intensities for appropriate categories using the gLMS. Tastants were administered by a sip-and-spit procedure while odors were given in odor jars. Results showed that responsiveness to the tastes and odors were generally correlated, although the degree of correlation was much greater for the pairs within a modality than between modalities. Importantly, responsiveness to odors varied greatly among individuals compared to that of tastes. In the presence of a congruent taste, however, responsiveness to the odors was significantly increased, in particular for those who have decreased olfactory sensitivity. These findings imply that an individual's responsiveness to 1 or 2 prototypical tastants or odor cocktails may be good predictors of a person's overall sensitivity for the given modality. The current data also suggest that an individual with low olfactory sensitivity may not recognize the reduced sensitivity when consuming foods. Acknowledgements: Oregon State University. FCOI Disclosure: None.

#23 **POSTER SESSION I**

Smell and Taste, Trait or State? The Influence of Circadian Rhythm on Chemosensory Thresholds

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Gustatory and olfactory sensitivity are presumed to be traits that vary according to individual difference characteristics. However, links between obesity and the timing of food intake raise the issue of whether odor and taste detection may also vary as a function of circadian processes. Twenty-one adolescents (ages 11-16y; 10 female) took part in a 28-hr forced-desynchrony protocol (FD) with 17.5 hours (h) awake and 9.5h of sleep. Odor threshold to PEA was measured using a staircase method with 16-concentrations (Sniffin' Sticks); threshold was calculated as the mean of 4 reversals. Bitter, sweet, salty, and sour taste strips at 4 concentrations were presented lowest to highest; detection was calculated as the first of 2 consecutive correct identifications. [N=18 for taste.] Odor testing preceded taste, began 1h after waking, and repeated at 3h intervals for 6 trials each FD cycle. Circadian phase was determined by dim light melatonin onsets (DLMO). Data were binned by phase (60-degree bins). Odor threshold showed a significant effect of circadian phase, with best detection 60 degrees before DLMO (late afternoon) and worst at 60-120 degrees after DLMO (middle of night/early morning). Only bitter taste showed a significant main effect of circadian phase, with best detection at 120 degrees after DLMO (early morning) and worst detection 120 degrees before DLMO (midday). Detection of the other tastes showed no effects of phase. These preliminary data are the first to show that odor threshold and bitter taste detection are influenced by circadian timing. Our future research will explore how circadian fluctuations in chemosensory sensitivity affect food choice and intake in obese and normal weight adolescents. Acknowledgements: NIDDK grant DK101046. FCOI Disclosure: None.

#24 POSTER SESSION I

Low spontaneous amygdala activity is associated with increased taste intensity perception in humans

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Patients with unilateral resection of the amygdala for pharmacologically intractable epilepsy paradoxically become more sensitive to taste (Small et al., 1997; 1999; 2001). This suggests that the amygdala exerts an inhibitory influence, which when removed enhances sensitivity to afferent taste signals. To test this hypothesis we used real time fMRI to measure spontaneous amygdala activity and timed the delivery of gustatory stimuli to periods of high and low activity. We predicted that the perceived intensity of a salty stimulus (0.18 M NaCl) would be influenced by the state of amygdala activity such that saltiness would be perceived as more intense during low compared to high bouts of spontaneous activity. As predicted, the same salty stimulus was rated as more intense during low compared to high spontaneous activity in four out of five subjects and the difference in spontaneous activity correlated positively with the ratings (r=.927, p=.023). In contrast, although activity in the insula covaried with amygdala response, it was not significantly associated with intensity ratings (r=.622, p=.262). We estimated dynamic causal models for a network consisting of amygdala and insula and tested families of models: those that have information flowing from amygdala to insula (option 1), from insula to amygdala (option 2) and in both directions (option 3). There is a posterior probability of 0.95 for option 1, suggesting changes in activity in the amygdala cause changes in activity in insula. These results suggest that the amygdala exerts a tonic inhibitory influence on the primary gustatory cortex to modulate overall sensitivity to taste. Acknowledgements: R01DC006706. FCOI Disclosure: None.

#25 POSTER SESSION I

Nostril-specific perceptual learning of enantioselectivity

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Optical isomers with almost identical physical and chemical properties could possess different smells to the human nose. Such enantioselectivity in olfaction is partially genetic but can also be acquired through learning, reflecting a high degree of plasticity in our olfactory representations. It is nevertheless under debate whether this plasticity originates in the central or the peripheral components of the olfactory system. Here we show that learning induced perceptual gain, as indexed by the discrimination of odor enantiomers, is largely specific to the trained odor pair and restricted to the trained nostril. Some degree of transfer is observed to an untrained yet structurally similar enantiomeric pair. Our results argue that peripheral mechanisms play a key role in the 'tuning' of olfactory coding by experience. Acknowledgements: This work was supported by the National Basic Research Program of China (2011CB711000) and the National Natural Science Foundation of China (31422023 & 31100735). FCOI Disclosure: None.

#26 POSTER SESSION I

Effects of learning and neuromodulation in a computational model of olfactory bulb and cortex

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Using a combined computational model of olfactory bulb and cortex, we investigate the effects of odor learning as well as cholinergic and noradrenergic modulation on odor processing and memory in olfactory cortex. We first show that olfactory bulb acetylcholine (ACh) modulates the sparseness and coherence of odor representations, leading to better readout and higher learning rates in olfactory cortex. ACh in olfactory cortex allows the formation of attractor memories by enhancing synaptic plasticity and neural excitability. Noradrenergic (NE) modulation in the OB modifies signal to noise ratio by decreasing spontaneous activity and enhancing odor responses. In olfactory cortex, NE modulates excitatory synaptic transmission as well as pyramidal cell and interneuron excitability and further enhances signal to noise ratio. To date, modeling results strongly predictive of behavioral results in the lab. Our model also shows how changes in PC neuronal parameters, experimentally shown to be induced by olfactory rule learning, modulate speed of acquisition and memory performance. We are currently exploring how regulation of ACh and NE occurs, how these two neuromodulators interact and how feedback projections from the PC to OB further modulate olfactory learning. Acknowledgements: R01 DC009948 F32 DC011974. FCOI Disclosure: None.

#27 POSTER SESSION I

Role of Basal Forebrain Cholinergic Neurons in Olfactory Learning

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The ability of the olfactory system to represent sensory cues is strongly influenced by neuromodulators released in response to a constantly changing external environment. Of particular interest

is the neuromodulator acetylcholine (ACh), which has been linked in several brain regions with attention and cue detection. The olfactory bulb and piriform cortex receive abundant cholinergic innervation from the basal forebrain (BF) and it has been suggested that in these regions ACh is required to generate a more efficient olfactory coding necessary for olfactory learning and memory. How BF neurons are activated during active olfactory learning, however, remains unknown. Here, we performed electrophysiological recordings from the BF of awake and freely moving animals exposed to the associative learning paradigm go-no go. This task studies the capacity of a water-deprived rodent to discriminate between two odorants (a rewarded and a non-rewarded odor). To study the exclusive contribution of cholinergic release to olfactory learning at the end of the behavioral session we used selective optogenetic stimulation to identify cholinergic neurons of the BF. Specifically, we stereotactically inserted an optetrode (composed of an optical fiber and four tetrodes) into the BF of animals expressing channelrhodopsin under the control of the choline acetyltransferase promoter, an enzyme that is exclusively expressed in cholinergic neurons. Cholinergic neuron identification was performed by applying blue light directly into this region and by performing off-line spike sorting analysis. Our preliminary results show that cholinergic neurons are recruited prior the cue is delivered and after the odor is presented, suggesting that precise temporal cholinergic release is necessary for proper olfactory learning. Acknowledgements: This work was funded by DC004657 and DC000566.
FCOI Disclosure: None.

#28 **POSTER SESSION I**

Licking activity in the Davis rig is modulated by olfactory conditioning

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The Davis Rig is an apparatus primarily used for analyzing ingestive behavior of liquid substances in small rodents. In this rig, a series of small sipper bottles are mounted in a block so that any one of them can be positioned in front of a drinking slot of a cage. The Davis Rig has been used both to look at ingestive behavior as it relates to stimulus preference, but also as a measure of taste aversion. Previous studies have shown reduced intake of a stimulus when that stimulus has been paired with a malaise-inducing stimulus, for example, lithium chloride (LiCl). To investigate the use of a Davis Rig in aversive olfactory conditioning, we designed a relay device that operates off a 2V signal from the Davis rig, so that every time an animal licks, an air pump pushes odorized air through a tube positioned above the sipper tube opening. Twelve mice were conditioned in one of three experimental groups. One group was exposed to isoamyl acetate in a Davis rig during which time they were injected with 0.3M LiCl. Another group was exposed to isoamyl acetate in a Davis rig during which time they were injected with physiological saline, while a third group was placed in a Davis rig without an odor and injected with LiCl. Two days following conditioning, animals were water deprived and tested 24 hours later in the Davis rig. During testing, all groups were presented with isoamyl acetate while they sampled water and licking behavior was measured. Animals in the first group, which had both isoamyl acetate and LiCl showed an increase in the amount of water consumed compared to the other two control groups. These data suggest that the Davis rig can, in fact, be used for olfactory conditioning, in that animals will associate a conditioned odor with the malaise-inducing effects of LiCl. Acknowledgements: DC 011579. FCOI Disclosure: None.

#29 **POSTER SESSION I**

The Olfactory Tubercle Encodes Odor Valence in Behaving Mice

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Sensory information acquires meaning to adaptively guide behaviors. Despite odors mediating a number of vital behaviors, the components of the olfactory system responsible for assigning meaning to odors remain unclear. The olfactory tubercle (OT), a ventral striatum structure that receives monosynaptic input from the olfactory bulb, is uniquely positioned to transform odor information into meaningful neural codes. No information is available, however, on the coding of odors among OT neurons in behaving animals. In recordings from mice engaged in an odor discrimination task, we report that the firing rate of OT neurons robustly and flexibly encodes the valence of conditioned odors over identity, with rewarded odors evoking greater firing rates. This coding of rewarded odors occurs prior to behavioral decisions and represents subsequent behavioral responses. We predict that associative coding in the OT is a critical mechanism whereby odors acquire meaning in the mammalian brain to guide goal-directed behaviors. Acknowledgements: This work was supported by grants from the National Science Foundation (ISO-1121471), Alzheimer's Association (14-305847), and the Mt. Sinai Healthcare Foundation.
FCOI Disclosure: None.

#30 **POSTER SESSION I**

The coding of odors by single neurons in the olfactory tubercle of mice engaged in a fixed-interval reinforcement task

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Olfactory structures have the capacity to encode both the chemical identity of an odor, and through learning, an odor's valence. Recent work from our lab demonstrates that the olfactory tubercle (OT), a ventral striatum structure that is part of the olfactory network, robustly and rapidly encodes the

valence of odors while mice are engaged in a learning-based odor discrimination task. It still remains unknown, however, in what ways OT neurons encode odors independent of acquired behavioral significance. Therefore, we recorded single-neuron activity from the OT of male c57bl/6 mice as they engaged in a fixed-interval head-fixed task wherein they received a water reward during the inter-trial intervals of pseudorandomly presented odors. In this design, we were able to investigate the coding of odors while still ensuring the mice were attending to a structured operant task similar to that used in odor-learning paradigms. We observed that OT neurons as a population displayed low levels of spontaneous activity. The bulk of neurons displayed significant odor-evoked responses and were recruited uniquely by differing odors. Indeed, in this behavioral context, single neurons were variably tuned, with some representing a narrow-, and others a wide- range of molecularly diverse odors. These results, taken into consideration with our recent findings, suggest that the OT has the capacity to encode both odor identity and odor valence, depending upon the behavioral context. Acknowledgements: This work was supported by National Science Foundation Grant IOS-1121471.
FCOI Disclosure: None.

#31 **POSTER SESSION I**

Large Bilateral Gustatory Cortex Lesions Significantly Impair Taste Sensitivity to KCl and Quinine but Not to Sucrose in Rats

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Recently, we reported that bilateral gustatory cortex (GC) lesions significantly impair taste sensitivity to salts in rats. Here we sought to extend the range of tastants tested to include sucrose and quinine in rats with ibotenic acid-induced lesions in GC (GCX) and in sham-operated controls (SHAM). Presurgically, on a single occasion, immediately after drinking 0.1 M NaCl (15 min), rats received either a LiCl or saline injection (i.p.), but postsurgical tests indicated a weak conditioned taste aversion even in the SHAM LiCl-injected rats. The rats were then trained and tested in a gustometer to discriminate a tastant from water in a two-response operant taste detection task. Psychometric functions were derived separately for each tastant (sucrose, KCl, and quinine, in series) by lowering the stimulus concentration across test sessions. A mapping system was used to determine, in a blinded fashion, acceptable placement, size and symmetry of the bilateral lesions (~90% damage to GC on average). For KCl, there was a significant difference between GCX (n=22) and SHAM (n=13) rats indicated by a rightward shift (ΔEC_{50}=0.57 $\log_{10}$ units, $p< 0.001$) in the psychometric function, replicating our prior work. There was a significant lesion-induced impairment (ΔEC_{50}=0.41 $\log_{10}$ units; SHAM [n=12], GCX [n=19], p=0.006) in quinine sensitivity as well. Although taste sensitivities to KCl and quinine were attenuated, impairment with one stimulus was not significantly correlated with that of the other. Interestingly, unlike what was observed for KCl and quinine, taste sensitivity to sucrose was comparable between GCX (n=25) and SHAM (n=13) rats. Apparently, the degree to which the GC is necessary for the maintenance of normal taste detectability depends on the chemical and/or perceptual features of the stimulus. Acknowledgements: Supported by NIH R01-DC009821 (ACS) and NIH T32-DC000044 (MBB).
FCOI Disclosure: None.

#32 **POSTER SESSION I**

Functional Circuitry of Gustatory Cortex During Active Tasting

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Neurons in gustatory cortex (GC) of alert animals respond to taste stimuli with temporally rich patterns of firing activity that convey information on taste identity and palatability. Although response dynamics are believed to emerge from neural interactions within gustatory circuits, little is known about the GC circuitry responsible for their genesis. Much of the difficulty in studying GC circuits lies in the difficulty of directly accessing this area. We developed a novel lateral surgical preparation that provides direct access to GC for visually targeted recordings in awake head-restrained mice engaged in an active tasting paradigm. This preparation allows consistent animal-to-animal targeting of specific GC subdivisions and accurate depth recordings across cortical layers. Using linear electrode arrays, we have recorded single-units across all cortical layers (layers II/III n=9, layer IV n=5, layer V n=9, layer VI n=4), in spatially targeted subdivisions of GC as mice actively sample stimuli representative of four basic taste qualities (salt, sour, bitter, sweet). Recording locations and depths were verified by post-hoc fluorescent histology. 85% of single-units were responsive during licking (22/26), with 54% (12/22) showing significant taste-specific responses. Response dynamics across neurons are diverse, with subsets of neurons showing sparse and phasic responses to taste stimuli, sustained responses during licking, and clear anticipatory activity ~250ms prior to the first lick during spout movement. This experimental approach will allow us to map the response dynamics observed in actively licking mice onto different layers and subdivisions of GC, and represents the foundation for an in depth circuit-level study of GC taste coding in alert animals. Acknowledgements: DC012543 F-32-DC012461. FCOI Disclosure: None.

#33 POSTER SESSION I

Effects of the Gustatory Cortex on Temporal Coding in the Nucleus of the Solitary Tract of the Rat

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Previous work in our lab has shown that spike timing of taste-evoked spike trains in single cells in the nucleus of the solitary tract (NTS; the first relay in the central gustatory pathway) can convey more information about taste quality than spike count alone. Here, we studied how the centrifugal input from the gustatory cortex (GC) shapes the temporal pattern of taste responses in the NTS. We infused the GC of naïve rats under ketamine (100mg/kg)/xylazine (14 mg/kg) anesthesia with an AAV encoding a channelrhodopsin-2 -EYFP transgene. Following recovery of 2-4 wks, we anesthetized each rat with urethane (1.5 gm/kg) and located the taste-responsive portion of the NTS with a tungsten recording electrode using 0.1 M NaCl as a search stimulus. Once a taste-responsive cell was isolated, neuronal responses to repeated presentations of prototypes of the 5 basic tastes: 0.5 M sucrose, 0.1 M NaCl, 0.01 M HCl, 0.01 M quinine HCl, and 0.1 M MSG +0.01 M IMP were recorded. Each trial consisted of 10 s distilled water, 5 s tastant, 5 s pause, 20 s distilled water rinse. During a random half of the trials, GC axon terminals within the NTS were excited with 25 Hz pulses from a fiber optic cable coupled to a 488nm laser. Tastant delivery and spike timing were collected using Spike2 software (CED, Inc.). Preliminary data revealed that disruption of normal GC signaling to the NTS via optogenetic stimulation of GC axons produced a stimulus-specific reduction of the short interspike intervals produced by a taste stimulus in the first 2 s of response. These data point to a novel influence of the GC on taste responses in the NTS: modification of the temporal arrangement of spikes. The mechanisms and implications of this influence for both neural representation of taste and behavioral taste reactivity are under study. Acknowledgements: NIDCD Grant RO1 DC006914 to PMD. FCOI Disclosure: None.

#34 POSTER SESSION I

Dynamics of ongoing and evoked neural activity in the gustatory cortex

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Neural ensembles in the sensory cortex generate complex patterns of activation in response to sensory stimulations. Such evoked patterns have been traditionally considered distinct from ongoing activity during inter-trial periods. However, recent studies suggest a potential link between the two. Here, we elucidate such relationship in the case of multi-electrode recordings in the gustatory cortex (GC) of alert rats. Taste-evoked ensemble activity in the GC can be characterized in terms of sequences of metastable states, where each state is represented by a quasi-stationary firing rate pattern across neurons. State sequences have been previously considered for their role in memory, decision-making, olfactory and taste coding. Yet, very little is known about the mechanisms responsible for their generation at the network level. We show that state sequences can be observed not only following taste administration, but also during inter-trial periods, in the absence of overt stimulation. We characterized single neuron ongoing activity as attaining 3 or more firing rates across states ("multi-stability"). We present a recurrent spiking network model that accounts for both the observed multi-stability in single neuron firing and the network generation of state sequences in the absence of stimulation. When probed with external stimuli, the model predicted the quenching of single neurons multi-stability into bi-stability and a reduction of trial-by-trial variability. At the population level, the model predicted that stimuli reduce the dimensionality of neural trajectories in the space of ensemble firing rates. All predictions were confirmed in the data. Altogether, our results provide a unifying and mechanistic framework that accounts for both ongoing and evoked neural dynamics in GC.
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FCOI Disclosure: None.

#35 POSTER SESSION I

Stimulus temperature and concentration interact to influence the gustatory response to sodium in the mouse brain stem

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Taste sensitive neurons throughout the gustatory neuraxis respond to oral somatosensory stimuli, like touch and temperature. For example, sucrose sensitive cells in the rostral nucleus of the solitary tract are sensitive to warm solutions and show a reduction in response onset latency when sweet solutions are warmed (e.g. 37° C). Previous studies have shown that epithelial sodium channels in the tongue, which provide gustatory information about sodium to central taste neurons, are largely temperature sensitive, with cool solutions increasing the activity of sodium currents. To investigate the potential influence of temperature on neural activity for sodium taste, we made extracellular recordings from single neurons in the rostral nucleus of the solitary tract of anesthetized C57BL/6J mice during oral application of temperature-and concentration-varied tastants. Stimuli included purified water and a concentration series of sodium chloride (in M): 0.003, 0.006, 0.01, 0.018, 0.032, and 0.056 presented "whole mouth" at 5, 15, 25, and 35° C.

Preliminary analyses of 15 sodium-oriented neurons show that the slope of the mean concentration response function was significantly greater than 0 for all temperatures ($p < 0.003$). The slope for the concentration response function for 35° C (0.4), however, was significantly steeper than slopes measured at lesser temperatures ($p < 0.001$). This was due to suppression of sodium activity at lower concentrations by warming. These data suggest temperature induces systematic effects on unit activity to sodium chloride in sodium-oriented neurons, indicating temperature is a parameter of the neural processing for sodium taste. Acknowledgements: DC011579. FCOI Disclosure: None.

#36 **POSTER SESSION I**

fMRI investigation of the effects of Capsaicin as taste enhancer

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Because cardiovascular diseases seem to be associated with high dietary sodium intake interest in other possibilities to increase the perception of savory food has increased. Recent research suggests that adding capsaicin to a salt solution increases the intensity of salt perception proposing the possibility to use capsaicin as a taste enhancer. Other studies suggest that capsaicin has a certain satiety effect and therefore may also reduce appetite and promote a heathy habit. Guided by this evidence we set up an experiment to investigate the different brain networks involved in the mechanisms associated with integration of taste-pungency sensation in the mouth. Twenty-four healthy young participants (mean age±s.d.=26±3 years) were invited for a gustatory fMRI experiment. Four taste conditions were presented independently: capsaicin (0.9 µM); salt (NaCl, 237.7 mM); iso-volume mixture of salt (NaCl, 64,3 mM) and capsaicin (0,9 µM); and artificial saliva. The concentration levels were set from a psychophysical test included in a prior training session of the population indicating an iso-intense perception between the solutions of salt and mixture (paired t-test (17)= 1.667; p=0.1139). The fMRI results were assessed with ROI specified in the primary and secondary taste areas (insula/frontal operculum and prefrontal cortex, respectively; contrast: tastant vs. artificial saliva; p(FWE-corr)< .05; # of cluster>5). We observed a common brain activity in a site of the insula with maximum in the MNI coordinate (-33, -16, 19). Interestingly the area of activation was bigger when stimulated by the mixture compared to salt and capsaicin alone with the following ranking: Mix>NaCl>C (relative number of voxels, 12> 8> 6). This suggests that low doses of capsaicin increase brain activation to salty stimuli. Acknowledgements: National Natural Science Foundation of China (81270392) and the National Basic Research Program of China (2012CB517805). FCOI Disclosure: None.

#37 **POSTER SESSION I**

Investigating the Trigeminal network using fMRI and Co_2 stimulation

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Introduction: Chemosensory processing is partly based on the interaction between the olfactory and the trigeminal systems. The olfactory system mediates the quality aspect of an odor whereas the trigeminal system conveys sensations such as burning, pungency, stinging etc. Although the olfactory system has received much attention, the neural substrate of intranasal trigeminal stimulation remains poorly understood. The goal of this fMRI study is to delineate the spatial and temporal dynamics of trigeminal network using Independent Component Analysis (ICA). Method: Eleven normal subjects completed the CO_2 fMRI paradigm at 3.0T. CO_2 without change of airflow (6 L/min) was sequentially delivered to either the right, left, or bilateral nostrils with an inter stimulus interval of ~18 sec, and for a total duration of ~10 min. Results: Out of magnet testing reported a success rate of more than 85% to localize CO_2. Consistent with previous research, ICA detected spatial maps containing the brain stem, thalamus, anterior and dorsolateral orbitofrontal cortex and insula. It also detected a map containing POC which has previously been implicated in processing chemosensory stimuli from both olfactory and trigeminal stimulation. Conclusion: Our novel trigeminal fMRI paradigm presented here seems to detect robust activation in brain regions common to both networks. This overlap demonstrate that only when we understand the integration of olfactory, trigeminal and gustatory network processing can we start to fully understand how we process food and flavor. Acknowledgements: This research was supported by the department of radiology, College of Medicine, Penn State University. FCOI Disclosure: None.

#38 **POSTER SESSION I**

Resting state functional connectivity of intranasal olfactory and trigeminal brain networks

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Intranasal chemosensory stimulation results in diverse central processing of the incoming information depending on the relative concentrations of olfactory-specific and trigeminal-specific elements, and yet produces a unified perceptual experience. We studied resting state functional connectivity of these brain systems to determine whether they share network nodes that would allow for sensory integration. 16 healthy adults participated in an eyes closed resting state fMRI study (180 volumes; TR = 2 sec). Preprocessing included motion correction and global signal regression, smoothing with 8 mm3 FWHM, and transformation into MNI space. Seed time courses were extracted in MNI space, defined from coordinates that

purportedly anchor olfactory and trigeminal systems in activation studies [1-2]. Seeds comprising the olfactory network (ON) included piriform cortex and oribtofrontal cortex. Seeds comprising the trigeminal network (TN) included anterior insula and cingulate cortex. Results are reported at $p < .001$, minimum cluster of 20 contiguous voxels. ON showed strong functional connectivity to thalamus, medial PFC, caudate, nucleus accumbens, parahippocampal gyrus, and hippocampus. The TN showed strong functional connectivity to precuneus, thalamus, caudate, brainstem red nucleus, and cerebellum. Right hippocampus was more strongly correlated to the ON than TN. In summary, ON and TN share common nodes in the thalamus, caudate and insula, that may foster sensory integration for perceptual experiences. ON showed stronger functional connectivity to the medial temporal lobe memory system, supporting the relation between olfaction and memory systems. The TN resembled networks involved in central processing of experienced pain, and did not reveal any functional connectivity to networks for conscious memories. Acknowledgements: Supported by the Pennsylvania State University Department of Radiology. FCOI Disclosure: None.

#39 **POSTER SESSION I**

The Effect of Temperature on Umami Taste at the Tongue Tip and in the Whole Mouth

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It is well-known that temperature can affect sweet taste perception in humans, but whether temperature also affects umami taste is unknown. Because the GPCRs for umami and sweetness have in common the temperature-sensitive cation channel TRPM5, we hypothesized that the thermal sensitivity of umami taste would be similar to the thermal sensitivity of sweet taste. 47 Ss (19M, 28F) served in a preliminary session in which the sensitivity to MPG was assessed by dipping the tongue tip into 56, 100, and 200 mM solutions at 21°C and rating umami, sweet, sour, salty, and bitter taste intensity on the gLMS. Ss also sipped samples of 200 mM MPG at 10°, 21°, and 37°C to assess the effects of temperature in the whole mouth. 33 Ss who reliably perceived umami on the tongue tip returned for two sessions in which the effect of temperature on both the sensitivity and adaptation to MPG were measured. The results showed that (1) in both the whole mouth and on the tongue tip, cooling to 10° but not 21°C significantly reduced umami taste intensity compared to 37°C; and that (2) cooling did not affect umami taste adaptation on the tongue tip. The effect of cooling on umami taste intensity was similar to previously reported effects on sweet taste intensity and thus is consistent with the TRPM5 hypothesis. However, the absence of an effect of temperature on umami taste adaptation differs from our recent evidence that cooling increases the rate of sweet taste adaptation. This implies that adaptation occurs at least in part at a stage in transduction prior to TRPM5 (e.g., in the Venus flytrap domain), where cooling may have a greater effect on sustained activation of the T1R2-T1R3 sweet receptor compared to the T1R1-T1R3 umami receptor. Acknowledgements: Supported in part by NIH grant DC05002. FCOI Disclosure: None.

#40 **POSTER SESSION I**

The Effect of Menthol on Flavor Intensity and Nicotine Irritation in an E-cigarette

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A longstanding question that is of increasing interest given the growing popularity of E-cigs is whether menthol can mask, or attenuate the sensory irritation of inhaled nicotine. E-cigs offer a unique aerosol delivery system that enables study of this question independently of the other irritants found in tobacco smoke. In this preliminary study we recruited 32 adult cigarette smokers (16F, 16M) to sample aerosolized e-liquids (70% PG-30% VG) containing 5 different pseudorandomized concentrations of nicotine (0, 6, 12, 18, 24 mg/ml) with and without 2 concentrations of l-menthol (0.5% and 3.5%) delivered using V2™ Cigs (VMR). Ss attended 2 sessions, one for each concentration of menthol, and exposures with and without menthol were blocked within session to avoid carryover effects across trials. After each inhalation Ss rated overall sensation intensity, coolness/coldness, harshness/irritation on the gLMS, and liking/disliking on the LHS. The main findings were that (1) ratings of harshness/irritation were unaffected by 0.5% menthol but were significantly reduced by 3.5% menthol at the highest nicotine concentration (24mg/ml); (2) at the 2 lowest nicotine concentrations 3.5% menthol significantly increased ratings of harshness/irritation and dominated ratings of overall intensity; (3) nicotine did not significantly reduce menthol coolness; and (4) menthol tended to increase liking only for smokers who disliked the no-nicotine and low nicotine aerosols. These findings demonstrate that menthol can in fact suppress airway irritation from inhaled nicotine, but that its effects on E-cig flavor are more complex and include contributions of its own sensory irritancy and cooling to overall intensity and liking. Acknowledgements: NIH grant P50 DA036151. FCOI Disclosure: None.

#41 **POSTER SESSION I**

The evaluation of enhancing effect of spilanthol on carbonation bite by half-tongue method

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Part of the refreshing sensation from carbonated beverages comes from the characteristic bite of carbon dioxide (CO2). There is a strong demand for new ways to enhance carbonation bite, both to decrease gas pressure in plastic bottles and to lower gas pressure of carbonated juice products. After screening a

number of herbs, spices, and extracts for their potential to enhance carbonation bite, we conducted a more focused experiment on spilanthol. To generate objective data, we used a spatial, 2-alternative forced-choice (split tongue) procedure. During each trial, subjects' tongues were pretreated with two filter paper disks (one on each side). One disk contained a sub-threshold amount of spilanthol (3.8 nmol/3.14 cm2). The other disk was a blank. The side pre-exposed to spilanthol was counter-balanced. After 30 seconds of pre-treatment, subjects removed the disks and dipped their tongues into CO2 solution (15 °C, 0.27kgf/cm2) for 20 seconds. Subjects were instructed to choose the side that yielded stronger bite, guessing if uncertain. In a control experiment, subjects dipped their tongues in H_2O (15 °C) instead of CO_2 solution. Sensation on the side pre-treated with spilanthol was chosen more often when subjects dipped their tongues in CO_2 solution (14/18 subjects, p=0.0309 according to a two-sided binomial test). When subjects dipped in water, no pre-treatment effect was observed (10/18 subjects, n.s.). Taken together, the results suggest that spilanthol pre-treatment enhanced carbonation bite, but did not produce noticeable pungency in the absence of carbonation. This result is consistent with recent findings that spilanthol sensitizes CO_2 sensitive neurons. Acknowledgements: This research was supported by Ogawa & Co., Ltd. FCOI Disclosure: None.

#42 **POSTER SESSION I**

Developmental changes in the response profiles of rat trigeminal neuronal populations to capsaicin, nicotine and cooling

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There is considerable remodeling within the trigeminal during perinatal development. The purpose of study is to examine the differential sensitivity of trigeminal neurons to some prototypical irritants and innocuous cooling in neonatal (2-3 days) and adult (5 weeks) rats. We used intracellular calcium imaging to exam neuronal sensitivity to capsaicin, nicotine and physical cooling across the 5 weeks of development. The concentration-response function for capsaicin was slightly different between in neonatal and adult trigeminal neurons (EC50s: 55.6±10.9nM, n=112 and 72.5±4nM, n=137, respectively), while for S-nicotine the concentration-response (0.1-1000uM) functions were similar between the two groups (neonate: n=174 and adult: n=219). The numbers of neurons sensitive to distribution to capsaicin, cooling and nicotine was similar between neonatal and adult. However, neuronal subtypes, based the different combinations of sensitivities, were different in neonates and adults, with cool sensitivity more prevalent in neonate nociceptors than in the adult. Moreover, cooling thresholds were lower in neonate than in adult neurons. This may reflect different thermoregulatory demands at different ages. Acknowledgements: This research was supported in part by Philip Morris, Co. FCOI Disclosure: None.

#43 **POSTER SESSION I**

Trigeminal convergence onto oral sensory neurons in the mouse nucleus of the solitary tract associates with gustatory and thermal tuning

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Fibers of the mandibular branch of the trigeminal nerve (V3) contributing to oral cutaneous sensation project onto neurons in gustatory-sensitive region of nucleus of the solitary tract (NTS), implicating this structure in the integration of taste and oral somatosensation during flavor. However, the pattern of V3 termination onto NTS neurons is poorly understood. Here we studied associations between the oral sensory tuning of thermo-gustatory NTS neurons and their receipt of input from V3. Extracellular recordings (spikes) were made from single NTS units in anesthetized C57BL/6J mice under oral adaptation to 35°C. Stimuli included oral delivery of 25°C tastants (in M: 0.1 NaCl, 0.5 sucrose, 0.01 quinine, and 0.01 citric acid), temperature-adjusted water (5, 15, 25, 35 and 55 °C), and electrical stimulation (1-30 Hz, 10-150 μA) of V3. Cells were classified as receiving input from V3 (V3+) or not (V3-) based on an ability, or lack thereof, to orthodromically follow stimulation of the ipsilateral V3. As positive control, we analyzed V3- neurons only in mice where additional V3+ NTS cells were encountered. Thirty of 59 (51%) taste-responsive NTS units were excited by stimulation of V3. A greater ($P < 0.01$) proportion of NaCl "best" neurons were V3+ (61%) compared to sucrose best cells (32% V3+). Moreover, V3+ neurons displayed greater ($P < 0.05$) entropy in gustatory tuning compared to V3- cells; V3+ neurons were more broadly-tuned across tastants. Considering thermal tuning, responses to oral stimulation with 5°C and 15°C were greater in V3+ compared to V3- neurons ($P < 0.05$). These data demonstrate relationships between thermo-gustatory tuning and receipt of trigeminal input in NTS neurons that may pertain to their neuronal function. Acknowledgements: NIH DC 011579 (CHL).
FCOI Disclosure: None.

#44 **POSTER SESSION I**

Capsaicin, nonivamide and trans-pellitorine decrease free fatty acid uptake without TRPV1 activation and increase acetyl-coenzyme A synthetase activity in Caco-2 cells

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Capsaicin, the most abundant capsaicinoid in red pepper has been associated with hypolipidemic effects in rats. However, mechanistic studies exploring the effects of capsaicinoids on lipid

metabolism are scarce. Here, we studied the effects of the capsaicinoids capsaicin, nonivamide, the aliphatic alkamide *trans*-pellitorine and vanillin, as a basic structural element of all capsaicinoids, on fatty acid uptake in differentiated Caco-2 cells. A dose-dependent inhibition of intestinal fatty acid uptake was shown for capsaicin (IC_{50} 0.49 µM) and nonivamide (IC_{50} 1.08 µM) at a concentration range of 100 nM to 100 µM, whereas *trans*-pellitorine reduced fatty acid uptake by 14.0±2.14% only at the highest test concentration of 100 µM. Since vanillin was not effective, a pivotal role for the alkyl chain with the acid amide group in intestinal fatty acid uptake is hypothesized. Mechanistic studies further revealed that the inhibition of fatty acid uptake by capsaicin, nonivamide and *trans*-pellitorine was neither associated with TRPV1 activation, nor with an activation of the epithelial sodium channel (ENaC). Since paracellular transport mechanisms or the transport of small molecules like glucose were not affected, a direct effect of capsaicin, nonivamide and *trans*-pellitorine on fatty acid transport proteins 2 and 4 was postulated, which was confirmed in a time-course gene expression experiment using RT-qPCR. In addition, an increased acetyl coenzyme A synthetase activity points to an increased fatty acid biosynthesis to compensate decreased fatty acid uptake. Acknowledgements: The financial support by the Austrian Federal Ministry of Economy, Familiy and Youth and the Austrian National Foundation for Research, Technology and Development and the Symrise AG is gratefully acknowldeged. FCOI Disclosure: The authors S. Widder, J.P. Ley and G.E. Krammer are employed at the Symrise AG.

#45 **POSTER SESSION I**

The Detection of Chemical Irritants by the Earthworm, Lumbricus terrestris: Electrophysiology and Immunohistochemistry

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Charles Darwin said of the earthworm: "Without the work of this humble creature, who knows nothing of the benefits he confers upon mankind, agriculture, as we know it, would be very difficult, if not wholly impossible" (1881). Earthworms can change the physical, chemical and biological properties of soil, which may have significant effects on plant growth. The earthworms' movement through soil is dependent, in part, on the chemicals in the soil. Like most organisms, earthworms are repelled by toxins and irritants. However, little is known about how earthworms detect aversive compounds. We have recently begun investigating this question using a variety of techniques. Here we report our preliminary electrophysiological and immunohistochemical results. Chemically receptive neurons in the epithelium send their axons through segmental nerves to the ventral nerve cord. Each segment contains 6 segmental nerves, 3 on each side. Using standard whole-nerve electrophysiological recordings, we have demonstrated that earthworm epithelial receptor neurons respond to acetic acid and allyl isothiocyanate delivered to the epithelium in Ringer's solution. They also differentiate between solutions of different pH. Preliminary immunohistochemistry experiments demonstrate that some earthworm epithelial cells are positive for TrpA immunoreactivity. Other cells appear to be positive for g-protein, G q/11 immunoreactivity. We are continuing our experiments to determine what other compounds, both natural and man-made, may repel earthworms and what the cellular mechanisms responsible for repellency may be. Acknowledgements: Funded by the Department of Biology, Wake Forest University.
FCOI Disclosure: None.

#46 **POSTER SESSION I**

The Detection of Chemical Irritants by the Earthworm, Lumbricus terrestris: Behavior and Scanning Electron Microscopy

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Aristotle referred to earthworms as 'the intestines of the soil'. They keep the soil aerated and full of nutrients. They are often used as biomarkers for healthy soil – the more worms present, the healthier the soil. Chemicals in the soil that are repellent to earthworms could therefore impact an area's ability to grow plants. Although previous behavioral and toxicity studies examined the effect of aversive compounds on earthworms, little is known about how such compounds are detected. We recently began investigating this question using a variety of techniques. Here we report our preliminary anatomical and behavioral results. Two behavioral assays showed that worms can detect and avoid acetic acid (AA) and allyl isothiocyanate (AITC). The T-maze assay consists of a funnel connected to a T-connector. A worm is placed into the funnel and a bright light causes it to move into the T, which has filter paper in both arms, one soaked with water, the other with test compound. Worms significantly moved into the arm containing the water. In the other assay, worms were placed into a clear plastic drinking straw, open at both ends and with a hole punched into the middle. A Q-tip dipped in water or test compound was touched to the anterior, posterior, or middle segments of the worm and the amount of contraction was measured. The anterior and posterior of the worm were sensitive to AA and AITC, but the middle segments were not. This corresponds with our preliminary scanning electron microscopy analysis that shows numerous apparent sensory receptor organs on the anterior and posterior, but few, if any, on the middle segments. We are continuing our experiments to determine what other compounds, both natural and man-made, may repel earthworms and what the cellular mechanisms responsible for repellency may be. Acknowledgements: Funded by the Department of Biology, Wake Forest University. FCOI Disclosure: None.

#47 POSTER SESSION II

Histamine Enhances the Antennal Lobe's Ability to Process Natural High Frequency Stimuli Encountered During Odor Guided Flight

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Active sampling often imposes a periodic structure to sensory stimuli and nervous systems must cope with this stimulus structure. For example, sniffing in mammals and wing-beating in the moth Manduca sexta are behaviors that cause oscillatory airflow over their sensory arrays. Many sensory systems exploit corollary discharge (CD) mechanisms to filter such reafferent signals or otherwise enhance the structured sensory input to optimize sensory processing. CD circuits have been identified in two olfactory pathways, however their role in sensory processing are not clear. In Manduca two histamine (HA) immunoreactive cells project from flight sensory-motor centers to the antennal lobe (AL), and furthermore AL neurons express HA receptors. Therefore, we sought to determine the computational significance of the MDH circuit by means of an ablation study, pharmacology and a suite of analytical techniques. We find that ablating the MDH circuitry reduces the animal's ability to track periodic stimuli, as measured by power spectra and information. Next, using HA and the HA receptor antagonist cimetidine, we show that HA mediates the ability of AL neurons to entrain to periodic stimuli presented at the wingbeat frequency. Using the same pharmacological methods and spatial-temporal measures of distance, we furthermore show that HA enhances while cimetidine diminishes the ability of the AL network to produce odor specific neural representations. This is consistent with psychopharmacological studies showing that blockade of this CD circuit disrupts measures of olfactory acuity. These results support the interpretation that this CD circuit enhances the AL's ability to process high frequency stimuli as experienced during flight when wing beating produces a significant self-generated input. Acknowledgements: RDC009417 to KCD.
FCOI Disclosure: None.

#48 POSTER SESSION II

Antennal lobe local interneurons display heterogeneity of co-transmission

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All neural networks rely on local interneurons to refine the transfer of information between processing layers. These local interneurons endow networks with a wider dynamic range and thus allow for flexibility in information processing. Understanding the mechanisms by which local interneurons refine processing has been challenging due to the remarkable diversity of their morphology, physiology and transmitter content. In the antennal lobe of Manduca sexta, local interneurons do no co-vary in terms of their morphology, physiology or GABA-immunoreactivity. In other words, morphological categories of local interneurons do not predict physiological characteristics or transmitter content. We explored another feature of local interneurons: co-transmission of different neurotransmitters. We sought to determine if local interneurons expressing one transmitter consistently expressed other transmitters allowing local interneurons to be categorized based on the co-transmission of signaling molecules. Using immunocytochemistry we determined that populations of local interneurons expressing any of several neuropeptides had equal proportions of cells expressing GABA. Thus expression of any given neuropeptide was not predictive of co-transmission with GABA. RT-PCR was used to verify the expression of each neuropeptide receptor by neurons contributing to the antennal lobe network. This suggests that local interneurons represent a completely heterogeneous population and a lack of redundancy is likely an integral aspect of the lateral interactions exerted by this highly diverse population of neurons. Acknowledgements: R03 DC013997 and start-up funds from WVU ECAS to AMD.
FCOI Disclosure: None.

#49 POSTER SESSION II

Noradrenergic modulation of information processing in the male accessory olfactory bulb

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The accessory olfactory system (AOS) is an olfactory subsystem that, in rodents, is involved in the detection and interpretation of odorants that carry a social meaning. The first site of information integration in the AOS is the accessory olfactory bulb (AOB). The neuromodulator noradrenaline (NA) is known to regulate activity within this brain region (Araneda & Firestein, 2006), but its effects on odorant information processing have not been systematically assessed. We examined the effects of NA on AOB activity through extracellular recordings of AOB principal neurons in an ex vivo preparation of the male C57/Bl6 and B6D2F1 AOS. This preparation allows for the delivery of stimulus to the intact vomeronasal sensory organ, while performing recordings in a functionally connected AOB. We measured neuronal spiking activity to various vomeronasal odorants before, during, and after applying 10 µM NA (pre-, intra-, and post-drug periods, respectively). In accordance with published results (Brennan & Keverne, 1997), we observed suppression of certain odor responses following repeated odorant stimulation in the presence of NA. Intriguingly, this suppression was stimulus-specific: in our battery, cells that responded to female mouse urine exhibited suppression in the presence of NA ($p < 0.05$). In cells that did not respond to female mouse odorants, significant suppression did not occur. In cells responsive to female mouse urine, the suppression appears to develop with repeated stimulus delivery. These data suggest that NA may not act as a simple gate to odorant suppression as previously suggested, but instead has odorant-specific effects on

AOB information processing. Acknowledgements: This work was supported by a UT Southwestern Institutional Predoctoral Training Award from the National Institute of Neurological Disorders and Stroke to WID (T32NS069562-05) and National Institute on Deafness and Other Communication Disorders award (R00DC011780) to JPM. FCOI Disclosure: None.

#50 **POSTER SESSION II**

Serotonin Modulation of Mitral Cells of the Main versus Accessory Olfactory Bulb

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Because mitral cells of the main olfactory bulb (MOB) typically project one apical dendritic tuft into a single glomerulus, whereas those of the accessory olfactory bulb (AOB) extend several apical dendrites, differential anatomical features across the two systems may lead to functionally distinct information coding. Recent studies have demonstrated that MOB and AOB mitral cells have distinct intrinsic membrane properties but differential neuromodulation has not been well explored. Herein, we investigated a widely-distributed CNS modulator, serotonin (5-HT), for its ability to modulate the biophysical properties of mitral cells, using an *in vitro*, brain slice approach in postnatal 15-30 day mice. Using a whole-cell configuration in current-clamp mode, bath application of 20 µm 5-HT elicited a 4.6 ± 3.1 (Mean ± SD) fold increase in firing frequency of mitral cells in the MOB with a mean onset of 18.2 s. In contrast, 40 µm 5-HT was necessary to elicit consistent neuromodulation in the AOB and with a longer mean onset of 102.6 s. Moreover, AOB neuromodulation appeared to exhibit heterogeneity, where 6% of tested cells were excited (3/50), 78% were inhibited (39/50), and 16% were unresponsive (8/50) to 5-HT. In contrast, 97% of recorded mitral cells in the MOB (88/91) showed an enhanced firing frequency in response to 5-HT. While the mean membrane capacitance (117.1 ± 24.2 pF for MOB, 84.3 ± 29.9 pF for AOB) and input resistance (80.6 ± 21.7 MΩ for MOB, 162.4 ± 67.2 MΩ for AOB) were significantly different, 5-HT modulation across the two systems was reversible and could be blocked by a $5\text{-HT}_{1\text{-}2}$ receptor antagonist. These data suggest that serotonergic pathways may be largely excitatory for MOB mitral cells but largely inhibitory in the AOB. Acknowledgements: This work was supported by RO1 DC003387 from the NIH at the NIDCD. FCOI Disclosure: None.

#51 **POSTER SESSION II**

Smelling without serotonin

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The neurotransmitter serotonin (5-HT) is a powerful modulator of olfactory neural activity and is considered integral for state-dependent changes in olfaction. While several lines of evidence suggest 5-HT is essential for olfactory perception, it remains unknown whether, or if at all, disruption of postnatal 5-HT synthesis affects olfaction. This is in part due to a lack of specificity and efficiency of traditional techniques to disrupt 5-HT transmission. To overcome these limitations, we established a conditional genetic approach that targets postnatal 5-HT synthesis in mice – robustly eliminating whole brain 5-HT levels in a matter of weeks. Using this powerful model, we investigated the behavior of 5-HT depleted and control mice during learning and performance of an olfactory Go-No/Go task. Based upon previous physiological and pharmacological evidence, we hypothesized that adult 5-HT is necessary for normal odor-guided behavior. Surprisingly, the absence of 5-HT had no effect on the ability of mice to learn and perform the odor-based task. 5-HT-depleted mice had similar rates of learning in each phase of the task compared to control-treated mice. Additionally, when comparing correct odor discriminations, 5-HT depleted mice had similar levels of odor acuity. Further, odor reversal learning was identical between 5-HT depleted and control-treated mice. While no direct measures of sniffing behavior have been performed to date, odor sampling times were similar across animals. Relatedly, mice lacking 5-HT displayed normal, coordinated nose-poking behavior and movement from port-to-port suggesting the mice maintained intact motor function throughout the task. These results suggest that adult 5-HT is not required for the basic aspects of olfactory function. Acknowledgements: This work is supported by National Science Foundation grant IOS-1121471 (D.W.W.) and NIH grants F30MH099704 (M.S.W.), R01MH062723 (E.S.D.), P50MH096972 (E.S.D.), and T32GM007250 (CWRU MSTP). FCOI Disclosure: None.

#52 **POSTER SESSION II**

Differential serotonin action on two classes of glomerular inhibitory interneurons

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Glomeruli are the first site of synaptic integration for olfactory signals. Serotonin (5HT) acting on 5HT2A receptors excites external tufted cells (ETCs), key excitatory neurons in the

glomerular circuit (Liu et al., 2012). Here we investigated the action of 5HT on the two major inhibitory glomerular neuron types, GABAergic periglomerular cells (PGCs), which form intraglomerular synapses and GABAergic/dopaminergic short axon cells (SACs), which form intra- and interglomerular projections. Using mice that express GFP in GAD65- or TH-positive neurons, we measured whole cell responses to 5HT of PGCs and SACs, respectively. 5HT evoked direct depolarizing currents in SACs, but not in PGCs. The current was mediated by $5HT2_C$ receptors, as it was blocked by the specific $5HT2_C$ receptor antagonist, SB248084, but unaffected by the $5HT2_A$ receptor antagonist 4F4PP. Serotonin increased spontaneous excitatory and inhibitory postsynaptic currents (sEPSCs and sIPSCs) in both cell types. The sEPSC increase was largely blocked by 4F4PP, indicating increased excitatory drive from ETCs. sIPSC increases were also attenuated 4F4PP, as well as by fast glutamatergic blockers (APV and DNQX), but not by SB248084, implicating increased excitatory drive from ETCs onto interneurons. The net functional effect of 5HT was increased spike rate in SACs, and decreased firing in PGCs. This should enhance intra- and interglomerular inhibition by SACs. Finally, 5HT appears to directly modulate glomerular dendrodendritic synapses independently of action potentials. In the presence of TTX, 5HT significantly increased the frequency of IPSCs in ETCs, SACs and PGCs, indicating a net increase of GABA release and glomerular inhibition. Acknowledgements: NIH NIDCD 005676 and 19015. FCOI Disclosure: None.

#53 **POSTER SESSION II**

Dopamine and GABA modulation of neural transmission in the olfactory bulb in lampreys

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In vertebrates, goal-directed locomotor behaviors, such as homing, predator avoidance or food and mate searching can be triggered by olfactory inputs. The neural substrate underlying olfactory-motor behaviors was uncovered in lampreys (Derjean et al. 2010 PLoS Biol 8(12): e1000567). It consists of a specific neural pathway, extending from the medial part of the olfactory bulb (OB) to the posterior tuberculum. The inputs are then relayed to the Mesencephalic Locomotor Region and eventually reach reticulospinal (RS) cells that constitute the main descending pathway generating swimming in lampreys. This pathway is dedicated to action by rapidly generating motor responses to olfactory cues. Modulatory mechanisms act on this pathway and will introduce variability in the behavioral responses. In this study, we examined the possibility that dopamine and GABA modulate this pathway by using anatomical (tracers and immunohistochemistry) and physiological (intracellular recordings) techniques. Dopamine and tyrosine hydroxylase immunoreactive fibers were present in the OB, but were most abundant in the medial part. GABA immunofluorescence showed dense innervation throughout the OB. Physiological experiments were carried out in the *in vitro* isolated brain of the lamprey. The olfactory nerve was stimulated electrically and synaptic responses were recorded in RS cells. Injections of dopamine in the OB modulated the synaptic responses. Injections of the $GABA_A$ antagonist gabazine into the OB considerably amplified or unmasked excitatory responses of RS neurons to the stimulated olfactory nerve. Taken together, our results show that olfactory inputs are modulated directly in the OB by dopaminergic and GABAergic neurotransmitter systems. Acknowledgements: GLFC 8400272, CIHR 15129, NSERC 217435, FRSQ 5249. FCOI Disclosure: None.

#54 **POSTER SESSION II**

Enzymatic processing regulates synaptically released dopamine in the olfactory bulb

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The efficacy of neurotransmission depends on multiple factors including presynaptic release of transmitter vesicles, post-synaptic receptor populations, and clearance/inactivation of the transmitter from the synaptic cleft. In the olfactory bulb (OB), short axon cells (SACs) form an interglomerular circuit that utilizes GABA and dopamine (DA) as co-transmitters. Selective optical activation of SACs causes GABA and DA co-release, resulting in a fast, postsynaptic GABA inhibitory response and a slower G-protein coupled DA excitation. In most DAergic systems, synaptically released DA is cleared by the dopamine transporter (DAT). However, our previous studies indicated high levels of metabolic DA breakdown products in the OB suggesting that enzymatic catalysis may predominate over re-uptake. To assess this possibility we quantitatively measured the amount of the DA breakdown enzyme catechol-O-methyl-transferase (COMT). Compared to the striatum, the brain's richest source of DA synapses, the OB contains 50% more COMT per mg of tissue. Furthermore, the OB contained dramatically less DAT compared to striatum, supporting the idea that the primary mechanism for DA clearance is COMT enzymatic breakdown rather than DAT recycling. To functionally test the role of COMT in the inactivation of synaptically released DA we employed fast scan cyclic voltammetry in a line of mice expressing cre under the control of the tyrosine hydroxylase promotor to selectively express cre-dependent channel rhodopsin AAV in SACs. Optical stimulation evoked a robust DA voltammetry signal demonstrating DA release in the glomerular layer. Addition of the COMT inhibitor, tolcapone, increased the DA signal ~2-fold. Taken together, these data indicate that the OB preferentially utilizes enzymatic inactivation of synaptically released DA. Acknowledgements: Supported by NIH NIDCD 005676 and 019015. FCOI Disclosure: None.

#55 POSTER SESSION II

The Effect of Dopamine on Investigation of Novel and Habituated Odors

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In rodents, the vomeronasal organ detects conspecific and heterospecific chemosignals and relays information to the medial amygdala via the accessory olfactory bulb. Anterior and posterior regions of medial amygdala (MeA, MeP) project to preoptic and hypothalamic areas involved in production of social behaviors. Responses in the medial amygdala may represent an evaluation of chemosignals, categorizing stimuli based on salience. In mice, immediate early gene FRAs expression in MeA increases after exposure to conspecific and heterospecific stimuli, while exposure to conspecific stimuli increases FRAs expression in MeP only. The main intercalated nucleus (m-ICN), a group of mostly GABAergic neurons lateral to MeP, may modulate MeP activation by preventing response to non-relevant stimuli. For heterospecific stimuli, the mouse m-ICN is active while MeP is suppressed and the inverse is true for responses to some conspecific stimuli. D1 receptors on GABAergic intercalated cell clusters (ITC) have been implicated in modulation of basolateral and central amygdala activity in the fear conditioning circuit. To test the effects of dopamine (DA) on chemosignal recognition, we habituated mice to conspecific or heterospecific odors, administered a DA agonist or antagonist systemically and then tested responses to the same or opposite odor category. We observed time spent sniffing the odors before and after drug administration. As expected, mice receiving vehicle displayed habituation to the first odor with increased interest in a novel odor. While the agonist did not affect this behavior, mice receiving the antagonist did not show increased interest in a novel heterospecific odor, suggesting DA may be involved in chemosignal processing. Its locus of action is currently under study. Acknowledgements: NIDCD grants R01-DC005813 and T32-DC000044. FCOI Disclosure: None.

#56 POSTER SESSION II

Olfactory Metacognition in Patients with Parkinson's Disease

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It is well known that patients with Parkinson's Disease (PD) suffer from olfactory impairment, but it is not clear as to whether patients are aware of any such declines in olfactory functioning. Since PD is a slow and progressive disorder that is correlated with olfactory loss (Ansarai & Johnson, 1975), it is possible that these patients would therefore be subject to errors of over-estimation of olfactory ability (White & Kurtz, 2003). Twelve non-demented PD patients and ten age-matched control participants were each given an objective measure of olfactory functioning (the UPSIT, Doty et al, 1984) and a subjective questionnaire asking them about their olfactory function generally, as well as to self-rate their ability to smell each of 20 odors; 12 of these odors were also assessed on the UPSIT. All of the PD patients showed impaired olfactory ability, as did 4 of the controls. Only errors of over-estimation of olfactory ability were observed in either group: 73% in patients with PD and 30% in controls. When the 12 odors common to both the subjective questionnaire and UPSIT were compared, PD patients showed less metacognitive awareness of their ability than controls on 10 of them. Self-rated olfactory ability and UPSIT scores were significantly correlated in controls ($r = .69$, $p = .03$) but not in patients with PD ($r = .33$, $p = .30$). These results support the idea that olfactory metacognition is often impaired even in controls recruited for normosmic ability (Wehling et al, 2011), and indicate that Parkinson's patients exhibit over-estimation of their olfactory ability at a rate that is generally higher than controls. These findings imply that PD patients, unaware of their olfactory deficit, are at greater risk of harms normally detected through olfaction, such as smoke or spoiled foods. Acknowledgements: Supported by NSERC grant 355938-08 to JD and by Le Moyne Research and Development Sabbatical Award to TLW. FCOI Disclosure: None.

#57 POSTER SESSION II

Decreased Dopamine Receptor D2 Binding Potential is Associated with Better Olfactory Function

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The main aim of this study was to investigate the relation between D2 receptor binding potential (D2R-BP) and olfactory function in a sample of healthy adults. Positron emission tomography (PET) was used to estimate D2R-BP in brain regions involved in olfaction. The radioligands [^{11}C]Raclopride and [^{11}C]FLB 457 were used to estimate binding in striatal and extrastriatal regions, respectively. Fifteen participants (41-65 years of age) were assessed across five different olfactory tasks: olfactory threshold, odor discrimination, episodic odor memory, as well as free and cued odor identification. Correlations (age and/or education held constant) between olfactory function and D2R-BP were not uniform, neither within the targeted brain regions, nor across olfactory functions. Odor discrimination proficiency was significantly correlated with D2R-BP in the associative striatum (AST; $r = .58$) and the amygdala ($r = -.57$). Episodic retention of odors was negatively associated with D2R-BP in the left ventral striatum ($r = -.55$), the amygdala ($r = -.67$), the left side of the parahippocampal region (PH; $r = -.56$) the medial occipital cortex ($r = -.57$) and the left anterior cingulate cortex (ACC; $r = -.57$). Free odor identification was significantly correlated with D2R-BP in the right associative striatum ($r = .63$), the ACC ($r = -.58$), the right medial temporal lobe (MTL; $r = -.55$), the PH ($r = -.59$), the limbic lobe ($r = -.54$)

and the left insular cortex (r = -.54). Overall, these results suggest that lower D2R-BPs in specific regions of the brain is associated with better olfactory function. Acknowledgements: Swedish Research Council 421-2011-1792 and The Swedish Foundation for Humanities and Social Sciences M14-0375:1.
FCOI Disclosure: None.

#58 **POSTER SESSION II**

GABAergic Modulation of Olfactory Bulb Responses to Pheromones and Amino Acids in the Sea Lamprey (Petromyzon marinus)

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Pheromones and amino acids can elicit a behavioral change in the sea lamprey, via an olfactory-motor pathway that transits through the medial region of the olfactory bulb, as well as by a GABA modulated glutamatergic pathway from the lateral olfactory bulb region to brainstem locomotor control centers via the lateral pallium and the posterior tuberculum. While olfactory sensory neurons are located throughout the main olfactory epithelium, pheromones are most strongly detected in the dorsal olfactory bulb region, and amino acids in the lateral olfactory bulb region. The response magnitude may be also affected by the inhibitory GABAergic system whose cell fibers extend into all layers of the olfactory bulb. How GABAergic modulation affects pheromone and amino acid-evoked responses in the sea lamprey was studied by comparing bulbar local field potential responses to odors before, during, and after the local injection of gabazine (SR-95531), a selective GABAA receptor antagonist. Upon gabazine injection into the olfactory bulb, an increase in average maximum peak amplitude was observed in response to three individual pheromones and an amino acid mixture in the dorsal and lateral bulbar regions respectively. Recovery can be seen in all cases. Based on these findings we conclude that GABA modulates responses in the olfactory bulb by modifying peak amplitude of pheromone and amino acid odor responses. These data support findings that endogenous GABA modulates olfactory-mediated locomotor control. Acknowledgements: GLFC 8400272, NSERC 03916. FCOI Disclosure: None.

#59 **POSTER SESSION II**

Asymmetric functional deficit in the primary olfactory cortex of early-stage Parkinson's disease

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The motor symptoms are usually asymmetric at the diagnosis of early-stage idiopathic Parkinson's disease (PD). Pathological assessment has found greater neuronal loss in the substantia nigra (SN) contralateral to the initial symptom onset side. Olfactory dysfunction is a prodromal symptom of PD and prevalent in up to 96% of patients. PD pathology initiates in the olfactory bulb and anterior olfactory nucleus years earlier than in the SN. However, the relationship of olfactory dysfunction with the clinical status of the disease is not clear. Applying a newly developed fMRI technique that can detect odor-induced primary olfactory cortex (POC) activation to the study of 20 early-stage PD patients and 8 age-/gender-matched healthy control subjects, we found that, while the patients' sniffing function in the POC was relatively intact, their odor-induced POC activation was significantly weaker, and such an activation decrease was more severe in the brain hemisphere correspondent to the initial motor symptoms. In contrast, the healthy subjects did not show significant asymmetry in the POC activations. Anatomically the POC structures are paired, and there is no evidence of left-right asymmetry in the normal anatomy or functions of any of these areas. The observed asymmetry of functional deficit in the POC was consistent with the hemisphere-specificity of motor deficits in the early-stage PD. Further studies of this asymmetric function deficit in the central olfactory system may be able to clarify the relationship of olfactory dysfunction with the clinical status of PD. Acknowledgements: The DANA Foundation.
FCOI Disclosure: None.

#60 **POSTER SESSION II**

Drinking espresso influences vigilance – but not olfactory function in healthy subjects

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Objectives: Caffeine, a widely used drug, is known not only to influence the circulatory system or the metabolic systems but also to have effects on the central nervous system. While in animal studies caffeine has shown a positive effect on olfactory function this effect has never been systematically examined in humans. It was therefore the aim of our study to examine the effect of caffeine on olfactory function in humans. Methods: In a randomised study olfactory function using the Sniffin´Sticks test battery in sixty-six healthy volunteers (35 men, 31 women, mean

age: 42 years) was examined twice, once before and 30 minutes after the intake of an espresso, either containing caffeine or being decaffeinated. All participants rated their olfactory function, vigilance state and nasal breathing ability using visual analogue scales. Moreover, vigilance before and after caffeine intake was evaluated usisng the d2 test. Results: Odor thresholds in both groups (caffeine, n= 35; decaff, n= 31) did not differ significantly, neither did the subjective ratings of olfactory function, vigilance or or nasal breathing. In addition, vigilance measured with d2-test did not differ significantly between both groups. Changes of ratings of nasal breathing and olfactory function correlated slightly (r=.27, p=0.03) as well as ratings of olfactory function and general concentration ability (r=.26, p=0.36). Vigilance in all subjects improved during the second test, independent of caffeine intake. Conclusion: In healthy subjects olfactory function is rather stable and is not influenced by a single dose of caffeine. Moreover, in these subjects higher vigilance does not correlate with better olfactory function. Acknowledgements: University funds to AWL. FCOI Disclosure: None.

#61 **POSTER SESSION II**

Short-term effect of caffeine on olfactory function

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Introduction: The purpose of this study was to investigate the potential effects of caffeine in patients with olfactory loss. The suggested mechanisms of action consist in the non-selective blocking of adenosine receptors as well as in phosphodiesterase inhibition. Materials and Methods: Olfactory function was tested twice in 76 patients with olfactory loss. The participants were divided into two groups: one received espresso with caffeine (65 mg/cup), the other espresso without caffeine (placebo). Before and 30 minutes after espresso consumption olfactory function was assessed for phenyl ethyl alcohol odor threshold, and odor discrimination. Results: Across all participants, in comparison to placebo there was no significant effect of caffeine on olfactory function, regardless whether the cause of the olfactory loss was an acute infection of the upper respiratory tract or sinunasal disease. Conclusions: These results indicate that the phosphodiesterase-inhibitor/adenosine-receptor agonist caffeine has little or no short-term effect on olfactory function in patients with olfactory loss. Acknowledgements: University funds. FCOI Disclosure: None.

#62 **POSTER SESSION II**

Taste in a Competitive Environment: A Field Study

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Taste perception is modulated by a variety of extraneous influences, such as emotional manipulation or acute stress. To evaluate the effect of commonplace emotional variation on taste function, basic taste intensity ratings and hedonic evaluations of ice cream samples were collected from approximately 550 attendees following Cornell University men's hockey games spanning the 2013-2014 season. This period encompassed 4 wins, 3 losses, and 1 tie. Since different outcomes at competitive sporting events are known to induce varying affective dimensions, this field study presented a unique environment to evaluate the effect of real-life emotional manipulations on the taste system, where previous research in this field focused on extraneous manipulation within a laboratory environment. Regression analysis revealed that positive emotions correlated with enhanced sweet perception while negative emotions were associated with heightened sour taste. As positive affect grew, hedonic ratings for an ice cream flavor that was less liked overall selectively increased. The results of this field study indicate that everyday emotional manipulations in the form of pleasantly or unpleasantly perceived events may influence sweet, sour, and creamy perception, driving hedonics for less acceptable foods. These results suggest that such modulation in taste perception could promote emotional eating. Acknowledgements: CALS startup funds. FCOI Disclosure: None.

#63 **POSTER SESSION II**

Stress-Related Body Odor Heightens Attention- and Empathy-Related Brain Activity

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Simulation-theories assume, that the perception of other's emotions and one's own feelings share a common neural substrate, the mirror neuron system. The induction of empathy for pain using depictions of painful and non-painful situations is a common approach linking the mirror neuron system to social perception. The current study tested, whether body odor of stressed individuals can modulate the mirror neuron system's response to depictions of painful and non-painful actions. Applying a within-subject design, participants (N = 22) observed pictures of painful and non-painful actions, while an odor was presented via a constant flow olfactometer. Odors were sampled using cotton pads while donors participated in a simulated job interview (modified Trier Social Stress Test; stress odor) or cycled on a stationary bike (neutral odor). Pure cotton was used as control. μ-Activity (8 – 12 Hz), which is inversely correlated with the activity of the mirror neuron system, was measured via

electroencephalography. The μ-activity at central electrodes (C3, Cz, C4) and μ-activity at occipital electrodes (O1, Oz, O2), which served as a control for effects of attention, were calculated using wavelet transformation. As expected, stronger suppression of power in the 8-12 Hz band at central and occipital electrodes in response to painful compared to non-painful actions was observed in the context of cotton control ($p < .01$). However, neutral pictures elicited stronger suppression in the context of stress odor compared to cotton control ($p < .05$) at left electrodes (C3, O1). These results indicate that stress body odor leads to stronger suppression in the 8-12 Hz band and thereby might prime attention allocation and empathy in otherwise neutral situations. Acknowledgements: The study was entirely supported by the Heinrich-Heine-University Duesseldorf. FCOI Disclosure: None.

#64 **POSTER SESSION II**

Rapid Stress System Drives Chemosignaling of Fear in Humans

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Humans can register another person's fear not only with their eyes and ears, but also with their nose. Previous research has demonstrated that exposure to body odors from fearful individuals elicited implicit fear in others. The odor of fearful individuals appears to have a distinctive signature that can be produced relatively rapidly, driven by a physiological mechanism that has remained unexplored in earlier research. The apocrine sweat glands in the armpit that are responsible for chemosignal production contain receptors for adrenalin. We therefore expected that the release of adrenalin through activation of the *rapid* stress response system (i.e., the sympathetic-adrenal medullary system) is what drives the release of *fear* sweat, as opposed to activation of the slower stress response system (i.e., hypothalamus-pituitary-adrenal axis). To test this assumption, sweat was sampled while eight males prepared for a speech (adapted Trier Social Stress Task). Compared to baseline and the "slow stress" condition, the fast stress condition resulted in marginally higher heart rates ($M = 91.91$ beats/min, $SD = 25.75$), $F(2,14) = 5.21$, $p = .052$, and more axillary sweat production ($M = 226.3$ mg, $SD = 142.62$), $F(2,14) = 14.55$, $p < .001$. Importantly, exposure to sweat from participants in the fast stress condition induced in female receivers (N = 31) a simulacrum of the senders' state, evidenced by increased activity of the *medial frontalis*, $F(8,240) = 2.46$, $p = .040$, and *corrugator supercilii* muscle over time, $F(8,240) = 3.27$, $p = .013$ (i.e., the emergence of a fearful facial expression) and faster classification of emotional facial expressions, $F(2,60) = 3.24$, $p = .046$ (i.e., vigilant behavior). Hence, the fast stress system drove the release of what has been labeled "fear chemosignals". Acknowledgements: This research was supported by a TALENT grant from the Netherlands Organization for Scientific Research (406-11-078/MaGW). FCOI Disclosure: None.

#65 **POSTER SESSION II**

Chemosensory Anxiety Signals Prime Defensive Behavior in Prepubertal Girls

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Chemosensory anxiety signals effectively prime motor responses related to withdrawal behavior, such as the startle reflex, in adult humans. The reproductive status, however, affects odor perception, especially regarding social chemosignals. The current study thus aimed to investigate whether chemosensory anxiety signals would augment withdrawal-related motor responses in prepubertal girls. Using cotton pads, axillary sweat was collected from 28 men while waiting for an important oral examination (anxiety odor), and during ergometer training (sport control odor). Using a constant-flow olfactometer, odor samples as well as pure cotton pad samples (cotton control odor) were presented to 10 prepubertal girls aged 9-13 years (M = 11.25, SD = 1.25) for 3000 ms during inhalation. White noise bursts of 102 dB(A) served as startle probes, and startle responses were recorded via electromyography of the orbicularis occuli muscle. The prepubertal girls showed larger startle amplitudes to probes presented in the context of chemosensory anxiety signals as compared to a context of sport control odor ($p < .01$) as well as cotton control odor ($p = .03$). This effect was not attributable to differences in detection rates or hedonic ratings. The results show that in prepubertal girls, similar to adults, chemosensory anxiety signals are able to prime defensive motor behavior. This effect likely resembles a transmission of stress from the signal sender to the perceiving individual, as demonstrated in most species. Thus, chemosensory communication supporting individual and group survival is independent of the reproductive status in humans. Acknowledgements: The study was supported by university funds (Christian-Albrechts-University Kiel, Heinrich-Heine-University Duesseldorf). FCOI Disclosure: None.

#66 **POSTER SESSION II**

Nosewitness Testimony: Effects of Emotional Stress

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Each individual has a unique body odor (BO), similar to a fingerprint. However, in forensic research, identification of culprit BOs has been performed by trained dogs, but not by humans. It is established that emotion can aid encoding and later retrieval. It has also been indicated that stranger BOs are

processed by neural networks also involved in the processing of potential threats (Lundström et al., 2008). We introduce the concept of nosewitness identification and present the first experimental results on BO memory in witness situations involving violent crimes. Two experiments indicated that BO associated with male characters in authentic videos could later be identified in BO line-up tests well above chance. Moreover, culprit BO in authentic and emotional crime videos could be identified considerably better than the BO of a male person in neutral videos. This indicates that nosewitness identification, in contrast to typical eyewitness identification (e.g., Houston, Clifford, Phillips, & Memon, 2013), benefits from emotional encoding. Altogether, the study testifies to the virtue of body odor as a cue to identify individuals observed under negative emotion. Acknowledgements: This study was supported by the Swedish Research Council (Grant 421-2012-1125), the Bank of Sweden Tercentenary Foundation (Grant P12-1017 M. J. Olsson), and from FCT, Portugal (Sandra Soares. FCOI Disclosure: None.

#67 **POSTER SESSION II**

Sex differences in effects of infant odor memory on fear conditioning in adult rats

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In rodents, previous studies have revealed that odors associated with traumatic events during the neonatal period affected their emotionality and anxiety-like behaviors in adulthood. In this study, we investigated whether an odor that was presented rat offspring during neonatal maternal separation (MS) affected their approach to the odor, and the acquisition and retrieval of fear memory in adulthood. Wistar-Imamichi dams and their pups were divided into four groups [MS, MS+odor, no MS (NMS), NMS+odor]. Pups in MS and MS+odor groups were daily separated from their mothers for 3 hours during postnatal days (PND) 2–14. Pups in MS+odor group were exposed to 2-phenylethanol during MS. Pups in NMS+odor group were exposed to the same odor in their home cage with their mother. The odor approach test and contextual fear conditioning were conducted on PND 44-54. In the odor approach test, a cup containing with 2-phenylethanol was placed in the center of an open field, and the time taken by the rats to explore the cup was measured. While the females in MS+odor group spent less exploring the odor than the females in the other groups, the exploration time of the males in MS+odor group was longer than the males in the other groups. For contextual fear conditioning, both conditioning (two 0.2-mA footshocks) and the retention test (24h after the conditioning) were conducted under the odor presentation. In the retention test, the freezing levels of the males in MS and MS+odor groups were lower than males in NMS and NMS+odor groups. However, females in MS+odor group showed higher freezing levels than the females in the other groups. These findings suggest that an odor associated with traumatic events during infancy can induce behavioral alterations in adulthood, and that these effects vary between sexes. Acknowledgements: Japan Society for the Promotion of Science to K.Y. (24530909), Y.I. (24653209) and S.A. (26590175). FCOI Disclosure: None.

#68 **POSTER SESSION II**

Orofacial inflammation induced temporal anxiety phenotypes in mice

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Orofacial inflammation could seriously affect the life quality as well as emotion, including neuroinflammatory pain, eating disturbances, speak disruptions, and even sleep disorders. We compared the emotion influence of two experimental orofacial inflammation: pulpitis (mechanically exposing pulp of left maxillary first molar to the oral environment), and masseter inflammation (unilaterally injection of Complete Freund's Adjuvant) in mice. In the short-term(1 and 3 days after the surgery), both of the two inflammation group decreased the food intake, water consumption and body weight. On the post-operation day 7 and 14, compared with sham group, the inflammation groups showed more anxiety-like phenotypes in the test of open field, elevated plus maze, and duration of appetite inhibition. Ibuprofen could partially relieve the negative behaviors. In the c-fos study, the immunopositive cells was significantly increased in CeA and PBN regions, suggestting the Orofacial inflammation induced abnormal behavior is more similar as visceral pain rather than somatic. Acknowledgements: National Natural Science Foundation of China (Grant No.81371949) Key Program in Medicine Research of Shanghai Science and Technology Commission (Grant No.13411951200). FCOI Disclosure: None.

#69 **POSTER SESSION II**

Free Radical Scavenging Activity in Saliva after a Stressful Task and The effect of Fragrance

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Stress can be defined as a physical, chemical, or emotional factor that leads to biological or mental strain. It can affect our daily life in many ways. Stress may be linked to the onset of degenerative diseases like obesity, high blood pressure, high cholesterol, as well as sleep disorders, depression, and anxiety. Biomarkers are released into the body in response to stress. Alpha amylase, cortisol, immunoglobulin A and chromogranin A are some examples currently used in stress research. They can be measured in saliva produced in response to a stressful task. Oxidative stress

refers to the increased production of reactive oxygen species such as free radicals and peroxides in a biological system. The healthy, normal human body produces molecules that have Free Radical Scavenging Activity (FRSA) which can protect the body from oxidative stress. FRSA can also be measured in saliva. In this study, we examined the FRSA response to a psychosocial stress task and compared it to alpha amylase and cortisol response. We also investigated the effect of smelling fragrance during the stress task. FRSA of saliva was measured using the 1,1-diphenyl-2picryhydrazyl (DPPH) method. We found that, in a laboratory setting, a psychosocial stress task caused a 91.3% increase in salivary FRSA (p=0.01472). All three biomarkers measured, FRSA, alpha amylase, and cortisol increased in response to stress. Additionally, smelling fragrance during the stress task reduced the increase of FRSA, alpha amylase and cortisol levels in saliva versus the control. We conclude FRSA can be another useful biomarker to study the effects of psychosocial stress. Acknowledgements: Takasago International Corp. United States. FCOI Disclosure: None.

#70 **POSTER SESSION II**

Tumor Necrosis Factor-Alpha Antagonist Suppresses Inflammatory Reaction and Ameliorates Olfactory Nerve Recovery Following Injury

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We previously reported that recovery in the olfactory system depends on severity of local injury and anti-inflammatory treatment with steroid or anti-interleukin-6 antibody is effective in improving recovery outcome after olfactory nerve transection. Recently, it is reported that tumor necrosis factor-alpha (TNF-alpha) plays an important role in inflammatory reaction and TNF-alpha antagonist suppresses inflammatory reaction. Actually, etanercept, a TNF-alpha antagonist, is used for clinical treatment of refractory inflammation as the rheumatoid arthritis. The present study was designed to investigate if etanercept is also useful for functional recovery in the olfactory system following injury. We made a model of severe injury by performing olfactory nerve transection using a rigid stainless steel blade in transgenic (OMP-tau-lacZ) mice. Etanercept was injected intraperitoneally just after the nerve transection. Histological assessment of recovery within the olfactory bulb was made at 5, 14, 42 and 70 days after injury. X-gal staining was used to label the degenerating and regenerating olfactory nerve fibers and immunohistochemical staining was used to detect the presence of reactive astrocytes and macrophages. Etanercept-injected animals showed significant smaller areas of injury-associated tissue, less astrocytes and macrophages, and an increase in regenerating nerve fibers in a dose-dependent manner. These findings suggest that TNF-alpha antagonist can be useful as a therapeutic drug for olfactory dysfunction by head injury. Acknowledgements: This work was supported by JSPS KAKENHI Grant Number 26462579. FCOI Disclosure: None.

#71 **POSTER SESSION II**

Intermale aggression induces Arc expression in a select population of accessory olfactory bulb interneurons

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The accessory olfactory system regulates sex-typical behaviors in rodents. The accessory olfactory bulb (AOB) circuit is the first to process sensory information from the vomeronasal organ (VNO), making it a critical link between odor sensation and downstream behavior circuits. Little is known about how AOB circuit elements respond to salient behavioral events, and how those changes may impact future behaviors. We investigated the AOB circuit response to intermale territorial aggression using the immediate early gene *Arc*, which indicates recent activity and is a putative regulator of AOB plasticity. We found that adult resident C57BL6/J males significantly upregulated Arc expression in internal granule cells (IGCs) by 334% compared to controls following a 10-minute exposure to a novel Balb/cJ male intruder. This effect peaked 2 hours after behavior and returned to baseline by 4 hours. 88.7% of labeled cells were found in the posterior AOB, and soiled bedding alone was sufficient to induce *Arc* expression. *Arc* transcription was not induced above baseline in Trpc2-/- mice, which lack functional VNOs, suggesting that VNO input is needed. We used transgenic mice that express GFP in *Arc*-transcribing cells (Arc-d4EGFP-BAC mice) to investigate the physiological effects of *Arc* transcription. We targeted patch clamp recordings to GFP+ and nearby GFP- IGCs in the hours after behavior. We found a group of Arc-expressing IGCs that exhibited a fast-spiking phenotype when depolarized by a current injection. This suggests that these IGCs either undergo a plastic change following exposure to a novel male odor, or that *Arc* induction is specific to this fast-spiking population. These data represent a step towards understanding sensory-mediated plasticity in circuits that guide innate, sex-typical behaviors. Acknowledgements: This work was supported by UT Southwestern startup funds awarded to JPM, R00 DC011780 (NIDCD) awarded to JPM, and T32-DA07290 (NIDA) awarded to HLC. FCOI Disclosure: None.

#72 **POSTER SESSION II**

Taste Signaling Pathways in the Regulation of Gut Inflammation

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Peripheral gustatory system protects our body's homeostasis by selecting nutritious foods and avoiding poisonous or harmful substances. Recent studies indicate that the taste system is involved in much more than sensing food flavor, and taste receptors have been localized in several extra-oral sites, including intestine, pancreas, lungs and testes, suggesting that taste system is utilized by the body for multiple purposes. One of newly revealed interesting and important functions of taste receptors is

to modulate immune reaction/inflammation and sense and respond to microbes. Studies have demonstrated that taste receptor cells contain pathogen recognition components such as Toll-like receptors and produce many immune molecules including cytokines, chemokines, and antimicrobial proteins. Additionally, our preliminary study has revealed the existence of taste signaling transduction molecules, T1R3 and gustducin, in gut mucosal immune cells. In this study, we investigated the roles of taste signaling pathway in regulation of gut mucosal immunity and inflammation using gustducin-/- (KO) mice in dextran sulfate sodium (DSS)-induced colitis model. DSS is a chemical colitogen that can cause intestinal epithelial damage and inflammation. To determine whether gustducin deficiency increases the sensitivity of mice to DSS-induced colitis, we gave gustducin KO mice and wild-type controls 3% DSS in drinking water for 7 days to induce colitis. Our preliminary results show that KO mice had increased weight loss, diarrhea, intestinal bleeding over the experimental period compared to wild-type mice. KO mice also showed more gut inflammation, tissue lesion, and leukocyte infiltration in colon mucosa. These results suggest that taste signaling pathways may play a role in regulating gut inflammation. Acknowledgements: This study was supported by National Institutes of Health/National Institute of Deafness and Other Communication Disorders grants DC010012 (H.W.), DC007487 (L.H.), and P30 DC011735 and by the National Science Foundation Grant DBJ-0216310. FCOI Disclosure: None.

#73 **POSTER SESSION II**

Transgenic Models for Studying the Roles of Inflammatory Cytokines in Taste Disorders

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Taste disorders are frequently associated with inflammatory conditions, such as infections and chronic inflammatory and autoimmune diseases. Our recent studies suggest that inflammation can be a driving factor for the development of taste dysfunction in some diseases. However, the molecular pathways that contribute to the pathogenesis of taste dysfunction remain largely unknown. Inflammatory cytokines are important mediators of inflammation, and several of these cytokines are strongly induced in subsets of taste bud cells under various disease conditions. To further investigate the roles of inflammatory cytokines in taste disorders, we developed transgenic mouse lines using the tetracycline-dependent gene expression system to induce the expression of inflammatory cytokines in taste tissues. We obtained double transgenic mouse lines which express the reverse tetracycline-controlled transactivator (rtTA) under the control of Keratin 5 (K5) promoter and inflammatory cytokines under the control of a tetracycline-responsive promoter element. After administration of doxycycline (a tetracycline analog), these transgenic mice express inflammatory cytokines, such as tumor necrosis factor (TNF) or interferon-g (IFN-g), in K5-positive taste progenitor/stem cells. We also generated triple transgenic mouse lines that express TNF or IFN-g, under the control of doxycycline, in T1R3-postive or PKD2L1-positive taste receptor cells. Our results show that induction of TNF in K5-positive cells strongly attenuates the number and size of taste buds in circumvallate and foliate papillae. The number of Gustducin-positive taste receptor cells was also reduced by TNF induction. These results suggest that inflammatory cytokines, such as TNF, may play important roles in the pathogenesis of taste disorders. Acknowledgements: This study was supported by National Institutes of Health/National Institute of Deafness and Other Communication Disorders grants DC010012 (H.W.) DC013177 (L.H.) and P30 DC011735 and by National Science Foundation grant DBJ-0216310. FCOI Disclosure: None.

#74 **POSTER SESSION II**

Bitter taste is regulated by tumor necrosis factor

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Patients with inflammatory diseases often experience taste alterations. Previously, we showed that tumor necrosis factor (TNF), a potent proinflammatory cytokine, is preferentially expressed in a subset of type II taste cells. The level of TNF in taste cells can be further induced by inflammatory stimuli. However, it remains unclear whether TNF plays a role in regulating taste responses. In this study, we carried out gustatory nerve recordings and taste behavioral tests in wild-type (WT) and TNF-deficient (TNF-KO) mice. Nerve recording experiments showed that the chorda tympani nerve is less responsive to bitter compounds in TNF-KO mice than in WT mice, while its responses to sweet, umami, salty, and sour compounds are comparable in TNF-KO and WT mice. Additionally, the results of the two-bottle preference tests and brief-access gustometer assays also showed that TNF-KO mice are less sensitive to the bitter compound quinine than WT mice. We further showed that TNF receptors, TNFR1 and TNFR2, are both expressed in taste buds cells. Together, these results suggest that TNF signaling may preferentially modulate bitter taste responses and may contribute to taste dysfunction associated with inflammation. Acknowledgements: NIH P30DC011735 (Robert F. Margolskee). FCOI Disclosure: None.

#75 **POSTER SESSION II**

Expression and functions of the GFL-Ret signaling pathway in the development of the peripheral taste system

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In the peripheral taste system, neurotrophin-4 and brain-derived neurotrophic factor, signaling through the TrkB receptor, are important mediators of axon guidance and survival of

chemosensory geniculate neurons projecting to the anterior tongue. While the functions of the neurotrophins in the development of the peripheral taste system have been extensively explored, it is unknown whether additional families of neurotrophic factors are involved in this process. Furthermore, it is unknown whether distinct subpopulations of geniculate neurons exist that can be delineated based on their dependence on different neurotrophic factors, as in other sensory populations. In this study, we provide evidence that the glial cell line-derived neurotrophic factor (GDNF) family ligands (GFLs), signaling through the receptor tyrosine kinase, *Ret*, have an important role in the development of the peripheral taste system. Utilizing Ret reporter lines, we observed that Ret is expressed in a subset of taste receptor cells, a subset of chemosensory and somatosensory axons innervating fungiform papillae, and in a subpopulation of geniculate ganglion neurons. Upon analysis of both *Ret* germline knockout mice and neuron-specific Ret conditional knockout mice, we observed a loss of fungiform taste buds. Furthermore, genetic deletion of *Ret* resulted in a significant reduction of Phox2B expression in the geniculate ganglion. Collectively, these data indicate that Ret is required for the development of geniculate ganglion neurons, and may represent a novel subpopulation with unique functional properties. Ongoing experiments are analyzing the expression of Ret at additional developmental time points, identifying the spatiotemporal expression of the GFLs, and examining whether innervation patterns are altered in *Ret* knockout mice. Acknowledgements: Cure Huntington's Disease Initiative University of Michigan Rackham Merit Grant NIDCR Grant 1F30DE023479-01A1. FCOI Disclosure: None.

#76 **POSTER SESSION II**

Type III, sour-responsive taste cells are preferentially innervated by nerve fibers expressing the serotonin receptor, 5-HT$_{3A}$

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Type III taste cells respond to sour stimuli and release serotonin (5-HT) as well as GABA and norepinephrine. Surprisingly, the role of each of these neurotransmitters in the transmission of sour taste information onto afferent nerve fibers is unclear. In the present study, we used a transgenic mouse line in which GFP is under the control of the 5-HT$_{3A}$ promoter (5-HT$_{3A}$-GFP) to identify a subpopulation of afferent nerve fibers that expresses 5-HT$_{3A}$ receptors and innervates taste buds. Using quantitative immunohistochemistry, we found that nerve fibers expressing 5-HT$_{3A}$-driven GFP preferentially contact Type III taste cells (transducing sour) over Type II taste cells (responding to either sweet, umami or bitter) in all taste fields ($p < 0.01$). Moreover, immuno-electron microscopy demonstrates conventional synapses between Type III taste cells and 5-HT$_{3A}$-GFP labeled nerve fibers, supporting our light microscopic data. Since 5-HT$_{3A}$-GFP fibers preferentially innervate Type III cells, we investigated whether they project centrally to restricted regions of the rostral nucleus solitarius, the primary taste nucleus of the brainstem. We find that 5-HT$_{3A}$-GFP fibers preferentially terminate in the rostral-lateral and lateral parts of the rostral central subnuclei. Finally, we quantified c-Fos staining (a neural activity marker) in response to 30 mM citric acid stimulation via intraoral cannulae and found the greatest number of acid-evoked c-Fos positive cells in areas of the nTS that also show the densest 5-HT$_{3A}$ GFP staining. Together, these data suggest that transmission of sour taste information involves communication between Type III taste cells and 5-HT$_{3A}$ afferent nerve fibers. Acknowledgements: These studies were supported by NIH NIDCD grants R01DC012931 and P30DC004657. FCOI Disclosure: None.

#77 **POSTER SESSION II**

Functional expression of umami taste receptor T1R1/T1R3 in neutrophil

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The G protein-coupled receptor (GPCR) complex T1R1/T1R3 is an amino acid receptor that was discovered in gustatory neurons as a detector of the umami flavor. Since amino acids are mainly generated by food digestion, umami taste receptor expression has been extensively studied in the organs involved in food recognition, intake, and digestion. Furthermore, it has been reported that the increased amino acids throughout the body caused by the injury and infection. Neutrophils play an important role in the initiation of innate immunity against infection and injury. Although many different types of GPCRs are functionally expressed in neutrophils, no reports have demonstrated functional expression of umami taste receptor in these cells. In this study, through RNA sequencing and quantitative RT-PCR analysis, we observed that mouse neutrophils express the umami taste receptor heterodimeric T1R1/T1R3. We also found that stimulation of mouse neutrophils with L-alanine or L-serine, which are ligands for the umami taste receptor, elicited not only ERK or p38 MAPK phosphorylation but also neutrophil chemotactic migration. Moreover, addition of L-alanine or L-serine markedly reduced the production of several cytokines including TNF-α induced by lipopolysaccharide through inhibition of NF-κB activity or STAT3 phosphorylation in neutrophils. Our findings demonstrate that neutrophils express the umami taste receptor, through which tastants stimulate neutrophils, resulting in chemotactic migration, and attenuation of LPS-induced inflammatory response. These findings suggest a novel insight into the functional role of the umami taste receptor in mouse neutrophil. Acknowledgements: This research was supported by National Research Foundation of Korea (2013R1A1A2009145) & Korean Health Technology R&D Project, Ministry for Health, Welfare & Family Affairs (HI13C0423). FCOI Disclosure: None.

#78 POSTER SESSION II

Identification of taste stem/progenitor cells and useful CreERT2 strain to generate conditional gene knockout in adult taste buds

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Lineage tracing is a powerful method to test whether the cells expressing a gene of interest would be stem/progenitor cells, and identifying stem/progenitor cells by this method simultaneously reveals the existence of very useful CreERT2 mouse strain to generate conditional gene knockout in tissue(s) of interest by inducing Cre-mediated recombination in their stem/progenitor cells. Several CreERT2 strains were used for lineage tracing to find taste stem/progenitor cells, but no strain sufficiently induced recombination in stem/progenitor cells. We conducted lineage tracing using *Sox2-CreERT2* knock-in and *Rosa26-tdTomato* reporter strains and found that about 6 months after the tamoxifen injection almost all taste bud cells were labeled with tdTomato as well as non-gustatory oral epithelial cells. Considering the half-lives of taste cells that are about 20 days or shorter, these results suggest that Sox2+ cells that give rise to taste bud cells for long enough are taste stem/progenitor cells. Then, to evaluate the availability of *Sox2-CreERT2* strain to induce a conditional knockout of a gene of interest in taste buds, we compared the expression of Ascl1 in taste buds of Ascl1(*neoflox/neoflox*); *Sox2-CreERT2* and Ascl1(*CreERT2/neoflox*) mice after tamoxifen injection. The Ascl1 expression in taste buds of Ascl1(*CreERT2/neoflox*) mice decreased shortly after tamoxifen injection, but it recovered over time. Contrary, Ascl1 expression was not observed even 7 weeks after the tamoxifen injection in the Ascl1(*neoflox/neoflox*); *Sox2-CreERT2* mice. These results indicate that *Sox2-CreERT2* strain is a useful genetic tool to generate conditional knockout of a gene of interest in taste buds. Together, we found taste stem/progenitor cells and useful CreERT2 strain for molecular genetic studies in taste. Acknowledgements: Supported by Monell Institutional Grants (IM) and NIH P30DC011735 grant (Robert F Margolskee). FCOI Disclosure: None.

#79 POSTER SESSION II

Organization and Synaptic Dynamics of Inhibitory Circuitry Connected to Projection Neuron Populations in the Mouse Rostral Nucleus of the Solitary Tract as Revealed Through Laser Scanning Optogenetic Stimulation

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The rostral nucleus of the solitary tract (rNST) is the target of gustatory primary afferents from the oral cavity. Thus, the rNST is in a prime position to control all aspects of gustatory-related behavior, from perception (parabrachial nucleus [PBN] projections) to reflexes (reticular formation [RF] projections). However, the intrinsic circuitry involved in transforming incoming sensory information into output action potential trains remains relatively unstudied. Using a mouse line expressing channelrhodopsin under the control of the vesicular GABA transporter promoter, we investigated the organization and dynamics of local inhibitory connectivity. Fluorescent microbeads were injected into either the PBN or RF to label neurons projecting to either of these second-order gustatory targets. Local GABAergic circuits were activated with a custom-made laser scanning photostimulation system while recording the resulting inhibitory synaptic currents in an *in vitro* slice preparation. The spatial distributions of inhibitory connectivity for rNST were compared using a normalized linear distance algorithm. RNST-RF projection neurons received inhibitory connections from rNST regions further from the cell soma than rNST-PBN projection neurons. The dendritic morphology of rNST projection neurons as well as the optically evoked synaptic amplitude and rising slope were only moderately correlated to the spatial distribution of inhibitory connectivity. This indicates a specificity of inhibitory interneuron axon targeting rather than a stochastic connectivity. Together, these results suggest the presence of distinct local circuits capable of modulating these diverse rNST output pathways. Acknowledgements: NIH NIDCD Grant DC009982 (RMB), NIH NIDCD Grant R03 DC014306 (JAC), NIH NIDCD Grant T32 DC000011 (JAC). FCOI Disclosure: None.

#80 POSTER SESSION II

Localization of GABA within Crh- or Sst-expressing axon terminals in the PBN

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Corticotrophin-releasing hormone (Crh) is a neuropeptide that diminishes food intake, while the neuropeptide somatostatin (Sst) increases intake and taste preference. Previous work has shown that Crh and Sst cells of forebrain origin project into the taste-sensitive area of the parabrachial nucleus (PBN); this project aimed to investigate synapses in the PBN that contain these neuropeptides and determine if there is a correlation with expression of the inhibitory neurotransmitter GABA. Sst or Crh terminals in the PBN were labeled by crossing Sst-cre or Crh-cre mice with Ai9 TdTomato fluorescent reporter mice. In Sst/TdTomato mouse tissue, seventy-five percent of Sst synaptic terminals (27 of 36) in the PBN contained GABA. In contrast, only 8 of 28 (28%) Crh/TdTomato synaptic terminals in the PBN contained GABA. Additional experiments using viral delivery of fluorescent reporter identified Sst cell types in the central nucleus of the amygdala as a major source of GABAergic input to the PBN. In all cases, the majority of postsynaptic targets did not contain GABA, which likely reflects direct synaptic contacts on PBN projection neurons. These results indicate a mechanism whereby activation of Sst CeA cell types can monosynaptically inhibit PBN neurons and gate the relay of taste signals through the PBN. Acknowledgements: University of Louisville School of Medicine Bridge Award. FCOI Disclosure: None.

#81 POSTER SESSION II

Taste-evoked responses in the nucleus of the solitary tract of 129.B6-Tas1r3 congenic mice

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The *Tas1r3* gene encodes the T1R3 protein, which forms part of a taste receptor that binds sweet compounds. Allelic variation in *Tas1r3* between inbred mouse strains is associated with differences in preferences for and peripheral taste nerve responses to sweeteners. For example, a strain of 129.B6-*Tas1r3* congenic mice has been generated on a 129P3/J (129) genetic background, but with insertion of a small donor fragment from C57BL/6ByJ (B6) mice that includes *Tas1r3*, and the effect of this gene insertion was to increase sweetener preferences in 129 mice to levels similar to those found in B6 mice. In the current experiment, we sought to determine the effects of variation in *Tas1r3* on the strength of the central neural signal evoked by sweeteners. Neural responses were compared between littermates from the 129.B6-*Tas1r3* segregating congenic strain that did or did not have one copy of the B6 allele for *Tas1r3* (B6/129 and 129/129 mice, respectively). The activity of individual neurons in the nucleus of the solitary tract (NST) was isolated in anesthetized animals, and an array of diverse taste compounds was applied to the mouth. Responses to 500 mM sucrose were significantly larger in B6/129 than 129/129 mice. These data provide evidence that *Tas1r3* influences the size of the neural response to sucrose in the first central relay for taste information, and they suggest that the B6 allele for *Tas1r3* may promote ingestion of sugars by increasing their perceived intensity.
Acknowledgements: This work was supported by NIH grants R03 DC005929 to SAMc and R01 DC00882 to AAB.
FCOI Disclosure: None.

#82 POSTER SESSION II

Conditional Knockout of ENaCα Produces Altered Terminal Field Organization of Primary Afferent Terminal Fields in the Mouse Gustatory Brainstem

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The developing nucleus of the solitary tract (NTS) is especially plastic to global experimental manipulations, such as early dietary alterations and postnatal taste nerve injury. We sought here to examine the terminal field organization of the chorda tympani (CT), greater superficial petrosal (GSP), and glossopharyngeal (IX) nerves in mice where only sodium taste was experimentally eliminated throughout development. This was done through a conditional knockout of the alpha subunit of the epithelial sodium channel (ENaCα) in mouse taste buds. Functionally, Chandrashekar et al. (2010) showed a selective lack of functional amiloride taste responses in the CT in mice where ENaCα was conditionally knocked out in taste buds. We crossed homozygous floxed ENaCα mice (Scnn1α$^{lox/lox}$) with mice in which the cytokeratin 19 gene drove expression of Cre-recombinase in taste bud cells. We explored the terminal field organization of the CT, GSP, and IX terminal fields (and their overlapping fields) in adult mice where ENaCα was knocked out during early prenatal development. Data from these mice were compared with their littermates that had the Scnn1α$^{lox/lox}$ gene but not the *cytokeratin 19* gene (controls). Terminal field volumes for each nerve and all of the overlapping fields in knockout mice were at least 200% greater than the respective volumes in control mice ($p < 0.05$). Therefore, the conditional knockout of ENaCα had a profound effect on the terminal field development of nerves that carry sodium taste to the brainstem (i.e., CT & GSP) and also impacted the terminal field of the IX, which does not carry the same salt message to the brain. This suggests a dynamic interaction among the three terminal fields during development that is activity dependent.
Acknowledgements: NIH R01 DC00407.
FCOI Disclosure: None.

#83 POSTER SESSION II

Differential Inhibitory Effects on NST Taste Neurons Studied by Optogenetics

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A significant proportion of neurons in the rostral nucleus of the solitary tract (rNST) is inhibitory, but little is known of their function. We used transgenic mice that expressed the light-sensitive protein, ChR2, (along with EYFP) under the control of GAD65, a synthetic enzyme for the inhibitory neurotransmitter, GABA. The effects of network inhibition on NST gustatory responses were studied by recording taste activity in the presence and absence of light pulses (5ms/10Hz) delivered through optical fibers coupled to single-unit recording electrodes. Preliminary results suggest widespread effects of activating inhibitory, GAD65-expressing neurons (ANOVA, stimulus: P =.008; light: P=.043). A marginal interaction between stimulus and light (P=.07) was suggestive of differential effects. When stimuli were classified as "best" or "2nd-best" for a given neuron, the proportional suppression was greater for the 2nd-best (68%) than the best (18%) stimulus (P=.0002); this also tended to be the case for the absolute decrement; spikes were depressed by 1.6 vs 5.7 Hz, respectively (P=.08). These observations echo those reported by Smith and Li (1998) where the $GABA_A$ antagonist, bicuculline, suppressed sideband more than optimal responses. These results suggest that an important function of inhibition in rNST is to modulate breadth of tuning, perhaps to control stimulus discriminability. In addition, inhibitory effects may be differential according to chemosensitive neuron type; e.g. light suppressed sucrose responses in "sucrose-best" neurons by 41% but NaCl responses by just 5% in "NaCl-best" neurons. Parallel *in vitro* experiments are currently exploring differential effects of

inhibition on rNST responses of different magnitudes evoked by electrical stimulation of the solitary tract. Acknowledgements: NIH RO1 DC000416 NIH R21 DC013676 Seed Grant Funds, OSU College of Dentistry. FCOI Disclosure: None.

#84 **POSTER SESSION II**

Electrical Stimulation of the Anterior but not the Posterior Gustatory Cortex Elicits Taste Reactivity Behaviors in Conscious Rats

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In adult rats, the gustatory cortex (GC) extends about 2.0mm in the anterior-posterior plane within the insular cortex. Dorsal-ventrally the GC includes the granular (GI), dysgranular (DI), and dorsal and ventral agranular (AID and AIV) insular cortices. Although there is evidence that different tastes activate different populations of neurons along the GC's anterior-posterior axis, little is known about the behavioral effects of neurons along this plane. Therefore, in the current study, neurons in the GC were stimulated with bipolar electrodes in conscious rats while taste reactivity (TR) behavioral responses were assessed. The electrodes were implanted into the right anterior, central, or posterior GC under pentobarbital anesthesia in 26 male Wistar rats. After a week of recovery and adaptation to the behavioral arena, TR behaviors were videotaped while weak (40μA, 0.4msec, 50Hz) or strong (200μA) current was used to activate GC neurons. The number and location of activated neurons were determined using Fos immunohistochemistry. Strong stimulation of the central GC (n=5), which activated neurons throughout the insular cortex, elicited both ingestive and aversive TR behaviors compared to unstimulated controls ($p's < 0.01$, n=10). Weak stimulation of the anterior GC (n=7) activated significantly fewer neurons but also increased both ingestive and aversive TR behaviors. On the other hand, weak stimulation of the posterior GC (n=4) activated numerous neurons in the insular cortex but did not elicit TR behaviors. Linear regression analysis suggested that the numbers of Fos immunoreactive neurons in the GI and DI cortices were positively related to the number of TR behaviors performed. These data suggest that neurons in the anterior dorsal GC influence the performance of TR behaviors. Acknowledgements: NSF RUI IOS-1145132. FCOI Disclosure: None.

#85 **POSTER SESSION II**

Parabrachial nucleus projections to ventral tegmental area transmit taste information to the reward system in mice

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The brain reward system responds to sensory information (environmental cues, rewarding stimuli), including taste. Previous studies indicate activation in the reward pathway, including the ventral tegmental area (VTA), after consumption of a palatable meal (Park and Carr, 1998), and taste-evoked dopamine release in the nucleus accumbens is compromised if the parabrachial nucleus (PBN) is lesioned (Hajnal and Norgren, 2006). It is therefore likely that gustatory signals reach the VTA through a projection from the PBN. We used neuroanatomical techniques to determine whether a direct PBN-VTA projection exists and if it functions to transmit gustatory signals in C57BL/6J mice. Anterograde and retrograde neural tracers were injected into the PBN and VTA, respectively. Visualization of labeling in either area confirmed the presence of a direct projection. Following stimulation (using intraoral delivery or in freely licking animals) with 1 M sucrose, a subset of PBN neurons were double labeled with retrograde tracer (from the VTA) and Fos immunoreactivity. These data suggest that the activation of some PBN projection neurons is associated with orosensory stimulation with a sweet taste compound. In the VTA, tyrosine-hydroxylase expressing (i.e. dopaminergic) neurons were co-labeled with sucrose-elicited Fos, and Fos-positive cells were more numerous in wild-type than TRPM5 KO mice, suggesting that many of these downstream dopaminergic neurons are activated due to mice tasting the rewarding sucrose rather than a viscerosensory effect. We conclude that in mice the PBN-VTA projection functions as a relay of taste into the reward circuit. Acknowledgements: This work was supported by the National Institutes of Health grant number R01 DC000353-30. FCOI Disclosure: None.

#86 **POSTER SESSION II**

Electrogustometric statistical evaluation of taste

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Electrogustometry allows eliciting taste sensation in humans and taste nerve responses in rodents by iontophoresis. In anodal stimulation, minute currents move the cations of the subject's saliva including Na+ and H+ toward his/her receptors. Cathodal stimulation in infra-threshold solutions including saccharin or cyclamate anions, etc. elicits sweet taste. Data obtained with a lab-made Electrogustometer including a constant current generator showed taste deficits after chemotherapy, dental desafferentations, stapedectomy and smoking. The recovery of these deficits can be precisely measured over time and seems to vary with the density of papillae: one month at the tip of the tongue (high density), one year at dorsal loci (low density). This quantitative, precise and reproducible electrogustometric threshold evaluation showed a need for methodological and technical improvements. We have developed a digital, 7.5x6x2.5 cm electrogustometer, which is software controlled via a tablet or smartphone. Three protocols are included: 1) a standard free monadic presentation, 2) the same one, associated to a staircase procedure and the calculation of the P50 for detection threshold,

and 3) the same in which the monadic presentation is replaced by a forced-choice pair comparison. The latter allows double blind experiment. A single threshold assessment requires about 10 stimulations and about 3mn. A disk electrode (8mm d.) mounted on a spring to ensure the contact of a constant surface without pressure is compared to a sphere. On-going experiments evaluate protocols, within-subject and across-experimenters reproducibility. With a smart and quick tool, we expect to be able to track the variations of taste sensitivity to various ions under the control of identified modulatory agents. Acknowledgements: Acknowledgements to JY Tiercelin and P Parra, Service Prototypage, CNRS, Gif sur Yvette, France. FCOI Disclosure: None.

#87 **POSTER SESSION II**

Gustatory event-related potentials elicited by different salt tastants

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Introduction: Sodium intake has been associated with increasing risk of chronic diseases like hypertension, stroke or cardiovascular disease. Therefore several campaigns aim to reduce dietary sodium intake and alternatively promote the intake of potassium - a salt also evoking a savory taste similar to sodium chloride, without containing sodium. Aim: In the present study we aimed to investigate the neural activity in the human brain elicited by different savory conditions. We used liquid solutions of potassium chloride (KCl) and sodium chloride (NaCl) for stimulation and recorded gustatory event related potentials with a high density electrodes set-up. Methods: 28 participants were included in the study (mean age±s.d. = 25±2.8 years). 3 tastants in liquid solution were used at iso-intensity: NaCl (295mM), KCl (352mM) and a 50-50 percent mixture of both solutions. The liquid stimuli were delivered by means of a computer controlled gustometer. 50 stimuli of each condition were presented (250 ms stimulus duration; 20s (range: 18-22s) inter-stimulus interval) and after filtering and manual artifact rejection, the grand average was computed for each condition. Results: Microstate segmentation on the grand average revealed similar topography maps for the first 300 ms, indicating similar brain processing up to this point. After 300 ms, substantial differences were detected both from microstate segmentation and a global map dissimilarity test, suggesting different brain structure involved in the late process. This was further confirmed by the analysis of the sources of the indicated maps. Conclusions: Although the neuronal processing seemed to be similar for the two salty stimuli, later processing differed significantly for the two salts, despite the fact they had been rated of similar intensity. Acknowledgements: Funding was provided by a University fund to AWL. FCOI Disclosure: None.

#88 **POSTER SESSION II**

Representation of multisensory signals in the gustatory cortex of rats before and after associative learning

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Recent experimental evidence demonstrates that neurons in the gustatory cortex (GC) are responsive to auditory cues predicting a given taste. However, other sensory modalities appear to be ecologically more relevant to taste than sounds. Indeed images, odors, and tactile stimuli can effectively trigger expectation of taste. Despite the growing evidence describing expectation-related activity in GC, little is known about single neuron responses to the full palette of sensory stimuli. We investigated the responsiveness of single neurons in GC to olfactory (O), visual (V), auditory (A), somatosensory (S) and gustatory stimuli in alert head-restrained rats. Nine rats were chronically and bilaterally implanted with movable bundles of electrodes in GC. In a first group of rats (naïve rats; n=4), orofacial and extracellular activity were recorded while sensory stimuli were randomly delivered without any association with taste. A second group of rats (trained; n=5) were classically conditioned with four sensory stimuli (O, V, A and S) predicting the availability of one taste (sucrose). During the entire training period (14 days) orofacial movements were recorded in order to ascertain the level and time-course of conditioning. At the end of the training, single unit responses to the conditioned cues were recorded from GC. The results showed robust multimodality of GC neurons in naïve rats (13.3, 13.1, 1.3, and 4.1% for O, S, V and A respectively). Specifically, odors and somatosensory stimuli appear to be the most effective. Learning increases the proportion of cue responsive neurons (30.8, 29.6, 7.4 and 15% for O, S, V and A respectively), but does not overturn the bias, unveiling a potential competition between sensory cues to gain associative value. Acknowledgements: this work was supported by SNSF (Swiss National Science Foundation) post-doctoral fellowship (P2GEP3_151816) to R.V. and by R01-DC010389 grant to A.F. FCOI Disclosure: None.

#89 **POSTER SESSION II**

Flavor Detection and Identification: A Decision-Theoretic Framework

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In 1956, WP Tanner (J Acoust Soc Am) set forth a framework, grounded in signal-detection theory, for relating identification (recognition) to detection of tones. The framework avoids

problems associated with comparing criterion-controlled detection thresholds to conventional identification/recognition thresholds, as the latter fail to adequately control criterion. Recently, LE Marks (AChemS 2014) adapted Tanner's theory to taste. Here we extend the adapted theory to multisensory flavor perception. In the current theory, the sensory effects of weak flavorants, olfactory as well as gustatory, are represented as overlapping distributions of events in an internal multidimensional space. The theory assumes that perceivers subdivide the space into regions associated with the possible identification responses. Detection sensitivity, D (akin to d'), reflects the difference between z-transformed probabilities of non-water responses to a given flavorant and non-water responses to water. Identification sensitivity reflects the portion of D attributable to correct identifications and corrected for false identifications of water. The present study asked subjects to identify 3 possible stimuli: water plus 2 non-water flavorants – where either both were olfactory (lemon, strawberry) or 1 was olfactory (lemon, strawberry) and 1 was gustatory (sucrose, citric acid). We tested all 5 possible pairs of non-water stimuli, analyzing results separately for each of 50 sessions. For both olfactory and gustatory flavorants, identification sensitivity was nearly continuous with detection sensitivity. Even flavorant concentrations below detection threshold produced positive levels of both identification and detection sensitivity, as previously reported with gustatory flavorants (Marks, AChemS, 2014). Acknowledgements: Supported by NIH grant R01 DC011823 to LEM. FCOI Disclosure: None.

#90 **POSTER SESSION II**

Effects of Lemon Flavor on Sour and Sweet Tastes, and Vice Versa

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Through experience, olfactory flavorants, such as lemon, may assume the capacity to create or enhance gustatory qualities, such as sour or sweet (e.g. Schifferstein and Verlegh, *Acta Psychol* 1996; Stevenson et al, *Chem Sens* 1999; Gautham and Verhagen, *Chem Sens* 2010). In this study, we mixed an olfactory flavorant, lemon (L) with gustatory flavorants, citric acid (CA) and sucrose (S), to ask how L affects sour and sweet tastes and how CA and S affect citrus flavor. Each of 12 subjects served in 3 counterbalanced conditions, rating the perceived sour, sweet, and citrus intensities on Labeled Magnitude Scales. Condition 1 combined each of 2 levels (zero and 1 non-zero concentration) of each of the 3 flavorants. Conditions 2 and 3 combined each of 3 levels (zero plus 2 non-zero concentrations) of L with each of 3 analogous levels of S (Condition 2) or CA (Condition 3). Statistical analysis (MANOVA) showed that in Condition 1, L significantly increased both sour taste ($p < .025$) and citrus flavor ($p < .001$) but not sweet taste. S significantly increased only sweet ($p < .001$) whereas CA significantly increased citrus ($p = .002$), sweet ($p = .002$), and sour ($p < .001$). In Condition 2, L significantly affected sour ($p = .014$) and citrus ($p < .001$) but not sweet, whereas S significantly affected only sweet ($p < .001$). In Condition 3, L significantly affected sour ($p < .001$) and citrus ($p < .001$) but not sweet. CA significantly affected sour ($p < .001$) and marginally significantly affected citrus ($p = .057$) but did not affect sweet. A critical step will be to parcel out the contributions of multisensory and decisional-cognitive processes to these bidirectional interactions. Enhancement of perceived citrus by CA, for instance, may reflect a sour component in citrus flavor. Acknowledgements: Supported by NIH grant R01 DC011823 to LEM. FCOI Disclosure: None.

#91 **POSTER SESSION II**

Sensory integration of olfactory and visual information in Aedes aegypti mosquitoes

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Olfactory navigation involves integration of multiple modalities, including visual and mechanosensory inputs. Whereas increasing work on sensory integration have been conducted, there has been little work on how olfactory input modulate visual behaviors in bloodhost-seeking mosquitoes. Using behavioral free-flight experiments in a wind tunnel, and tethered flight experiments in a LED arena, we examined how olfactory stimulation modified responses of *Aedes aegypti* mosquitoes to visual displays. First, mosquitoes tethered in a LED arena showed enhanced visual responses when stimulated with certain olfactory stimuli, such as 1-octen-3-ol or human scent. By contrast, other odorants, like benzyl alcohol, elicited little response. Additionally, olfactory stimulation enhanced stripe-fixation during closed-loop visual experiments. Finally, in free-flight assays mosquitoes only responded to visual stimuli when presented with an odor. Together, these results demonstrate that olfactory stimulation increases the salience of visual cues, and that this enhanced visual response is specific to the olfactory input channel. Acknowledgements: R01 DC013693. FCOI Disclosure: None.

#92 **POSTER SESSION II**

Olfactory and visual memory: Same or different?

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INTRODUCTION: It is often assumed that olfactory and visual memory rely on similar mechanisms. This assumption was tested in the experiment reported here. Odor memory performance

depends upon a variety of factors, such as how familiar and easy to name the odors are and may also depend upon the type of task used to probe it. With forced-choice tasks hits cannot be distinguished from correct rejections. This study compared olfactory and visual memory with a particular emphasis on memory task. METHODS: After a delay (15 mins, 48 hours or 1 week) participants completed one of two kinds of memory task (one-stimulus-at-a-time ("monadic") or 3AFC presentation) with common and uncommon odors and pictures, which they had been exposed to incidentally. RESULTS: As expected, memory declined with delay in both tasks and was better for pictures than odors and for common than uncommon items. Performance with common odors on the 3AFC task was better than predicted by chance (33.3%) for all three delays, whereas a signal detection analysis of monadic trials indicated that hit rates dropped to 50% (guessing) after a week, and correct rejections increased with delay. Hit rates for common visual stimuli stayed well above 50%. DISCUSSION: The results from the monadic task suggest that memory for common odors is based on novelty detection after 1 week and that the previously reported longevity of odor memory may be an artifact of using forced-choice tasks. Visual memory, on the other hand, seems to rely on genuine internal representations of memory targets. CONCLUSIONS: Odor memory is not functionally similar to visual memory. We find no support for the previously reported longevity of odor memory. Our results are in agreement with the recently proposed 'misfit theory of conscious olfactory perception' (MITSCOP). Acknowledgements: The study was supported by a Carthage College Faculty Research Scholarship and Creativity Grant. FCOI Disclosure: None.

#93 **POSTER SESSION II**

Complexity of Off Flavor in Orange Juice Made from Fruit Affected by the Huanglongbing Disease

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Citrus greening or Huanglongbing (HLB) is a devastating disease for the citrus industry. Not only do trees die within a few years of being infected, but fruit produced from diseased trees are small, misshapen and do not color properly. Early observations showed that orange juice made with HLB-symptomatic fruit (HLBs) was more bitter, metallic, and sour as well as less sweet than juice made with healthy-looking fruit. As the disease progressed in Florida, juice made with infected HLBs fruit was evaluated from different sources, cultivars and harvest maturities. A "typical HLB" off flavor was defined, but was not consistently described. Increased bitterness was due to increased limonoids, limonin and nomilin, with nomilin contributing to metallic taste, but it was also suspected that other flavonoids impart bitterness as well as astringency and harshness. Volatiles contribute to off-flavor or "flat" flavor, and while no new volatiles were identified, it is the difference in the volatile profile normally found in orange juice that contributes to a specific "off- or flat flavor". Acknowledgements: Citrus Research and Development Foundation. Florida Department of Agriculture and Consumer Services. FCOI Disclosure: None.

#94 **POSTER SESSION II**

Identification of the Off-odor in a Food Packaging Material Using 2D GC-MS/GC-Olfactory Analysis

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Food packaging companies often have odor panels to evaluate materials before selling their product. A Dow customer sought Dow's analytical help to identify an unusual odor generated in their flexible packaging. Using gas chromatography-olfactometry/mass spectrometry analysis, the volatiles from packaging with acceptable odor, as well as the failed material, were analyzed. A trace amount (ppt) of a compound causing a blueberry odor in the failed samples was detected. Through two-dimensional heart-cutting GC-MS experiments, the GC peak of interest was able to be isolated and a mass spectrum obtained. The compound causing the malodor in the failed sample was then postulated to be 2-ethyl-5,5-dimethyl-1,3-dioxane. The compound was then synthesized and confirmed by NMR, 1D and 2D GC-MS. This dioxane is not present in the Dow product and likely to have originated from the ink or film used in the packaging. This research demonstrates the power of combining two-dimensional separations, along with GC-olfactory analysis, to identify a critical trace level compound with a significant odor impact. Acknowledgements: Funding is through The Dow Chemical Company. FCOI Disclosure: None.

#95 **POSTER SESSION II**

Role of Intensates® Flavors in Flavor Perception and Salivation

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Intensates® flavors contain Sensates® ingredients which provide a 3rd dimension to the overall flavor experience. In addition to the traditional (expected) taste and aroma components of a flavor, the Sensates® ingredients deliver a trigeminal experience or chemesthetic benefit. The objective of this study was to determine the impact of different Intensates® flavors in chewing gum on dynamic flavor perception, salivation and chewing behavior. Naïve participants chewed on gum for 15 min and rated the intensity of flavor in 3 minute intervals while their chin position was tracked. Four Intensates® flavors (cooling, tingling, salivating and warming) were added to a citrus flavored gum and compared with a control flavored gum. The chin tracking showed that Intensates® flavors did not affect the chewing behavior (chew frequency, chew motion). Intensates® flavors increased flavor duration as measured by flavor intensity half time by 18-86% ($p < 0.001$). Intensates® salivation flavors had the smallest

effect and warming had the largest effect. Salivary flow rates were significantly affected ($P < 0.001$) by the Intensates® flavors, but saliva pH was not affected. The Intensates® warming flavors always had the higher salivary flow rate, while the Control gum had the lowest value. The number of swallows was higher compared to the control ($p=0.02$), particularly for 'cooling' and 'warming'. Thus the interaction between flavor perception and the chemesthetic effects of the Intensates® flavors increased the lastingness of the flavor during chewing despite a higher salivary flow rate compared to a control gum. FCOI Disclosure: MPM, LM, AJW funded by Takasago International.

#96 **POSTER SESSION II**

Salivary Precipitation Index (SPI) is a better predictor of oral astringency for tannic acid than for alum

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Salivary Precipitation Index (SPI) is a quantitative measure of salivary protein depletion, which reportedly correlates with individual differences in perceived oral astringency. SPI is calculated as the difference in total salivary protein before (S1) and after (S2) stimulation with tannic acid, with a greater absolute value (S2-S1) indicating greater protein depletion. Previously, others have reported that SPI predicts perceived astringency and liking of model systems (i.e. solutions of tannic acid) and real foods (i.e. wine and juices spiked with tannic acid). How this may generalize to other astringents is unknown. Here, the associations between SPI and perceived astringency and liking for a polyphenol (tannic acid) and a multivalent salt (ammonium aluminum sulfate; 'alum') were examined. Participants (n=84) indicated degree of liking and perceived intensity (astringency, bitterness, sweetness, and sourness) of tannic acid and alum in triplicate. Data were analyzed via linear regression, and as discrete groups for comparability with prior reports and to deal with potential nonlinearity in the relationship. In regression, the SPI predicted astringency for both stimuli, but the relationship was much stronger for tannic acid than for alum. For comparability with prior work, three discrete SPI groups were formed, with low (n=21), medium (n=42) and high (n=21) responders. The groups differed in their mean astringency for tannic acid; for alum, the same trend was apparent but the mean differences did not reach significance. SPI did not predict responses in the other attributes measured. As the relationship between protein depletion and perceived astringency appears to differ across stimuli, these data are consistent with the view that astringency may arise from more than one mechanism. Acknowledgements: Supported by funds from the Pennsylvania State University, the Pennsylvania Manufacturing Confectioners' Association (PMCA) and NIH grant DC0010904.
FCOI Disclosure: None.

#97 **POSTER SESSION II**

The Role of Salivary α-Amylase in Taste Perception and Oral Digestion of Starch

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Our previous study showed that humans can taste maltodextrins, primarily maltooligosaccharides (MOSs) present in the stimuli. However, MOSs are not abundant in human diet. Starch, in contrast, account for 60-70% of calories consumed. While it is speculated that starch cannot be tasted due to its insolubility/ bulky structure, starch is hydrolyzed to MOSs by salivary α-amylase during oral digestion. We hypothesized that taste responsiveness would be modulated by differences in sample preparation, tasting time, and amylase activity. Ss rated perceived taste intensities of 4 and 8% raw and cooked starch at 5, 15 and 35 sec. Meanwhile, Ss' saliva was analyzed for α-amylase activity. Results showed that taste responsiveness to all samples was below "barely detectable" and was not influenced by cooking, tasting time, nor level of amylase activity. The latter results were unexpected and prompted an *in vitro* study of starch hydrolysis. Since no tasting time effect was observed in the psychophysical study, we speculated that hydrolysis may occur even before 5 sec. Saliva was collected from Ss with high and low amylase activity and reacted with raw and cooked starch at 2, 15 and 30 sec. Hydrolysis products (DP1-8) were then quantified using HPLC. The results showed that cooking was effective in aiding hydrolysis, which occurred instantly. Total DP2-8 also increased with increasing reaction time. Further, Ss with high amylase activity had higher total DP2-8 than Ss with low activity. Total DP2-8, however, accounted for ~32% max of total hydrolysis products. The current study suggests that cooking, longer tasting time and higher level of amylase activity all help oral digestion of starch, although the amount of MOSs produced at the concentrations tested was insufficient to elicit robust taste responsiveness. Acknowledgements: Oregon State University. FCOI Disclosure: None.

#98 **POSTER SESSION II**

Salivary proteins alter the orosensory response to quinine

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It has been demonstrated that a subset of salivary proteins (SPs) alter acceptance of bitter diets. To determine whether induction of SPs affects the rat's orosensory responses to quinine, we recorded the unconditioned licking responses in a series of brief-access taste tests and, in a second group of animals, the responses of the chorda tympani nerve (CT), in the presence and absence of SPs. First, rats (n=12) were presented with 10 s access to five quinine solutions, in a semi-random order. Half the

animals were tested twice in the control condition, with SPs downregulated; the other half were tested once in the control condition and once while the SPs were upregulated. Curves were fit to the licking data for analysis. While there was no change in the control group's curves (p=0.46), there was a rightward shift of the curve in the experimental group (p=0.049) indicating that the quinine was perceived as less aversive in the presence of the SPs. In a second study, we used saliva as a solvent for 8 concentrations of quinine, which were applied to the tongue while recording multi-unit activity from the CT (n=5). Half of the saliva contained SPs while the remaining half was filtered of proteins. Consistent with our behavioral findings, the presence of SPs in the sample significantly decreased the CT response to quinine (p=0.001) compared to the protein filtered sample. Taken together these results suggest that these SPs reduce the orosensory response to quinine and may play a meaningful physiological role in diet acceptance. Acknowledgements: DC-012632. FCOI Disclosure: None.

#98.5 **POSTER SESSION II**

Menthol Attenuates Chemosensory Irritation and Elevates blood Cotinine in Cigarette Smoke Exposed Mice

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Addition of menthol to cigarettes may be associated with increased initiation of smoking, however, the mechanisms underlying this association are not known. Menthol, possibly through its effects on TRPM8 ion channels in cold-sensing peripheral sensory neurons, is known to inhibit the sensation of irritation elicited by respiratory irritants activating TRPA1, TRPV1 and other chemosensory irritant receptors. However, it remains unclear whether menthol modulates cigarette smoke irritancy and nicotine absorption during initial exposures to cigarettes, thereby facilitating smoking initiation. Using plethysmography in a C57Bl/6J mouse model, we examined the effects of L-menthol, the menthol isomer added to cigarettes, on the respiratory sensory irritation response to primary smoke irritants (acrolein and cyclohexanone) and smoke of reference cigarettes. We also studied L-menthol's effect on blood levels of the nicotine metabolite, cotinine, immediately after exposure to cigarette smoke. L-menthol suppressed the irritation response to acrolein with an apparent IC50 of 4 ppm. Suppression was observed even at acrolein levels well above those necessary to produce a maximal response. Respiratory irritation caused by cigarette smoke was significantly suppressed by L-menthol even at smoke concentrations as high as 300 mg/m3. L-menthol's effects were abolished by treatment with a selective inhibitor of TRPM8, the neuronal cold/menthol receptor. Inclusion of menthol in the cigarette smoke resulted in a ~1.5-fold increase in plasma cotinine levels over those observed in mice exposed to smoke without added menthol. These findings document that, L-menthol, through TRPM8, is a strong suppressor of respiratory irritation responses, even during highly noxious exposures to cigarette smoke or smoke irritants, and increases blood cotinine. Therefore, L-menthol, as a cigarette additive, may promote smoking initiation and nicotine addiction Acknowledgements: Supported by R01HL105635 (to SEJ. and JBM.) and P50DA03615 (SEJ and MRP).
FCOI Disclosure: None.

#99 **POSTER SESSION III**

Nkx2.2 is required for the generation of type III taste receptor cells

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Mammalian taste buds contain multiple types of taste receptor cells that respond selectively to particular tastants. For example, type II taste cells respond to sweet, umami, and bitter tastants. While type III taste cells respond to sour and salty compounds. Throughout adult life new taste receptor cells emerge from the surrounding basal epithelium, mature and senesce with a lifespan of ~1-2 weeks. Transcription factors determine the fates of various cell types, however, the identity of the transcription factors that control differentiation of taste cell types and subtypes remains elusive. We used single cell transcriptomics to identify transcription factors that are selectively expressed in type III taste cells. Nkx2.2, a member of the mammalian NK2 homeobox transcription factor family, was found by single cell transcriptomics to be more highly expressed in type III taste cells than in type II taste cells. Double immunofluorescence microscopy showed that Nkx2.2 was selectively expressed in PKD2L1-expressing type III taste cells, but not in TrpM5-expressing type II taste cells. To determine what role Nkx2.2 might have in taste cell function we examined Nkx2.2 knockout mice. Nkx2.2 knockout mice lacked type III taste cells (based on immunofluorescent staining for PKD2L1). Nkx2.2 knockout mice showed normal preference responses for sweet and bitter compounds and low concentrations of salts. However, unlike wildtype mice, Nkx2.2 knockout mice preferred sour stimuli and high concentrations of salts. We conclude that Nkx2.2 is required for the generation of PKD2L1-expressing type III taste cells. Acknowledgements: NIDCD/NIH grant R01DC03155 to RFM.
FCOI Disclosure: None.

#100 POSTER SESSION III

Osmotic-sensing in taste cells

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Osmotic sensing is an important mechanism for detecting and regulating fluid levels within a cell to maintain homeostasis. The best studied osmotic sensing mechanism is in renal cells and involves water entry through aquaporins (AQP), and activation of volume regulated anion channels. However, understanding details about osmotic sensing and its role in other tissues, such as taste, is limited. Tastants comprise stimuli that range from hypoosmotic to extremely hyperosmotic in nature, yet little is known about how stimulus osmolarity affects taste function. Our current research is focused on exploring the role of osmotic sensing in the peripheral gustatory system. Previously, taste buds have been shown to respond to hypoosmotic stimuli by eliciting anionic currents attributed to activation of the volume regulated anion channels (VRAC) associated with cell volume regulation (Gilbertson 2002). Isolated murine taste cells and Taste Bud Derived (TBD) cell lines were examined for expression of aquaporins by quantitative real time PCR. Similar to our previous findings (Watson et al. 2007), AQP2 and AQP5 were found to be expressed in taste cells and in the TBD cell lines. Calcium imaging was used to assess the ability of hypoosmotic stimuli to lead to changes in intracellular calcium ($[Ca^{2+}]_{in}$). TBD cells were loaded with fura-2 and exposed to solutions ranging from 230 to 310 mOsm. Hypoosmotic stimuli produced osmolarity-dependent increases in $[Ca^{2+}]_{in}$. Similar responses are being investigated in native taste cells. Given that TBD cells respond to taste stimuli, changes in osmolarity and express AQPs, it is anticipated that these cell lines will prove useful for studying osmotic-sensitive pathways in the taste system. Acknowledgements: UTA01082 from the Utah Agricultural Experiment Station. FCOI Disclosure: None.

#101 POSTER SESSION III

The Full Length TrkB Receptor is Expressed in Gustatory Neurons, while Taste Buds Only Express the Truncated (TrkBT1) Receptor

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BDNF maintains taste bud size, cell number, and innervation in adulthood by binding two receptors, TrkB and P75. BDNF primarily functions via the full-length TrkB receptor, while the truncated TrkB isoform (TrkBT1) can inhibit full length TrkB signaling by sequestering BDNF. We wanted to determine where (nerve fibers or taste cells) BDNF might be acting by determining which cells express these receptors. Using RT-PCR, we found that the TrkBT1 and p75 receptor are expressed in fungiform and circumvallate taste buds, while the full length TrkBT1 receptor was not. TrkBT1 is expressed at higher levels than p75 in fungiform taste buds ($p< 0.05$), but the reverse is true in circumvallate taste buds ($p< 0.01$). In contrast, in the geniculate ganglion, the full-length TrkB receptor was expressed at levels 9.2 times higher than the TrkBT1 receptor ($p< 0.001$). Total TrkB receptor expression was 6.3 times higher than p75 ($p< 0.001$). In TrkB-GFP mice, GFP was strong in nerve fibers but was also weakly present in cells within and around taste bud. Anti-TrkBT1 also labeled nerve fibers, taste cells, and perigemmel cells of fungiform taste buds. To determine if BDNF regulates its own receptor, we examined receptor expression in mice overexpressing BDNF. We found BDNF overexpression did not induce full length TrkB receptor expression in the taste bud. However, both total TrkB ($p< 0.05$) and the TrkBT1 receptor expression ($p< 0.002$) level were tripled in the circumvallate taste buds of BDNF overexpressing mice compared to wild type mice. Our data suggests that BDNF binds full length receptors on nerve fibers to maintain taste buds. The TrkBT1 receptor in cells within and around the taste bud is regulated by BDNF levels, and may function to sequester BDNF and prevent BDNF binding to full length TrkB and p75 receptors. Acknowledgements: NIH grant DC007176. FCOI Disclosure: None.

#102 POSTER SESSION III

The Molecular Mechanism of the Umami Taste Perception of L-Theanine

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L-Theanine is a unique amino acid present in green tea. It has been reported that L-theanine has a complicated taste, including umami, and that a synergistic effect of L-theanine and IMP on umami taste can be observed in humans and mice. However, its receptive mechanism in the oral cavity was not well understood. In this study, using a heterologous experimental system, we found that L-theanine activated the umami taste receptor T1R1+T1R3 in a concentration-dependent manner. Furthermore, the response of T1R1+T1R3 to L-theanine was synergistically increased by adding IMP. In previous in vivo studies, the synergistic effects of L-theanine and IMP were strongly observed in mice rather than in humans. Consistent with these results, the L-theanine response of mT1R1+T1R3 was strongly enhanced by the addition of IMP compared to the response of hT1R1+T1R3. The site-directed mutagenesis analysis revealed that L-theanine binds to the hinge region of the extracellular Venus flytrap domain of T1R1, which is known as the binding site for L-amino acids. These results strongly suggest that the umami taste of L-theanine, which was observed in both humans and mice, occurred via T1R1+T1R3. Acknowledgements: The work was supported in part by the Funding Program for Next Generation World-Leading Researchers from the Japan Society for the Promotion of Science (LS037). FCOI Disclosure: None.

#103 **POSTER SESSION III**

Effect of NaCl on taste preferences for L-valine in C57BL/6 mice

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NaCl is an important seasoning widely used in various foods. In addition to contributing saltiness, NaCl also modifies taste of other nutrients, including amino acids. NaCl was reported to enhance sweet taste of Gly, Ala and Ser (Ugawa et al., 1992), and to suppress bitterness of Phe and Ile (Ogawa et al., 2004). Many amino acids have complex taste. For example, humans perceive complex taste of Arg, which includes sweetness and bitterness (Kawai et al., 2012; Schiffman et al., 1981). We previously have shown that in C57BL/6 mice, NaCl influences the palatability of Arg, but this effect depends on the concentration of both Arg and NaCl. Val is also perceived by humans as bitter and sweet, depending on its concentration (Kawai et al., 2012; Schiffman et al., 1981). In the present study, we measured preferences for 0-50mM Val mixed with 0-200mM NaCl in C57BL/6J mice using the 2-bottle tests, in order to analyze the effects of NaCl on the taste of Val. Mice had moderate preference for water solutions of 3 and 10mM Val, and they were indifferent to 50mM Val. Mice were indifferent to water solutions of 5-100mM NaCl, and they had a weak aversion to 200mM NaCl. Addition of NaCl increased preference scores for Val depending on NaCl and Val concentrations. The strongest preferences were observed for 10mM Val + 50mM NaCl and 50mM Val + 100mM NaCl mixtures. These results show that NaCl increases the palatability of Val, probably by enhancing its sweet taste component and suppressing its bitter taste component. Acknowledgements: Supported by Fisheries Research Agency (Yokohama, Japan) research grant (YM) and NIH grant R01DC00882 (AAB). FCOI Disclosure: None.

#104 **POSTER SESSION III**

The release and re-uptake of glutamate in taste buds

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Glutamate has been proposed as a neuromodulator in taste buds. Vesicular glutamate transporters (VGLUTs) are expressed on the afferent fibers innervating taste buds and ionotropic glutamate receptors are expressed on Type III taste cells, suggesting that glutamate may be released from the afferent fibers via an axon reflex to modulate Type III taste cell activity. In addition, somatosensory fibers expressing TRPV1 enter taste buds and could also potentially release glutamate. We tested this hypothesis in a slice preparation of circumvallate papillae containing taste buds as well as gustatory and somatosensory nerve fibers. We measured the amount of glutamate released with UPLC-mass spectrometry in response to stimulation with KCl and capsaicin. Our results show that both KCl (55 mM) and capsaicin (10 μM) evoke a significant release of glutamate over stimulation with Tyrode's. Since taste cells lack expression of TRPV1 and VGLUTs, these data suggest the glutamate release is from nerve fibers and not taste cells. After release, glutamate is taken up by Excitatory Amino Acids Transporters (EAATs). To date, only EAAT1 (or GLAST) has been reported in taste cells. Our RT-PCR shows that other EAATs, namely EAAT2 (or GLT-1) and EAAT4 are also present in taste cells. Using immunohistochemistry and transgenic mice expressing GFP from the GLT-1 promoter, we found that GLT-1 is exclusively expressed in Type II cells and co-localizes with α-gustducin. In contrast, a specific antibody for EAAT4 shows expression in all cell types. These results confirm that glutamate is released from nerve fibers in response to depolarization and demonstrate that multiple glutamate transporters are present in the taste bud to regulate glutamate reuptake. Acknowledgements: Funded by NIH grants R01 DC012555 to SCK and P30 DC04657 to D. Restrepo. FCOI Disclosure: None.

#105 **POSTER SESSION III**

Shedding Light on Type III Taste Cell Function

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Type III taste cells in the mammalian taste bud respond to both sour and salty stimuli. Unlike Type II taste cells, Type III cells form classical synapses with afferent nerve fibers. The transmitter released at this synapse and its cognate receptors remain unclear. Studies of Type III cells in the intact bud are complicated, however, by the widespread effects of acid application (sour stimuli) on taste tissue. To circumvent this issue and isolate Type III cell activity, we have developed a Cre-dependent optogenetic system to modulate Type III cell activity using light as a stimulus. We created a knock-in mouse expressing Cre recombinase in Type III cells by inserting a bicistronic IRES Cre recombinase construct directly following the Polycystic Kidney Disease 2-Like 1 (*Pkd2l1*) stop codon. As PKD2L1 is expressed exclusively in Type III cells, this Cre construct allows for the expression of Cre-dependent channelrhodopsin-YFP or halorhodopsin-YFP specifically in Type III cells. Initial findings indicate that the expression of our construct is faithful, as channelrhodopsin-YFP and halorhodopsin-YFP are expressed in PKD2L1 immunoreactive Type III taste cells, with no expression in Type II taste cells. Light application to the tongue in an anesthetized PKD2L1-Cre, channelrhodopsin mouse produces a chorda tympani nerve response, indicating the functionality of

our system. Optogenetic modulation of PKD2L1 positive cells will allow us to examine Type III cell function in intact taste buds without the confounding effects of intracellular acidification. Acknowledgements: RO1 DC012555, T32 HD041697-13, P30 NS048154, 5P30 DC004657.
FCOI Disclosure: None.

#106 **POSTER SESSION III**

Distinctive Properties of a Proton Current Associated with PKD2L1 Expression in Taste Cells

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Sour taste is detected by taste receptor cells that respond to acids through yet poorly understood mechanisms. The cells that detect sour express the protein PKD2L1, which does not form the sour receptor, but nonetheless serves as a useful marker for sour cells. Using transgenic mice in which the PKD2L1 promoter drives expression of YFP (PKD2L1-YFP), we previously described an inward proton current in sour sensing taste cells from the posterior tongue (circumvallate papillae) and proposed that this current mediates sour transduction. A simple prediction of this hypothesis is that the proton current should be found in all taste cells that detect sour, and not in other cells that do not participate in sour taste. Here we show that a proton current is found in PKD2L1-expressing cells from the three major types of taste papillae: circumvallate, foliate, and fungiform. A similar current was not observed in a variety of non-sour taste cells, including a subset of spinal cord neurons that express PKD2L1 and that were previously shown to fire action potentials to strong acids. Additional characterization of the proton current shows that it is inhibited by Zn^{2+} in a pH-dependent manner, indicating that Zn^{2+} and proton compete for binding sites to the channel. The conductance shows no evidence of pH or voltage-dependent gating and thus may be open at rest. In support of this possibility, the resting cytosolic pH of PKD2L1-expressing taste cells bathed in a neutral saline solution was lower than the resting pH of non-PKD2L1-expressing cells. Together these data define a functional signature for the taste cell proton current that may aid in its molecular identification and indicate that its expression is mostly restricted to the subset of taste cells that detect sour. Acknowledgements: Research funded with NIH grant 1F32DC013971 awarded to J Bushman, and 5R01DC004564 and 1R01DC013741 awarded to E Liman.
FCOI Disclosure: None.

#107 **POSTER SESSION III**

Receptive field size, chemical responses and fiber conduction velocity of rat chorda tympani geniculate ganglion neurons

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Afferent chorda tympani (CT) fibers innervating anterior tongue taste receptors in fungiform papillae have neuron cell bodies in the geniculate ganglion. To characterize electrophysiological and receptive field properties, we recorded extracellular responses from single geniculate ganglion neurons to lingual application with chemical and thermal stimuli. We mapped receptive field size using electrical stimulation (1–10 μA) of individual fungiform papillae. Response latency to electrical stimulation was used to determine fiber conduction velocity. We have analyzed the responses of 18 neurons to room temperature 0.5M NaCl, 0.5M NH4Cl, 0.01N HCl, 0.03M citric acid, 0.02M quinine HCl and 1.0M sucrose, and to cold water at 4°C. Based on response profiles, 7 neurons were classified as narrowly-tuned, responding only to salts. Receptive field sizes of these neurons were larger, average of 7.3 papillae, and all were localized on the anterior-most CT innervation field. Conduction velocity was 1.1-1.8 m/s, characteristic of large C fibers. Eight neurons were broadly-tuned, responding to salts, acids and sucrose and had smaller receptive field sizes, average of 4.5 papillae, located over the entire CT innervation field. Conduction velocity was 0.7-1.9 m/s for 7 of these neurons (classified as small C fibers) and 2.5 m/s for one neuron (Aδ fiber). An additional 3 cells were classified as specific thermal neurons, responding only to cold water. Receptive field sizes were small, 1-2 papillae, and located around the tongue tip. Conduction velocity was 0.8-0.9 m/s, characteristic of small C fibers. The results suggest that receptive field size correlates with diameter of the afferent fiber and that specific thermal neurons exist in the geniculate ganglion with very small receptive fields. Acknowledgements: NIH NIDCD Grant DC009982. FCOI Disclosure: None.

#108 **POSTER SESSION III**

Using functional calcium imaging technique to study the taste representation in the geniculate ganglion

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In the taste end organ, the taste bud, Receptor (Type II) cells are believed to transduce sweet, bitter, or umami. Presynaptic (Type III) cells directly transduce sour, although via cell-cell connections, they respond to multiple tastes. Yet, despite this consensus about coding in the end organ, whether information downstream of taste buds is organized in a labeled-line or some other form of coding mechanism remains controversial. As a

major sensory ganglion of the gustatory system, the geniculate ganglion innervates fungiform and palatal taste buds. We have developed a novel approach allowing us to record Ca^{2+} activity directly from ensembles of neurons in this ganglion in live mice that express the Ca^{2+} indicator GCaMP3 in sensory neurons. In the search of how geniculate ganglion cells encode information from taste buds, we have recorded 101 neurons and found that 72% (n=73) responded to single taste while 28% (n=28) responded to 2 or more of sweet, salty, umami, bitter and sour tastes when presented at low-to-moderate concentrations (50% of EC50). To study if this pattern persisted at higher concentrations (i.e. quality invariance), we tested the same compounds at 1~1.5 x EC50. The proportion of multiple-taste responding neurons increased (51%; 79/155, $p< 0.0001$). There was no apparent topographical mapping of taste qualities onto the geniculate ganglion. Additionally, the set of neurons that responded to umami and sweet tastes overlapped considerably and the sets became indistinguishable when benzyl amiloride (benzamil, 1μM) was present. This is consistent with the finding that rodents confuse sweet and umami tastes in the presence of amiloride. Acknowledgements: NIH/NIDCD grant R21DC012746. FCOI Disclosure: None.

#109 **POSTER SESSION III**

Insulin-like growth factors are expressed at high levels in the taste system, but do not maintain taste bud structure

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Growth factors regulate growth and differentiation in many tissues and most of the growth factors regulating the taste bud have not been identified. In fact, some factor is released by nerve fibers and maintains taste buds. Hypothesizing that this was a growth factor, McLaughlin (2000) identified a number of growth factor receptors that were expressed at greater levels in taste buds than surrounding epithelia. From microarray data we determined that the ligands for 8 of these receptors were expressed at high levels in the E14.5 geniculate ganglia. RT-PCR confirmed that 4 of these ligands were expressed at high levels in adulthood. RT-PCR for the receptors in taste buds and ligands in the geniculate ganglion revealed that the combination of insulin-like growth factors (IGF1 and IGF2) in the ganglion and their receptor *Igf1r* in taste buds were expressed at the highest levels. To determine whether the Igr1r receptor maintains taste buds, we conditionally removed *Igf1r* from lingual epithelium using the K14 promotor (K14-Cre/$Igf1r^{lox/lox}$). While K14-Cre/$Igf1r^{lox/lox}$ mice had slightly fewer taste buds at P30 ($p< 0.05$), this difference was eliminated by P80. The IGF1R receptor was not required to maintain taste bud size or cell number and the number of PLCβ2 and Car4 positive taste receptor cells did not differ between genotypes. However, taste buds on the back of the tongue were larger ($p< 0.01$) and contained more cells ($p< 0.01$) than those at the tip, and this difference was eliminated in K14-Cre/$Igf1r^{lox/lox}$ mice. Contrary to previous reports, we found no difference in lingual epithelial thickness in either fungiform or filiform papillae. We conclude that although IGFs are expressed at high levels in the taste system, they play no role in maintaining adult taste bud structure. Acknowledgements: Supported by NIH grant DC007176. FCOI Disclosure: None.

#110 **POSTER SESSION III**

Taste responsiveness to sweeteners is resistant to elevations in plasma leptin

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Taste receptor cells express receptors for a number of bioactive peptides, including the hormone leptin. However, there is uncertainty about the relationship between plasma leptin and peripheral taste responsiveness in mice. Although some studies have reported that elevations in plasma leptin diminish responsiveness to sweeteners, another found that they enhanced responsiveness to sucrose. We evaluated the impact of plasma leptin on sweet taste in C57BL/6J (B6) and leptin-deficient ob/ob mice. Mice engineered to produce enhanced yellow fluorescent protein (EYFP) in cells that express the long-form leptin receptor (LepRb) showed EYFP expression selectively in Type 2 taste cells. However, leptin failed to activate a critical leptin-signaling protein, STAT3, in taste cells. Similarly, we did not observe any impact of intraperitoneal (i.p.) leptin treatment on chorda tympani nerve responses to sweeteners in B6 or ob/ob mice. Finally, there was no effect of leptin treatment on initial licking responses to several sucrose concentrations in B6 mice. We confirmed that basal plasma leptin levels did not exceed 10 ng/mL, regardless of time of day, physiological state or body weight, suggesting that taste cell LepRbs were not desensitized to leptin in our studies. Furthermore, i.p. leptin injections produced plasma leptin levels that exceeded those previously reported to exert taste effects. We conclude that any effect of plasma leptin on taste responsiveness to sweeteners is subtle and manifests itself only under specific experimental conditions. Acknowledgements: National Institute on Deafness and Other Communication Disorders (DC010110, DC010113). FCOI Disclosure: None.

#111 POSTER SESSION III

Hindbrain parabrachial nucleus lesions counteract hyperphagic responses to a NPY Y4 receptor agonist

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Central neuropeptide Y (NPY) receptor studies of feeding have traditionally focused on Y1/Y5 receptors, however recent work has identified a potential role for Y4 receptors. Although lightly expressed in the CNS, Y4 receptors are found in greater concentration around the hindbrain parabrachial nucleus (PBN), a nucleus strongly implicated in feeding control as emphasized by the recent finding that lesions of NPY/AGRP/GABA neurons projecting specifically to the PBN induce lethal anorexia (Wu et al., 2009). In rats fitted with third ventricle (3V) cannulas we evaluated the effect of bilateral ibotenic acid lesions of the PBN on licking for 0.2M sucrose after 3V injections of the Y4 ligand, rat pancreatic polypeptide (rPP; 0.09, 0.9 and 9nM/2μL), or vehicle. rPP significantly increased session intake and meal size in the sham lesion (n=13) and intact (n=13) control groups at the 9nM dose relative to vehicle (ps< 0.05). Intake increases were mediated via prolonged meal duration and increases in the number of bursts in the meal (ps< 0.05), which is consistent with a diminution of inhibitory postingestive feedback during the meal. This response pattern is also consistent with the pattern of licking microstructure responses we previously characterized after 3V NPY infusions (Baird et al., 2006). Rats with confirmed bilateral lesions of the PBN (n=9) failed to exhibit any intake increases or changes in licking microstructure after 3V injections of rPP. By contrast, rats with incomplete lesions of the PBN (n=11) showed significant hyperphagic responses to rPP (9nM dose). The PBN appears to be necessary for the expression of rPP hyperphagia, and Y4 receptors in the PBN may provide an important contribution to NPY-related food intake control mechanisms. Acknowledgements: NIH-DC07389 HHMI-52006280 Amherst College. FCOI Disclosure: None.

#112 POSTER SESSION III

Functional and Physical Mapping of PYY-Responsive Cranial Neuronal Network in Mice

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Recently we have described a novel role for the salivary hormone PYY in food intake and body weight maintenance, as well as in the modulation of bitter and lipid taste modalities. We have also identified a putative metabolic circuit associated with Y2R-positive cells in the oral cavity and extending through brainstem nuclei into hypothalamic satiety centers. To extend these findings, we have now conducted a mapping of the brain neuronal circuits activated in response to orally-administered PYY. Using fMRI, we tracked brain areas activated by exogenous PYY in WT or PYY KO mice. Using this approach, we now identified areas in the anterior and lateral hypothalamus significantly activated by PYY. To characterize PYY-specific neuronal networks and their respective connections, we utilized an attenuated Bartha strain of pseudorabies virus (PRV-152). This particular rPRV-EGFP produces infectious progeny that traffic into the somatodendritic compartment to move across synapses and spread retrogradely from post-synaptic to pre-synaptic neurons comprising a circuit. We have shown for the first time that topically applied rPRV infects taste receptor cells in the taste buds within circumvallate papillae as well as epithelial cells in the soft palate. To identify PYY-activated neurons, we used cFos as a surrogate marker to visualize the neurons by immunohistochemistry and confocal microscopy. Using retrograde tracing approaches, we mapped a PYY-responsive network originated in the sensory field of the soft palate and extending through the maxillary branch of the trigeminal nerve (CN-V) into the brain stem, hypothalamus, and amygdala. Thus, we have identified and characterized an alternative neuronal circuit that regulates ingestive behavior via the anorexigenic hormone PYY present in saliva.
Acknowledgements: R01 DC012819. FCOI Disclosure: S. Zolotukhin is a co-founder of Gila Therapeutics.

#113 POSTER SESSION III

Neuropeptide Regulation of the Olfactory Bulb

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The olfactory bulb (OB) receives afferent input from olfactory sensory neurons (OSNs) and uses its distinct laminar circuitry and neuronal subtypes to process odor information. The activity of the OB is circadian, and it is one of two brain regions that contains independent circadian neuronal clocks outside of the SCN (the other is the retina, a structure in vision parallel to the OB in olfaction). However, almost nothing is known about the cellular mechanisms that underlie the OB's circadian rhythms. A prominent cell type of the OB's circuitry is dopamine (DA) neurons found within the glomerular layer (GL) of the OB. DA is implicated in sharpening odor sensation and discrimination by presynaptically inhibiting the release of glutamate from OSNs. A second known endogenous regulator of the OB is vasoactive intestinal polypeptide (VIP), which has been implicated in regulating circadian activity within the OB. Unlike DA neurons, the circuitry of VIP neurons is not well established and is highly variable among mammalian species. Also, it is unknown whether VIP and DA are co-expressed by neuronal subpopulations and/or interact with each other to modulate OB activity. Hence, the goal of the present study is to test the hypothesis that subpopulations of OB neurons express DA, VIP, or both, and

that VIP regulates DA neurons (as it does in the hypothalamus) and/or DA regulates VIP neurons. To that end, we have immunohistochemical data that suggest that a subpopulation of rat DA neurons may also express VIP and that VIP receptors are highly expressed in the GL where OB DA neurons reside. We are currently examining whether DA modulates VIP neurons and whether VIP modulates DA neurons. Such interactions may contribute to the cellular mechanism underlying circadian activity of the OB. Acknowledgements: Florida State University and PQT. FCOI Disclosure: None.

#114 **POSTER SESSION III**

Glucose Entry Through GLUT4 in the Olfactory Bulb Subserves as a Signaling Molecule Independent from its Metabolic Function

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Because the olfactory system is directly affected by hormones and peptides related to food intake, we recently characterized a population of glucose-sensing mitral cells. Herein, we demonstrate that the Kv1.3 voltage-dependent potassium channel known to influence action potential frequency in mitral cells, may act in concert with glucose transporters to mediate olfactory-based glucose sensing or metabolic state. Using a heterologous expression system to co-express Kv1.3 channel with that of the insulin-dependent glucose transporter type 4 (GLUT4), we report that blocking Kv1.3 conductance using a pore mutation (W386F Kv1.3) caused an increase in transporter expression. *In situ* hybridization approaches supported co-localization of the channel and transporter in the mitral cell layer, which overlaps with previously reported Kv1.3 protein expression. The functional connection between the family of glucose transporters and Kv1.3 in shaping glucose sensitivity was sought using transporter inhibitors and non-metabolized glucose in conjunction with slice electrophysiology in mice. In current clamp mode, neurons that were reversibly excited by glucose were found to elicit a 29.6 percent decrease in firing frequency upon elimination of glucose. Bath addition of cytochalasin B decreased glucose-evoked increases in firing frequency by 34.2 percent. Unlike when glucose was eliminated, when 2-deoxyglucose was bath substituted for high glucose, no change was observed in the firing frequency of evoked action potentials (7.63 ± 3 Hz glucose vs. 7.82 ± 4 Hz 2-deoxyglucose). These data suggest that glucose sensitivity may be mediated by the family of glucose transporters in the olfactory bulb and that the neuromodulation may be dependent on the internalization of glucose and/or ATP utilization. Acknowledgements: This work was supported by R01 DC13080 by the NIH/NIDCD and a Robinson Endowment Grant from TMH Hospital.
FCOI Disclosure: None.

#115 **POSTER SESSION III**

Olfactory Behavioral Analysis of the Effect of Acute versus Chronic Intranasal Insulin Treatment

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Intranasal insulin delivery is currently being used in clinical trials to test for improvement in human memory and cognition, and in particular, for mitigating such for neurodegenerative diseases. Studies have reported the effects of short-term intranasal insulin treatment on various behaviors, but few have examined long-term effects. The olfactory bulb contains the highest density of insulin receptors in conjunction with the highest level of insulin transport within the brain. Previous research from our laboratory has demonstrated that acute intranasal insulin treatment (INI) enhanced both short- and long-term memory as well as increased two-odor discrimination. Herein, we investigated the behavioral effects of chronic INI. Adult mice were intranasally treated with 5µg/µl of insulin twice daily for one month. Metabolic assessment indicated no change in mean mass specific metabolism, ingestive behaviors, or body weight following INI, but there was a reduction in locomotor activity that was attributed to handling. Unlike acute INI that caused enhanced performance in a habituation/dishabituation task and increased two-odor discrimination, chronic INI did not enhance olfactometry-based odorant discrimination or olfactory reversal learning. Biochemical analyses of the olfactory bulb revealed that acute INI increased insulin receptor and Kv1.3 phosphorylation. Substrates for chronic INI induced phosphorylation are currently being determined. These data indicate that long-term administration of insulin appears to have no detrimental effects on olfactory function or learning. Acknowledgements: This work was supported by NIH grants T32 DC000044 and R01 DC003387 from the NIDCD.
FCOI Disclosure: None.

#116 **POSTER SESSION III**

Caffeine-induced activation of oral and gastric bitter taste receptors regulates gastric acid secretion

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The TAS2R family of receptors, responsible for bitter taste reception in oral taste buds, has been found in non-gustatory tissues including the gastric epithelia of mice and rats (Behrens, M. & Meyerhof, W. *Results Probl Cell Differ*, **2010**, 52, 87-99).

However, the role of TAS2Rs in regulating gastric functions, e.g. gastric acid secretion (GAS), is not yet known. Here, we hypothesize that TAS2Rs are involved in mechanisms regulating GAS. This hypothesis is supported by previous results demonstrating that bitter compounds of beer and coffee stimulate GAS (Walker, J. et al. J. *Agric Food Chem*. 2012, 60, 1405-12,Rubach, M. et al. Mol. *Nutr. Food Res.* **2012**). In this study we show that TAS2Rs mRNA is expressed in human gastric cells (HGT-1). We further investigated whether caffeine stimulates GAS via activation of oral and extra-oral bitter taste receptors in 10 healthy human volunteers by means of a Heidelberg capsule detection system. An amount of 150 mg caffeine was administered in 3 different ways: (1) encapsulated with 125 ml of water, (2) as solution (150 mg caffeine in 125 mL of water) that had to be swallowed, and (3) as solution (150 mg caffeine in 125 mL of water) that had to be spit out after swirling in the mouth. GAS was differentially regulated for these three ways of adminsitration. Activation of oral bitter taste receptors only (3) led to an inhibition of GAS, whereas activation of gastric TAS2Rs (1) had a stimulating effect. Administration of Homoeriodictyol, a bitter reducing compound (Ley, J.P. et al. J *Agric Food Chem*, 2005, **53**, 6061-6066), abolished the caffeine-evoked effect. In vitro experiments in HGT-1 cells demonstrated that TAS2R10 is involved in the stimulating effect of caffeine on mechanisms regulating GAS. In summary, bitter taste receptors are involved in regulating GAS. Acknowledgements: Christian Doppler Laboratory of Bioactive Aroma Compounds funded by the Austrian Federal Ministry of Economy, Family and Youth, and the Austrian National Foundation for Research, Technology and Development, and the Austrian Science Fund (FWF P23797). FCOI Disclosure: The authors S. Widder, J.P. Ley and J. Hans are employees at Symrise AG, Holzminden, Germany.

#117 **POSTER SESSION III**

Modulation of odor-cued memory processing by intranasal insulin

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According to recent research, the intranasal application of insulin reveals enhancing effects on delayed declarative memory performance in healthy subjects. We hypothesize that an intranasal insulin administration modulates neuronal processing of memory in general and olfactory memory in particular. Here we used functional MRI to examine brain activation during presentation and recollection of odors and pictures arranged in 3-D simulated mazes. Each subject participated in two experimental sessions in the MRI scanner during which they received either intranasally applied insulin (40 I.U.) or a placebo solution in a counterbalanced, double-blind manner. Each maze contained two parts: a learning trial during which subjects received eight odor or eight visual cues at different target locations and a recall trial during which subjects were re-exposed to these learned cues in a different order. The participant´s task was to remember the spatial location of the target cues in the maze. The preliminary behavioral results indicate that participants were significantly more accurate in remembering olfactory and visual stimuli during a recall task under the influence of insulin. A preliminary analysis of fMRI data revealed increased activation of the prefrontal cortex for the insulin vs placebo contrast ($p < 0.001$ uncorrected for whole-brain volume) and no activated voxels for the opposite contrast. The results point out that olfactory and visual memory processes are enhanced by an intranasal insulin application and this behavioral effect is mediated by a hyperactivation of a part of the prefrontal cortex. Future studies are needed to figure out, if odor-cueing in comparison to picture-cueing will help maximize the memory-enhancing properties of intranasal insulin in humans. Acknowledgements: Funding for this study was provided by the START program (691140) of the Medical Faculty of RWTH Aachen University and German Research Foundation (DFG, FR 3114/6-1) to JF. FCOI Disclosure: None.

#118 **POSTER SESSION III**

Optogenetic Activation of Pre-proglucagon Neurons in the Mouse Olfactory Bulb Controls Mitral Cell Activity

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Nutrients and hormones are able to modulate the activity of neurons involved in the transmission of olfactory input, suggesting an intimate link between the endocrine and olfactory systems. Using whole-cell electrophysiology in acute slices of the olfactory bulb (OB), we find that bath application of incretin (gastrointestinal) hormone glucagon-like peptide-1 (GLP-1) increases evoked firing frequency in mitral cells (MCs). Using a transgenic mouse that expresses YFP under the control of the preproglucagon (PPG) promoter, we discovered a population of GLP-1 producing neurons (PPG-neurons) in the granule cell layer. The expression of the glutamatergic marker GAD67 in these cells suggested a GABAergic activity. To study the effect of PPG-neurons activation on MCs, we established an optogenetic model by crossing mice expressing CRE-recombinase in PPG-neurons with those containing a floxed line expressing ChR2-YFP. The progeny successfully co-expressed ChR2-YFP in the PPG-neurons and extended dendritic terminations to the granule and glomerular cell layers. ChR2 expression was also confirmed by patch-clamp recording of light-induced TTX-insensitive inward currents from these neurons. Whole-cell, patch-clamp recordings from MCs revealed both inward and outward currents following light activation of PPG-neurons in the granule cell layer. Current-clamp recordings confirmed that PPG-neuron activation elicited inhibitory post-synaptic potentials (IPSP), which resulted in inhibition of MC activity. Applying the GABAA inhibitor, gabazine, suppressed the light-induced IPSPs and revealed light-induced excitatory

post-synaptic potentials (EPSP) in the MCs. These results demonstrate that PPG-neurons evoke a dual inhibition-activation, making them an interesting target to modulate olfaction. Acknowledgements: This work was supported by 14POST20380615 fellowship from the American Heart Association (AHA), R01 DC003387 from the NIH/NIDCD, and a Creative Research Council (CRC) award from FSU. FCOI Disclosure: None.

#119 **POSTER SESSION III**

Taste and palatability of Pyrophosphates by rats: a sensorial qualitative and quantitative approach

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Sodium pyrophosphates are well known ingredients used ubiquitously as cat food palatability agents. In 2010, Brand and Bryant (WO 2012/013480) demonstrated that at least the feline umami receptor was involved in their perception. Furthermore, preference tests and electrophysiology experiments on rats established pyrophosphates were highly palatable to them and suggested that this preference was not only due to their salty taste (McCaughey et al., 2007). Unfortunately, there is no method to evaluate cats' taste quality perception, but Palmer et al. reported in 2013 the development of a novel sensory assay capable of simultaneous measurement of taste quality and palatability with high throughput capacity, using rats in operant taste discrimination paradigm. We sought to further investigate taste and palatability of pyrophosphates using this technology to describe pyrophosphates taste quality. Five cohorts of four rats were trained to each discriminate one of the five basic tastes. Once ready, the rats were given series of sodium pyrophosphates solutions at various concentrations (2, 10 and 30mM) so as to identify their global taste profile. Then, the salty cohort was used to evaluate if pyrophosphates preference was mainly brought by their saltiness by evaluating the same solutions after inhibiting rats salty taste receptors with 100µM of amiloride. This study let us validate that sodium pyrophosphates are mainly perceived by rats as salty and significantly preferred to water. However, once the salty taste is inhibited, it is still preferred to water. We can therefore conclude that pyrophosphates have a distinctive taste besides the salty one, and that they might act on a separate gustatory transduction mechanism than those associated with the five prototypical taste qualities. Acknowledgements: Partnership between SPF and Opertech Bio. FCOI Disclosure: None.

#120 **POSTER SESSION III**

Nose Licking Good? – A Study on Taste Reactivity in Domestic Cats (Felis catus)

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We assessed behaviors indicative of how cats perceived different tastes. To this end, video footage and intake data from 13 adult, neutered cats during two-bottle preference tests using five concentrations of L-Proline (PRO) or quinine monohydrochloride (QHCl) versus water. Cats were singly housed during exposure to taste stimuli and were tested. Lick rate and volume consumed over 5 minutes was recorded while video was obtained. Cats preferred PRO at 50 and 500 mM. 50 different behaviors were analyzed to identify behaviors associated with preference. When sampling PRO at 50 mM, the cats kept their eyes only half open for a significantly longer proportion of the sampling time (P= 0.028) than when sampling from a water control; possibly indicating a behavior related to a pleasant taste experience. Also, the cats were found to lick their nose significantly more often (P= 0.011) during sampling of 500 mM L-Proline than when sampling the water control. The lick rate of QHCl was not significantly different from water at any concentration tested. However, whilst drinking 5 µM QHCl the cats were observed to fold one of their ears outward for a significantly longer period of time (P= 0.050) than when drinking from the water control. This suggests that while these concentrations of QHCl did not evoke aversion, they may be detected. This study described several affective taste reactions in cats and provides a basis for evaluating taste experience and comparing such responses to other taste qualities. Acknowledgements: The study was funded by AFB International. Funding to cover expenses for the visiting student was given by the Swedish Central Board of Study Support (CSN). FCOI Disclosure: None.

#121 **POSTER SESSION III**

Different food culture affects relationship between noticeability and intensity for taste

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Common foods consist of several taste qualities. Consumers perceive intensity of a particular taste quality after noticing it among other taste qualities, when they eat common foods. We supposed that the easiness of noticing a taste quality present in a common food, while eating, will differ among taste qualities which compose the common food. We, therefore, proposed a new measurement scale for food perception named "noticeability". Furthermore, we found that consumers' food

perception to common foods was modified by retronasal aroma. In order to examine whether retronasal aroma affects into relationship between noticeability and perceived intensity for taste, participants evaluated noticeability and perceived intensity of five fundamental taste qualities under open and closed nostril conditions using popular traditional Japanese confections called "yokan". We found the highest noticeability and perceived intensity values for yokan was sweetness, independent of the nostril condition. For sweetness, the correlation coefficients between noticeability and perceived intensity significantly decreased with retronasal aroma. In order to examine whether high familiarity with a common food was necessary for a correlating decrease between noticeability and perceived intensity, Japanese and Germans with different food culture, participated in the next experiment. We used again yokan that is familiar to Japanese but is unfamiliar to Germany. And we used marshmallow that is familiar to both Japanese and Germany. We found a significant correlating decrease between noticeability and perceived intensity for sweetness in response to the retronasal aroma of common foods, when Japanese ate marshmallow and yokan, and Germans ate marshmallow under the open nostril condition. Acknowledgements: This work was partially supported by Sapporo Bioscience Foundation.
FCOI Disclosure: None.

#122 **POSTER SESSION III**

Effect of Sucralose on Oral Glucose Tolerance

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Many studies have shown that sucralose ingestion by itself does not affect insulin or blood sugar. The purpose of this study was to determine whether ingested sucralose alters blood glucose and insulin responses to a glucose tolerance test. 12 human subjects were tested on four occasions, each spaced one week apart. On two occasions, subjects rinsed and consumed 75 g glucose dissolved in 300 mL water. On the other two occasions, subjects consumed a mixture of 75 g glucose and 2 g sucralose. Blood samples were drawn at the following time points: -20, -15, 10, -5, 0, 3, 6, 9, 12, 15, 30, 45, 60, 75, and 90 minutes. Plasma insulin peaked at 30 minutes for glucose and 45 minutes for sucralose + glucose, indicating a shift in insulin time course. Peak insulin was 69 uIU/ml for glucose and 86 uIU/ml for glucose + sucralose. Insulin from sucralose + glucose was significantly greater than insulin from glucose alone after 45 minutes ($p = 0.022$). Total insulin AUC from sucralose + glucose was 11.5% greater than AUC from glucose. Plasma glucose peaked at 30 minutes for glucose and 45 minutes for sucralose + glucose. Peak glucose was 142 mg/dl and 153 mg/dl for glucose and glucose + sucralose, respectively. The maximum concentration of plasma glucose was 7.75 % greater after consumption of the sucralose/glucose mixture compared to glucose alone ($p = 0.041$), suggesting glucose was metabolically generated to counter additional insulin. Blood glucose AUC was 2.8% greater from sucralose + glucose. These data show that consumption of sucralose in combination with glucose can elicit increases in blood insulin and glucose and, therefore, has clinical significance for regulating blood sugar. Acknowledgements: Funding by NIH DC02995. FCOI Disclosure: None.

#123 **POSTER SESSION III**

Human Differential Sensitivity to Fat Content in Milk

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Fat in food has positive palatability and can be highly reinforcing for animals and humans. National guidelines urge Americans to lower the fat in their diet to reduce metabolic and heart diseases. The preference for high-fat foods is presumed to be due to their metabolic effects, caloric value, and orosensory properties. Despite efforts by the food industry to find fat substitutes, low fat and fat-free foods are often judged less palatable. It is presently unclear whether individual sensitivity to fat content affects hedonic judgments and consumption of foods, and we know even less about human ability to discriminate fat levels in foods. The goal of the present study was to examine the differential sensitivity of individuals to fat content in milk. To do so, just noticeable differences (JND) were determined using a two alternative forced-choice (2AFC) staircase method. Subjects were asked to identify which of two samples contained more fat under red light condition while wearing nose clips. The fat concentrations ranged from 0.5% dairy fat (reference sample) to 8% with a 0.5% increment. Our results showed that about 25% of the subjects were not able to discriminate between a 0.5 % fat milk and a 8% fat milk. For other subjects, the JND was reliably as low as 1.5% fat. This illustrates the great range of sensitivities to fat content among participants. Similar tests of JND sensitivity with NaCl solutions confirmed our subjects' abilities to perform the task comparably. This study shows that a high percentage of people have great difficulty discriminating very large differences in the fat content of milk (skim milk vs. half & half). It would be of clinical interest to explore further the relationships among differential sensitivity to fat, fatty food preferences, and health outcomes. Acknowledgements: NIH DC02995. FCOI Disclosure: None.

#124 **POSTER SESSION III**

Effects of individual caffeine metabolism on coffee preference

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Genetic and environmental factors contribute to individual differences in caffeine sensitivity, and caffeine metabolism influences coffee consumption, but the effects of caffeine

metabolism on coffee preference are unknown. Relationships between caffeine metabolism, bitterness and coffee preference were investigated in 90 regular coffee consumers (Ss) who provided saliva samples after 12 hours caffeine abstinence, and at 30 and 90 min after ingestion of caffeine (100mg). A caffeine metabolism index (CmI) was computed as the ratio between caffeine in saliva at 90 min and at 30 min. Ss were grouped based on the CmI distribution median. The groups did not differ in measures of taste/oral sensitivity (fungiform papillae number; PROP taster status). Ss rated liking for, and sourness, bitterness and astringency of, six unsweetened and freely sweetened coffee samples varying in roasting degree, caffeine content and bitterness. They also rated the bitterness of 6 caffeine and 6 quinine (equi-intense) solutions. Finally, Ss choose coffee to drink on the basis of a label (strong vs balanced flavor) both after caffeine abstention and after no restrictions on caffeine intake. The CmI was strongly associated with the number of coffees consumed per day (r^2=0.93, p =0.033). The Low CmI (LCmI) group gave higher bitterness ratings than did High CmI (HCmI) Ss in both coffee (p =0.016) and caffeine (p = 0.023), but not quinine, solutions. LCmI also added more sugar to the coffee samples than did HCmI ($p \leq 0.0001$). Following caffeine abstention, Ss chose the "strong flavor" coffee independent of group, while without caffeine restrictions, LCmI preferentially chose the "balanced flavor" coffee. These results provide the first link between caffeine metabolism and bitterness perception, and thus to coffee-related behavior. Acknowledgements: This research received funding from illycaffè s.p.a, Italy. FCOI Disclosure: None.

#125 **POSTER SESSION III**

Design and Development of Drug-bound Nanoparticles Targeting the Olfactory Bulb to Regulate Metabolism

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This work highlights the development of a novel drug vector and delivery method targeted towards the Kv1.3 potassium channel, designed to modulate metabolism at the level of the olfactory bulb (OB). We have previously demonstrated that pharmacological block of Kv1.3 or gene-targeted deletion increases mass-specific metabolism in mice, while the later also prevents diet-induced obesity. Herein, we have examined the thermostability of the peptide and small molecule inhibitors MgTx, ShK-186, PAP-1 and TEA and their effectiveness to inhibit 80% of Kv1.3 voltage-activated currents as expressed in HEK-293 cells. Because MgTx had favorable stability, reduced K_d and acts to block the external vestibule of Kv1.3, we also tested its ability to inhibit outward currents in cells expressing *Shaker* family members Kv1.1 - Kv1.7. For a less invasive, alternative delivery method of Kv1.3 inhibitors to the OB, we have designed ZnS-overcoated, fluorescent quantum dots (QD) and Fe_3O_4 magnetic nanoparticles that can be intranasally delivered and allow peptide or small molecule inhibitors to be tracked and targeted to the OB. Towards this goal, we have visualized intranasally-delivered QDs in the mitral cell layer and conjugated a Kv1.3 peptide inhibitor to the QDs such that the conjugate retains the peptide's ability to block Kv1.3 in cell-attached patches. To further improve upon conjugation to nanoparticles, we designed methods to recombinantly express the inhibitor such that it has a higher affinity for nanoparticles and allows for greater control of valance and orientation of the bound peptide. Future *in-vivo* studies are planned following delivery of nanoparticle, Kv1.3-inhibitor conjugates to assess metabolism and olfactory acuity. Acknowledgements: This was supported by NIH grant RO1 DC13080 from the NIDCD, a Legacy Fellowship from the Florida State University and a grant award from the Bryan Robinson Endowment at Tallahassee Memorial Hospital. FCOI Disclosure: None.

#126 **POSTER SESSION III**

Marked fat preference deficits in P2X2/P2X3 and Calhm1 but not CD36 and GPR40/120 knockout mice

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There is considerable evidence for a gustatory component to dietary fat preferences in rodents. CD36 and the G-protein coupled receptors GPR40 and GPR120 have been implicated as fatty acid taste receptors. The role of these receptors in the preference mice display for a palatable soybean oil emulsion (Intralipid) was studied by comparing Intralipid vs. water preferences in CD36 knockout (KO), GPR40/GPR120 double KO (DoKO), and wildtype (WT) mice using 48-h 2-bottle tests. GPR40/120 DoKO mice did not differ from WT mice in their preference for Intralipid over water at 0.03% - 10% concentrations, while CD36 KO mice displayed a reduced preference only at 0.3% IL. However, CD36 KO and GPR40/120 DoKO mice consumed less Intralipid than WT mice at 1-10% concentrations. Other experiments studied lipid preferences in Calhm1 KO and P2X2/P2X3 DoKO mice; these strains have disrupted ATP signaling, which results in global taste deficits. Unlike WT mice, P2X2/P2X3 DoKO mice were indifferent to 0.3-2.5% Intralipid and consumed less 1.25 - 5% Intralipid than did WT mice. Calhm1 KO mice, unlike WT mice, were indifferent to 0.1 – 1% Intralipid and showed reduced preferences for 0.1% – 5% IL; they also consumed less 0.5 – 5% Intralipid than did WT mice. Calhm1 KO mice showed similar preference deficits in 3-min Intralipid vs. water tests. These data indicate that CD36 and GPR40/120 fatty acid receptors have limited or no role in lipid preference, although they influence total lipid intake. Yet, the substantial lipid preference deficits displayed by Calhlm1 KO and P2X2/P2X3 DoKO mice indicate significant gustatory involvement in fat preference. Taken together, the findings suggest the existence of additional lipid

sensors in taste cells. Acknowledgements: NIHDK Grant DK031135. FCOI Disclosure: None.

#127 **POSTER SESSION III**

Neural processing of calories is modulated by sensitivity to reward in the caudate and anterior cingulate cortex

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Some people are more sensitive to food rewards and more inclined to involve in (over)eating than others. A food's reward value may arise from its palatable taste but also, on a more basic level, from the calories it contains. Responses to foods/calories are found to be modulated by hunger state in several brain reward areas. In the current study we aimed to investigate in how far individual differences in reward sensitivity modulate neural responses induced by tasting calories during both hunger and satiety. Brain responses to tasting a sweet caloric (maltodextrin and sucralose) and a sweet non-caloric (sucralose) solution during hunger and satiety were measured in 30 normal weight (mean±SD BMI of 22.6±1.4 kg/m^2) participants using functional magnetic resonance imaging. Taste activation by sucralose was subtracted from taste activation by maltodextrin and sucralose to maintain only activation induced by calories. Reward sensitivity of participants was measured with the BAS Drive scale. BAS Drive scores correlated inversely with the brain response to calories in the right caudate (r = 0.6) and anterior cingulate (bilaterally) (left: r = 0.6, right: r = 0.6) during hunger. When participants were satiated, brain response to calories correlated positively with BAS Drive scores in the left caudate (r = 0.6). In conclusion, we found that neural responses by tasting calories are modulated by the personality trait reward sensitivity. Higher sensitivity to reward resulted in a diminished response to calories during hunger and an increased response during satiety. These findings indicate that the response to calories in people with higher sensitivity to reward is less adapted to internal hunger state. Further research is needed to elucidate how these findings relate to eating behavior. Acknowledgements: Supported by the European Regional Development Fund and the Dutch provinces Gelderland and Overijssel (Grant number 2011-017004). FCOI Disclosure: None.

#128 **POSTER SESSION III**

Nutrient Sensor in the Brain Directs the Action of Brain-Gut Axis in Drosophila

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Sugars in the natural environment can be detected through taste-dependent and taste-independent modalities. Taste-dependent modalities consist mainly of peripheral chemosensory neurons such as sweet taste receptors, which primarily detect the orosensory value of sugar (i.e. sweetness). Evidence of a taste-independent modality – a post-ingestive sugar sensor - that detects the nutritional value of sugar has been shown in insects and mammals. However, the identity of the post-ingestive sugar sensor and the mechanism by which animals respond to the nutritional content of sugar independently of orosensory value is not currently understood. Here, we show that six neurosecretory cells in the *Drosophila* brain that produce Diuretic hormone 44 (Dh44), a homologue of the mammalian corticotropin-releasing hormone (CRH), were activated by nutritive sugars that are present in the hemolymph and not by nonnutritive sugars. Dh44 neuronal cell bodies are located primarily in the *pars intercerebralis* and extend their dendrites to the dorsal region of the subesophageal ganglion zone (SEZ), and project their axons along the esophagus to innervate the gut. Flies in which the activity of these neurons or the expression of the Dh44 gene was disrupted failed to select nutritive sugars over nonnutritive ones after periods of starvation. Manipulation of the function of Dh44 receptors had a similar effect. Notably, artificial activation of Dh44 receptor-1 neurons dramatically increased the rate of proboscis extension reflex (PER) responses, promoting food intake, and excretion. Conversely, reduced Dh44 activity led to decreased excretion. Together, we propose that the Dh44 system directs the detection, ingestion, and digestion of nutritive sugar through a positive feedback loop to continue consumption of nutritive sugar. Acknowledgements: NIH grants (RO1 and K99- NIDCD, NIGMS, NIDDK) and Private Foundation grants. FCOI Disclosure: None.

#129 **POSTER SESSION III**

ANS Responses and Facial Experssions Differentiate Between the Flavor of Commercial Breakfast Drinks

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The high failure rate of new market introductions, despite initial successful testing with traditional sensory and consumer tests, necessitates the development of other tests. This study explored the ability of selected physiological and behavioural measures of the autonomic nervous system (ANS) to distinguish between repeated exposures to foods from a single category (breakfast drinks) and with similar liking ratings. In this within-subject study 19 healthy young adults sipped from five breakfast drinks, each presented five times, while ANS responses (heart rate, skin conductance response and skin temperature), facial expressions, liking, and intensities were recorded. The results showed that liking was associated with increased heart rate and skin temperature, and more neutral facial expressions. Intensity was associated with reduced heart rate and skin temperature, more neutral expressions and more negative expressions of sadness, anger and surprize. Strongest associations with liking were found after 1 second of tasting, whereas strongest associations with intensity were found after 2 second of tasting. Future studies

should verify the contribution of the additional information to the prediction of market success. Acknowledgements: N/A. FCOI Disclosure: None.

#130 **POSTER SESSION III**

OR1A1 regulates hepatic lipid metabolism by suppression of PPAR-γ via activation of HES-1 in cultured hepatocytes

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Olfactory receptors (ORs) comprise the largest G protein-coupled receptor gene superfamily. Recent studies indicate that ORs are also expressed in non-olfactory organs, including metabolically active tissues, although their biological functions in these tissues are largely unknown. In this study, OR1A1 expression was detected in HepG2 liver cells. OR1A1 activation by (−)-carvone, a known OR1A1 ligand, increased the cyclic adenosine monophosphate, but not intracellular Ca^{2+} concentration, thereby inducing protein kinase A (PKA) activity with subsequent phosphorylation of cAMP response element-binding protein (CREB) and upregulation of the CREB-responsive gene hairy and enhancer of split (HES)-1, a corepressor of peroxisome proliferator-activated receptor-γ (PPAR-γ) in hepatocytes. In (−)-carvone-stimulated cells, the repression of PPAR-γ reduced the expression of the target gene, mitochondrial glycerol-3-phosphate acyltransferase (mtGPAT), which encodes a key enzyme involved in triglyceride synthesis. Intracellular triglyceride level and lipid accumulation were reduced in cells stimulated with (−)-carvone, effects that were diminished following the loss of OR1A1 function. These results indicate that OR1A1 may function as a non-redundant receptor in hepatocytes that regulates the PKA-CREB-HES-1 signaling axis and thereby modulates hepatic triglyceride metabolism. Acknowledgements: This work was supported by the Basic Science Research Program of the National Research Foundation of Korea and the Ministry of Education, Science and Technology (no. 2013R1A2A2A01016176). FCOI Disclosure: None.

#131 **POSTER SESSION III**

Associations between ethanol perception and alcohol intake

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Ethanol elicits multiple percepts, including sweet, bitter, drying and burning sensations. Surprisingly, we are unable to find reports on how these sensations differ across concentration. Accordingly, we collected dose response functions for sweetness, bitterness, burning and drying across 5 ethanol concentrations and tested if individual differences in perception associated with alcohol use. Participants (n= 100; 33 men) rated sweetness, bitterness, drying and burning/stinging on a general Labeled Magnitude Scale for 4 to 48% v/v ethanol. Alcohol measures included intake of beer, wine, neat spirits and spirits with mixers in the last 30 days, total years drinking and drinking occasions. Each sensation increased with concentration, with large differences in the rate at which they increased; thus, the dominant sensation differed by concentration. For 4% ethanol, similar intensities were reported across qualities, whereas bitterness (7.1±1.0; 13.4±1.4) dominated for 8% and 16% ethanol, followed by burning (5.1±0.9; 12.6±1.3), drying (4.5±0.6; 8.9±0.9) and sweetness (4.5±0.7; 5.3±0.6). For 32% and 48% ethanol, burn dominated (29.4±2.0; 46±2.5), followed by bitterness (20.3±1.9; 25.3±2.5), drying (14.9±1.4; 22.0±2.3) and sweetness (4.5±0.8; 7.0±1.5). Notably, years of drinking and intake frequency varied significantly with burning from 48% ethanol. Our data are cross sectional so we cannot determine whether altered perception is an antecedent of use or if prior experience serves to depress ratings via a larger frame of reference. As the dominant sensation from ethanol differs over the range commonly seen in alcoholic beverages, this study informs future work on relationships between alcohol perception and use, misuse and abuse by suggesting measures of intake that ignore concentration may hide effects. Acknowledgements: Supported by the Pennsylvania State University and NIH Grant DCO10904. FCOI Disclosure: None.

#132 **POSTER SESSION III**

Determining the Detection Thresholds for Methyl Anthranilate (MA) in Water versus Wine

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Detection thresholds are often reported in the literature without details on the delivery matrix, or the exact method used to determine the threshold. Critically, the matrix strongly influences perception, both via physiochemical effects, like partition coefficients, and via differential activation of olfactory receptors, which may result in masking. Regarding foxy aromas in wines, reported thresholds for methyl anthranilate (MA) and 2-aminoacetophenone (2-AAP) differ drastically, despite very similar structures. Accordingly, we wished to test these compounds under the same conditions to confirm these differences. Here, detection thresholds for MA were determined in water versus wine using an ascending concentration series in a forced-choice paradigm (ASTM E679-04). Forty untrained panelists were asked to smell samples in standard ISO tasting glasses, and choose the different sample in each of six triads containing increasing MA concentrations. The test was conducted over 2 days in a counterbalanced design, comparing MA thresholds in water to those in Riesling. Initial analysis suggests the group threshold for MA in Riesling is at least an order of magnitude greater than in water. Analyses of individual best estimate thresholds suggest approximately 20% of individual judges were not able to correctly identify the spiked sample, even at the highest concentration. Work with 2-AAP under the same conditions is underway to further investigate prior reports that 2-AAP detection thresholds in wine are 2 orders of magnitude lower than for MA in wine. Understanding whether these difference are due to methodological differences between studies or structural differences are crucial in understanding the extent to

which the chemical structures of volatiles play a role in the aromatic profile of wine. Acknowledgements: Funds from The Pennsylvania State University. FCOI Disclosure: None.

#133 **POSTER SESSION III**

The Relationship between Taste Sensitivity for Monosodium Glutamate, Food Neophobia, and Daily Consumption of High-Protein Foods

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Umami is found in foods, beverages, and ingredients that are high in amino acids, such as meats, certain fresh fruits and vegetables, as well as foods that are aged, dried, cured, fermented, roasted, or toasted. The goal of the present study was to identify factors associated with the intake of high-protein foods that are rich in umami. To this end, college men and women between the ages of 17 and 21 years were tested by assessing their daily intake of umami-rich foods, their MSG taste detection threshold using a two-alternative forced-choice staircase procedure, MSG preference using a forced-choice tracking technique, food neophobia, using a standardized questionnaire, and demographic measures such as weight. Preliminary analyses revealed that the detection threshold of MSG in this sample was similar to that previously reported in adults. No differences were observed in the detection thresholds of or preferences for MSG between normal weight and overweight/obese adults. Although those who were more neophobic did not differ in their thresholds or preference for MSG, they consumed fewer high-protein foods, such as beef, nuts, cheese, peas, and seaweed (i.e., nori). Those with higher MSG thresholds, reported consuming more soup, such as Miso and Ramen. These results suggest that factors such as food neophobia, MSG thresholds may interact to predict consumption of certain foods that are rich in proteins and amino acids and are associated with a healthful diet. Acknowledgements: William and Mary Internal Funds. FCOI Disclosure: None.

#134 **POSTER SESSION III**

Food odors direct specific appetite

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Olfactory food cues have been found to affect appetite for specific products and taste categories (Jansen *et al.*, 2003; Rolls & Rolls, 1997; Ramaekers *et al.*, 2013). Food odors seem to increase appetite for congruent products and decrease appetite for incongruent products. We aimed to confirm this phenomenon for taste category (sweet/savory) and determine whether it extends to energy-density category (high/low). Fourteen healthy females (age 33±14; BMI 21±1) consecutively smelled four odors differing in energy-density and taste category, one non-food odor, and one odorless solution (control), in random order. Each odor was smelled for three minutes. Appetite for 15 food products was rated in the following two minutes. Appetite ratings after control odor were subtracted from appetite ratings after smelling the odors. Preliminary mixed model analyses revealed that appetite for a specific taste category (sweet, savory) was higher after smelling congruent odors than after smelling incongruent odors (both $p < .001$) or non-food odors ($p = .026$ for sweet; $p = .002$ for savory). Appetite ratings for both high- and low energy-dense products were significantly affected by the energy-density signaled by the odors (high/low/non-food; $p = .001$). More specific paired comparisons did not show significant differences between energy-density categories of the odors, but it was noticeable that appetite ratings for a specific energy-density category were highest after smelling the odors that were congruent to the product category. These preliminary results suggest that food odors can increase appetite of congruent products, in terms of both taste and energy-density category. This study will be continued in 16 additional participants and will look further into the influence of hunger state. Acknowledgements: This study was funded by NWO (The Netherlands Organization for Scientific Research), Veni grant nr. 451-11-021, awarded to SB. We would like to express our thanks to IFF and AllSens for supplying the odors.
FCOI Disclosure: None.

#135 **POSTER SESSION III**

Orbitofrontal Cortex Activation by Visual Only or Visual and Olfactory Food Stimuli Reflects Pork Sensory Specific Satiety

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Sensory specific satiety (SSS) is defined as the relative decrease in pleasantness for eaten food and is indicated by decreased brain activation in reward regions. We examined neural underpinnings of pork SSS during visual only cues or during visual and congruent olfactory cues via functional Magnetic Resonance Imaging (fMRI). Eighteen participants (11 female) were scanned during two sessions (both before and one hour after a pork-based standardised breakfast) while images of pork and three other food categories (visual only cues) were displayed, The post-breakfast session additionally presented combined visual and odour cues to probe SSS. A randomised repeated measures design was employed, additionally testing the influence of a pork-flavoured or a placebo pre-breakfast chewing gum treatment, separated by 1 week washout. Subjective liking for food cues were collected during scanning. Behavioural results showed that SSS was established after breakfast under flavoured chewing gum treatment in response to visual only food cues ($p < 0.05$). The SSS score was reinforced by the addition of congruent odour cues ($p = 0.01$). Brain imaging results showed the parahippocampal gyrus, hippocampus and lateral orbitofrontal cortex (OFC) were activated under visual only food cues. Amygdala and medial OFC (mOFC) were further activated in response to the combined visual and olfactory stimuli. The SSS calculated using bilateral mOFC signals were

correlated to the subjective SSS score (left mOFC peak at MNI (-8, 44, -18), r = 0.541, p = 0.001; right mOFC peak at (4, 40, -20), r = 0.428, p = 0.009). We conclude that SSS to pork was detectable under both visual and combined visual and olfactory cue paradigm at behavioural and neural level, and the mOFC activity may serve as an indicator for subjective SSS. Acknowledgements: Australian Pork CRC project 3A-107. FCOI Disclosure: None.

#136 **POSTER SESSION III**

One Fish, Two Fish, Orange Fish, Green Fish: Are Foods with Non-Traditional Colors Effective in Inducing Satiety?

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Objective: Using non-liquid foods, visual impact on sensory induced satiety has not been studied in children. This study attempted to determine if nontraditional food color influenced satiety in teenagers. Procedure: Twenty-three 8th grade students (13 girls, 10 boys) without diabetes, asthma, or allergies to crackers, subjectively and objectively normosmic (Pocket Smell Identification Test 3/3) and with positive hedonics towards Goldfish Snack Crackers were assessed. On an alternating counterbalanced basis, the subjects were presented 39 (100 Kcal) orange or green Goldfish Snack Crackers and rated them on a visual analogue scale, over a 30 minute period. Degree of satiety was determined under each condition using the method of Holt (1995) and significance determined using the Sign Test for paired differences. Results: The mean satiety value for orange Goldfish was 1.04, and for green, 1.07, an insignificant difference (p = 0.52). No effect of order of presentation on satiety was found (p = 0.83). In teenagers, traditional, as opposed to novel color, had no impact in this savory model. Conclusion: It was posited that color-flavor discordance would reduce satiety. Possible reasons for lack of such include: ambiguity of natural color, familiarity effect of alternative colors, savory rather than sweet food paradigm, and population age. Further investigation is warranted as to whether use of other food types (e.g., sweet) may have demonstrated such an effect. Acknowledgements: Smell & Taste Treatment and Research Foundation. FCOI Disclosure: ARH: has ownership in the Smell & Taste Treatment and Research Foundation and consults with multiple companies and health care facilities.

#137 **POSTER SESSION III**

Food odors trigger Drosophila males to deposit a pheromone that guides female oviposition decisions

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Animals utilize olfactory signals to directly interpret the external world. Pheromones are specialized animal-derived odorants that convey critical social information between members of the same species. However, connections between environmental olfactory stimuli and pheromone signaling remain largely unexplored. We have uncovered a new pheromone-signaling pathway in Drosophila. We have found that *Drosophila* males actively deposit the pheromone 9-tricosene upon food-odor stimulation. As monomolecular attractive food odorants were not sufficient to stimulate pheromone deposition, this suggests that attractive odors *per se* are not sufficient to trigger pheromone secretion, but instead it is the perception of food-odors that drives deposition. This male-specific pheromone modulates a number of Drosophila behaviors. For example, it acts as an aggregation pheromone for both genders, as an aphrodisiac to increase successful courtship, and as a cue to guide female oviposition decisions. We used genetic, molecular, electrophysiological, behavioral, and bioinformatics approaches to demonstrate that 9-tricosene activates evolutionarily divergent Or7a receptors. To our knowledge, this is the first insect cuticular hydrocarbon pheromone that has been linked to an Odorant Receptor. We also found that loss of Or7a+ neurons or the Or7a receptor abolishes aggregation behavior and oviposition site-selection. Therefore, 9-tricosene is an olfactory signal in *Drosophila* that influences social behaviors at food sites. These studies also link food-odor perception to male pheromone deposition, aggregation behavior and subsequent female decision-making. Acknowledgements: This work was supported by grants from the Whitehall Foundation (C.J.P.) and NIH NIDCD (R01DC013070, C.J.P.). FCOI Disclosure: None.

#138 **POSTER SESSION III**

HLA correspondence on partnership and sexuality

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Aim of the study was to investigate the relation between HLA type and partnership and sexuality. Several aspects of partnership were considered, depending on the correspondance of the couples in HLA class I (A, B, C) and class II (DP, DR, DQ) alleles. We could show that couples with matching HLA class I

alleles (B,C) rate their partners odor, their sexuality and their partnership significantly worse compared to couples with non-matching HLA B and C alleles. Consequently, non-matching couples had a stronger desire to have children than matching couples. Furthermore men who were heterozygous for HLA-A were evaluated better by their partners than homozygous ones. In contrast, we did not find evidence that class II HLA alleles influence partnership. Overall, the current study supports and extends previous work showing that HLA groups are of significance in partnership and sexuality. Acknowledgements: Smell & Taste Clinic, Department of Otorhinolaryngology TU Dresden. FCOI Disclosure: Prof. Dr. Thomas Hummel, Smell & Taste Clinic, Department of Otorhinolaryngology.

#139 **POSTER SESSION III**

Sex-Dependent Olfactory Sensory Neuron Physiology in Mice

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To explore the influence of sex on olfactory sensory processing, we performed in vivo widefield epifluorescence imaging in mice expressing synaptoHluorin (spH) under the olfactory marker protein (OMP) promoter. A cranial window was implanted dorsal to the main olfactory bulbs (MOBs) of anesthetized mice of both sexes. Optical spH signals were then acquired during the presentation of a panel of 4 odorants presented at 3 concentrations each. Odorant-evoked glomerular response maps in females contained a greater number of glomeruli receiving OSN input than in males. This difference occurred across a 4-fold range of concentrations, and was independent of the odorant-response selectivity of individual glomeruli within each odor map. The most discriminative glomeruli, which received input from OSNs stimulated by only 1 of 4 odorants, had larger response amplitudes in females than in males. Glomerular odor maps in females evolved on a faster time scale than in males, and also exhibited enhanced contrast between 2 esters. To evaluate the role of gonadal hormones in mediating these sex differences, we gonadectomized (or sham-gonadectomized) adult, OMP-spH mice, waited 2 weeks, and then imaged odorant-evoked OSN activity as described above. We observed different patterns of OSN input to the MOBs of male and female subjects that were gonadally-intact (the sham controls), replicating our initial findings. Interestingly, there was a robust reduction in the number of glomeruli receiving measurable odorant-evoked input in ovariectomized females relative to sham control females. Our data suggest that sex differences in olfactory capabilities may be partly influenced by sex-dependent functioning of OSNs, and further implicate the activational effects of circulating sex hormones as a mechanism for this dimorphism. Acknowledgements: This work was supported by DC013090, DC013090-02S1, and MH101293 to JPM and DC013719 to MDK. FCOI Disclosure: None.

#140 **POSTER SESSION III**

Scent on the Pathway to Fertilization? - Olfactory Receptors, G_{olf} and Adenylate Cyclase 3 Expression in the Male Reproductive System

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Expression of olfactory receptors (ORs) has been reported in non-chemosensory tissues since this gene family was identified 23 years ago (Buck & Axel, 1991). Apart from the olfactory system, the testes contain the highest number of "ectopically-expressed" ORs (Flegel et al., 2013). To explore the role of ORs in reproduction, we used a novel antibody to the family of rat ORs and commercial antibodies to G_{olf} and AC3 in an immunohistochemistry study of adult rat testes, epididymis and sperm. We proposed that finding co-expression of the 3 signalling components would provide additional evidence for the chemosensory function of ORs in reproduction. A degenerate peptide, corresponding to a conserved region of the rat OR-gene superfamily was produced in rabbit, designated fOR-AC1. On immunoblot analysis of olfactory neuroepithelium (ON) and testes, this antibody recognized a band of apparent molecular weight 55kDa. On immunohistochemical analysis of ON, fOR-AC1 labelled the ciliary layer in an almost continuous manner. In the reproductive system, we found ORs, G_{olf} and AC3 were expressed in spermatocytes and/or spermatids in testes and also localized to the head region of spermatozoa, the residual body and the spermatozoal tail. ORs were also expressed by epididymal stereocilia and interstitial tissue. In the epididymis, ORs, G_{olf} and AC3 were expressed in the head region of spermatozoa and in the connecting and middle piece of their tails. We concluded that ORs are likely to be signalling in sperm, via G_{olf} and AC3, and are possibly responding to molecules in the seminiferous tubules or female oviduct system, on the sperm's pathway to oocyte fertilization. Future studies are needed to identify such potential ligands and determine this receptor family's function in reproduction. Acknowledgements: This work was supported by a UNSW Australian Postgraduate Award and a UNSW Brain Sciences Top-Up Scholarship to Yuliya Makeyeva. FCOI Disclosure: None.

#141 **POSTER SESSION III**

Optogenetic inhibition of mitral cell activity in the accessory olfactory bulb reduces lordosis in estrous female mice

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In estrous female mice the expression of lordosis, a reflexive arched-back response to male mounts, is decreased after bilateral lesions to the vomeronasal organ (VNO) or the accessory

olfactory bulb (AOB). The AOB receives pheromonal inputs from the VNO and sends mitral cell (MC) axonal projections to forebrain targets controlling lordosis. We asked if optogenetic silencing of AOB MCs reduces the display of lordosis in females. Adult protocadherin21-Cre mice expressing cre-recombinase selectively in main olfactory bulb and AOB MCs were ovariectomized and given bilateral AOB injections of a cre-dependent ArchT and GFP vector (AAV8/Flex-ArchT-GFP; UNC Vector Core). ArchT is an outward proton pump that when activated by green light suppresses firing in mammalian neurons. Laser light (532 nm, 10mW at the fiber tip) delivered via an optical fiber implant on the midline above the AOB was used to inhibit MC firing. Females were given s.c. injections of estradiol benzoate and 48 hr later progesterone to induce behavioral estrus. Sexually experienced females received a series of five 20-min lordosis tests (Tests 1, 2 and 4 without any optogenetic stimulation and Tests 3 and 5 with the laser on continuously), and the lordosis quotient (LQ; number of lordosis responses/number of mounts received x 100) was determined for each test. Females with bilateral ArchT infections of the AOB (mean estimate of 260 GFP+ neurons/hemisphere) showed significant reductions in LQs during tests when the laser was switched on compared to tests with no laser. No optogenetic inhibition of lordosis was seen in mice with unilateral or low ArchT infection rates. Our results affirm the essential role of the VNO-AOB pheromone signaling pathway in the expression of lordosis behavior in female mice. Acknowledgements: Supported by NIH grant DC008962. FCOI Disclosure: None.

#142 **POSTER SESSION III**

Gender Difference in Chinese Adults with Postviral Olfactory Disorder

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Background: Postviral olfactory disorder (PVOD) is one of the most common reasons for acquired olfactory dysfunction, but there is little available information on this problem in Chinese adults who face different environmental exposures, life courses, and genetic susceptibility. We analyzed Chinese subjects with PVOD in order to determine whether demographic risk factors and clinical features are similar to Western populations. Methods: We examined data from 143 subjects with PVOD and 117 subjects with other olfactory disorders (non-PVOD; controls) presenting to two smell and taste centers in Beijing, China. We examined the age, duration and severity of olfactory loss in these subjects, including assessment of subjective and objective olfactory function (Sniffin' Sticks, Olfactory Event Related Potential [OERP], and olfactory pathway Magnetic Resonance Imaging [MRI]). Results: There were more women in the PVOD group compared to controls (70.6% vs. 42.7%, p< 0.001). In addition, we found an interesting age interaction: older subjects with PVOD were more likely to be women (79.5% vs. 61.4%, p=0.027). Consistent with this finding, the proportion of women with PVOD increased with the duration of olfactory loss p=0.032). There were no differences in the prevalence of abnormal OERP between subjects with PVOD and controls, although the female to male ratio was higher for those with PVOD (p=0.041). Lastly, women with PVOD were less likely to have olfactory pathway abnormalities by MRI compared to controls (p< 0.001). Conclusions: Chinese women face higher susceptibility to PVOD, a discrepancy that worsens with age and duration of loss. The similarity of our findings to prior data from Western nations supports a common etiology of PVOD that may be independent of environmental, cultural, or genetic differences. Acknowledgements: grants from the Natural Science Foundation, China (MD: 81271062) and Science and Technology plan project of Beijing (MD: Z121107001012041). FCOI Disclosure: None.

#143 **POSTER SESSION III**

Cortical feedback decorrelates olfactory bulb output in awake mice

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The olfactory bulb receives rich glutamatergic projections from the piriform cortex. However, the dynamics and importance of these feedback signals remain unknown. Here, we use multiphoton calcium imaging to monitor cortical feedback in the olfactory bulb of awake mice and further probe its impact on bulb output. Responses of feedback boutons were sparse, odor specific and often outlasted stimuli by several seconds. Odor presentation both enhanced and suppressed the activity of boutons. However, any given bouton responded with stereotypic polarity across multiple odors, preferring either enhancement or suppression. Feedback representations were locally diverse and differed in dynamics across bulb layers. Inactivation of piriform cortex increased odor responsiveness and pairwise similarity of mitral cells, but had little impact on tufted cells. We propose that cortical feedback differentially impacts the two output channels of the bulb by specifically decorrelating mitral cell responses to enable odor separation. Acknowledgements: NIH. FCOI Disclosure: None.

#144 **POSTER SESSION III**

The effects of specific inhibition of feedback projection from piriform cortex on the olfactory bulb

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The olfactory bulb (OB) is the first relay station of the olfactory system and plays important roles in odor information coding, processing and transmission. It receives input not only from the

olfactory sensory neurons, but also from the olfactory cortex and neuromodulatory inputs from other areas. The feedback projection from piriform cortex to the OB is critical for odor presentation and processing in the OB, however, the evidence of effects of specific inhibition of projections from piriform cortex to the OB is lacking. Here, we selectively express halorhodopsin in the piriform cortex pyramidal cells and test how specific inhibition of the projections affects the activities of mitral/tufted cells (M/Ts) in the OB. We find that light evoked inhibition of piriform cortex projections causes profound effects on the spontaneous activities of M/Ts: while in most of the M/Ts it decreases firing rate tonically, in a few cells it increased firing rate phasically. For odor stimulation evoked activity of M/Ts, inhibition of piriform cortex projections significantly decreases the response magnitude. Our results indicate that the piriform cortex can affect mitral/tufted cells, perhaps by a direct way, not only an indirect pathway via granule cells as reported in previous studies. Acknowledgements: NIH 5RO1DC00566 and 5RO1DC04657. FCOI Disclosure: None.

#145 **POSTER SESSION III**

Surprise alters primary sensory input to the brain

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Mammals learn about regularities in their sensory environment, thus establishing expectations that can influence neural processing of sensory stimuli. Here we show that violating expectations about the presentation of a tone cue can influence sensory processing of odors as early as the first synapse in the olfactory system. We performed wide-field *in vivo* imaging from awake, head-fixed OMP-synaptopHluorin mice. In these mice, expectations were established by repeatedly presenting a light-tone-odor sequence and then violated by omitting the expected tone while presenting the light and odor as usual. There was a suppression of odorant-evoked neurotransmitter release from OSNs during the surprising odorant presentation compared to the previous expected, tone-cued odorant presentation. This effect was not observed if mice were anesthetized or if the absence of the tone was unsurprising. Imaging of sniff-by-sniff calcium dynamics in OSN presynaptic terminals revealed that suppression of activity is present on the very first inhalation of odorant during the surprising trial. This suggests a $GABA_B$ receptor-mediated presynaptic inhibition, so we repeated the experiment in GAD2-GCaMP3 mice where we could visualize the activity of GAD65-expressing periglomerular interneurons. Consistent with this hypothesis, we observed an increase in GCaMP signals during the first inhalation of odorant on the surprising trial. Finally, we systemically blocked $GABA_B$ receptors with CGP35348 and observed removal of a tonic presynaptic inhibition of OSN terminals, after which the surprising omission of an expected tone no longer had any effect on odorant-evoked neurotransmitter release. These experiments suggest that expectation and surprise can shape sensory processing as early as the primary input into the brain Acknowledgements: This work was supported by NIH grants DC009442, MH101293, and DC013090 to JPM. FCOI Disclosure: None.

#146 **POSTER SESSION III**

Functional connections of medial amygdala circuits involved in evaluating chemosensory communication signals: An electrophysiological analysis

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Medial amygdala (Me) is a primary CNS area involved in analysis of chemosensory communication signals, however relevant circuits are not well studied. It receives projections from olfactory and vomeronasal systems in rodents and responds differentially to conspecific and heterospecific chemosignals that convey different meanings. Me projects to hypothalamic and preoptic circuits to engage appropriate behavioral responses. Our amygdala recordings follow up previous immediate early gene studies that implicate the amygdala's main intercalated nucleus (mICN) in producing characteristic responses to different chemosignals. mICN is one of several GABA-ir intercalated cell groups in the amygdala and appears to selectively inhibit the adjacent posterior medial amygdala (MeP), one of the primary output nuclei for chemosignal evaluation. This mechanism is similar to the regulation of basolateral and central amygdala by their adjacent (paracapsular) intercalated cell groups in the fear conditioning circuit – suggesting a modular organization in the amygdala. The Me/mICN circuit involved in chemosensory processing has not been directly studied. In new research using whole cell patch-clamp electrophysiology in hamster coronal and horizontal brain slices, we show a functional inhibitory connection between mICN and MeP, with hyperpolarization of MeP neurons viapharmacological and electrical stimulation of mICN. Furthermore, in horizontal slices, we show functional excitatory connections from the primary chemosensory-recipient zone of Me (anterior medial amygdala; MeA) to both mICN and MeP. Bath-applied dopamine (DA) significantly and reversibly reduces mICN hyperpolarization of MeP neurons. DA modulation of mICN could produce a brain state dependent bias in selection of behavioral response. Acknowledgements: Supported by NIDCD grants R01-DC005813, T32-DC000044 and funding from Florida State University. FCOI Disclosure: None.

#147 POSTER SESSION III

The olfactory thalamus: characterizing single-unit activity of the mediodorsal thalamic nucleus in behaving rats

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The thalamus is a key crossroad structure in the brain and is recognized as a major contributor to sensory perception, attention, sleep and arousal and memory. For all senses except olfaction, the information from the sensory neurons necessarily passes through a thalamic nucleus before reaching the primary sensory cortex. However, an olfactory thalamic nucleus exists: the mediodorsal thalamic nucleus (MDT) receives direct input from different olfactory structures including the piriform cortex (PCX), and in turn has bi-directional projections with the orbitofrontal cortex (OFC). Functionally, we have shown that, in urethane-anesthetized rats, MDT units respond to a wide variety of odorants and that odor stimuli induce a conjoint emergence of beta frequency oscillations in both the MDT and the PCX. Beyond this odor responsiveness, the precise role of the MDT in olfaction remains unclear. In fact, lesion studies in both humans and animal models suggest a role for the MDT in olfactory perception, odor discrimination, learning and attention. To investigate precisely the role of the MDT in olfactory processing, we recorded MDT single unit activity, using a multi-tetrode drive, in 8 rats performing a two alternative odor discrimination task. Our preliminary analyses demonstrate that a majority of MDT units modulate their firing rate during the task window. The MDT units seem to encode a variety of information. For example, a subset of MDT units modulate their firing rate before the nose poke/trial initiation, others show modulation during the sampling period as a function of the odorant, while others are modulated during sampling termination/decision making. Our initial analyzes thus reveal the involvement and the complex role of the MDT in olfactory processing. Acknowledgements: This work was supported by a grant DC003906 from the NIDCD to D.A.W and a Philippe Foundation award to E.C. FCOI Disclosure: None.

#148 POSTER SESSION IV

Interaction of the N-terminal Domain of Human T1R2 Taste Receptor with Brazzein, a Sweet-tasting Protein

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Brazzein is a small sweet-tasting protein found in the fruit of a West African plant, perceived through the activation of the T1R2/T1R3 heterodimeric sweet-taste receptor. T1R2 and T1R3 possess a large N-terminal domain (NTD) linked to a heptahelical domain by a cysteine rich domain (CRD). Molecular docking and mutagenesis studies have revealed that brazzein may interact primarily with The NTD of T1R2 (T1R2-NTD) and makes also favorable contacts with T1R3-NTD. In contrast, brazzein has been shown to require CRD of human T1R3 for receptor activation. To elucidate the contribution of T1R2-NTD to brazzein detection, we overexpressed T1R2-NTD in Escherichia coli as inclusion bodies. Human T1R2-NTD was refolded and characterized for its ability to interact with recombinant brazzein secreted by the yeast *Pichia pastoris*. Brazzein/T1R2-NTD interactions were measured using Bio-Layer Interferometry (BLI). This recent method is powerful for studying protein-protein interactions and measuring both affinity constants and kinetic parameters. BLI experiments were performed first by immobilizing T1R2-NTD onto a biosensor and measuring brazzein binding. In a second experiment, brazzein was immobilized and T1R2-NTD binding was followed using BLI. Both experiments showed that T1R2-NTD interacts with brazzein with a Kd value of approximately 30 µM. This affinity is in agreement with the capacity of brazzein to activate T1R2/T1R3 receptor heterologously expressed in HEK cells and with sensory experiments conducted on humans. This is the first demonstration that T1R2-NTD is able to interact with brazzein in absence of T1R3. To identify the binding site, site-directed mutagenesis will be conducted on both brazzein and T1R2-NTD. Acknowledgements: This work was supported by grants from the Regional Council of Burgundy France (PARI Agral 1) and the FEDER (European Funding for Regional Economical Development). FCOI Disclosure: None.

#149 POSTER SESSION IV

Gustatory synergy between sugars and amino acids in the yellow fever mosquito, Aedes aegypti

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Many insects including mosquitoes, butterflies, honeybees, flesh flies and ants have been shown to prefer diets that contain both sugars and amino acids. Fitness gains as enhanced longevity, flight capacity and fecundity, suggest an ultimate evolutionary mechanism for the preference of amino acid-rich sugar diets. However, the proximate mechanism(s) behind this preference has not yet been explored. In a recent study, the yellow fever mosquito, *Aedes aegypti*, was unable to detect individual amino acids unless combined with a behaviorally sub-threshold concentration of a sugar. This behavioral response to combination of two nutrients at individual sub-threshold concentrations indicates a synergistic proximate mechanism underlying the mosquito gustatory preference. To investigate this further, we conducted feeding bioassays and single-sensillum electrophysiological recordings using a combination of trehalose and various concentrations of either one of seven ecologically relevant amino acids (alanine, arginine, glycine, leucine, phenylalanine, threonine and valine). In feeding assays, females prefer and imbibe mixed diets in significantly higher volumes in a dose-dependent manner than trehalose alone for all amino acids except glycine & arginine. The single-sensillum recordings allowed us to generate a chemotopic map of the dorsal labellar

sensilla that describes five of the seven test mixed diets as eliciting responses in different sensilla, either increasing (leucine, valine & alanine) or decreasing (glycine & arginine) gustatory neuron firing rates. Our results indicate that the synergistic behavioral response of female *Ae. aegypti* to amino acid and sugar mixed diets is a result, at least in part, of combinatorial coding of the gustatory neuron input from the dorsal sensilla on the labellar lobes. Acknowledgements: The Crafoord Foundation grant (project number 19606000). FCOI Disclosure: None.

#150 **POSTER SESSION IV**

Taste cell-expressed carbohydrate-digesting enzymes contribute to gustatory responses to disaccharides

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The major sensor in mammals for sugars and non-caloric sweeteners is the combination of type 1 taste receptors 2 and 3 (T1r2+T1r3). This may be the only receptor of sweeteners, but in its absence (e.g. in T1r3 knockout mice), T1r-independent mechanisms function in taste cells to detect sugars. Several glucose transporters (GLUTs), sodium-glucose co-transporter 1 (SGLT1) and the ATP-gated K^+ (K_{ATP}) metabolic sensor are co-expressed in taste cells with T1r3. Thus, the taste cells that respond to sugars and sweeteners may contain two sweet-sensing pathways: T1r-dependent (T1r2+T1r3) for detecting sugars and sweeteners, and T1r-independent (GLUTs, SGLT1, K_{ATP}) for detecting sugars but not sweeteners. However, the T1r-independent pathway would only explain responses to monosaccharides (e.g. glucose and fructose); disaccharides such as sucrose and maltose are not substrates for transport by GLUTs or SGLT1. Thus, responses of T1r3 knockout mice to disaccharides may first require hydrolysis to monosaccharides. Using RT-PCR, qPCR, in situ hybridization and immunohistochemistry, we found that many intestinal enzymes involved in carbohydrate digestion, viz. alpha-amylase, maltase-glucoamylase and sucrase-isomaltase, are expressed also in taste tissue and taste cells. Treating the tongue with disaccharidase inhibitors decreased gustatory nerve responses to disaccharides but not to monosaccharides or sweeteners. In concert with amylase from the main and accessory salivary glands, these taste-expressed enzymes may locally produce monosaccharide substrates for the T1r-independent sugar-sensing pathway. The presence of two sugar-sensing pathways in the same sweet-responsive taste cells may underlie the unique sensory response to sugars and enable discrimination of sugars from non-caloric sweeteners. Acknowledgements: Supported by NIH grants R01DC03055, R01DC03155 and R01DK081421 to RFM. FCOI Disclosure: None.

#151 **POSTER SESSION IV**

Influence of saccharide length on detection of glucose polymers in humans

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Our previous study showed evidence that humans can taste glucose polymers independent of sweet taste. This supports the existence of a separate taste receptor for glucose polymers. However, glucose polymer preparations used previously consist of mixtures of mono-, di-, oligo-, and polysaccharides. Thus, it is unclear what the specific chain lengths are being detected by the hypothesized receptor. This study was thus designed (1) to prepare glucose polymer stimuli with specific ranges of degree of polymerization (DP), and (2) to identify the range of DP that can be tasted. Stimuli were prepared by fractionating commercial maltodextrin based on differential solubility in ethanol. Saccharide composition was quantified using HPLC. The following stimuli were produced: (S1) ~75% DP3-8, ~25% DP9+; (S2) ~25% DP3-8, ~75% DP9+; and (S3) 100% DP9+. Subjects then performed a discrimination task using a sip-and-spit procedure while wearing nose clips. Since salivary α-amylase was expected to hydrolyze glucose polymers and potentially alter the stimuli composition, Ss evaluated the stimuli (8 and 10%) and blank samples in the presence and absence of acarbose, an □-amylase inhibitor. Results showed that in the presence of acarbose, Ss were able to discriminate both concentrations of S1 but not S3. Discrimination improved in the absence of acarbose due to suspected hydrolysis of longer chain to shorter chain saccharides. These results suggest that optimal glucose polymer chain length that can be tasted is DP3-8. More studies are underway to confirm that DP 3-8 is mediated through a separate taste receptor, but not by the sweet receptor. Acknowledgements: Supported by university. FCOI Disclosure: None.

#152 **POSTER SESSION IV**

Dietary sugars downregulate intestinal sweet taste receptors leading to altered glucose absorption in mice

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Epidemiological data suggest that consumption of a high-fructose diet is associated with adverse metabolic effects, weight gain and obesity. Although, the pathophysiological mechanisms for these are largely unknown, dietary sugars activate sweet taste receptors (STRs) expressed in a variety of tissues, including the gastrointestinal (GI) tract. We hypothesize that STR may be a mechanistic link between the high consumption of fructose and adverse metabolic outcomes. Mice were placed on a high (60%) sucrose diet (HSD) or corn starch (60%) diet (CON) for one feeding cycle (overnight) and STR function was assessed. Mice lacking STR signaling (T1R2 knockout; KO) were used to evaluate the direct role of STRs in response to sugar feeding.

Intestinal expression of t1r2 and t1r3 (STRs) and glut2 (glucose transporter) genes was significantly downregulated in response to short-term HSD compared to CON. Mice on HSD also had lower plasma glucose excursions immediately (5-15min) after an oral glucose tolerance test that was due to reduced rates of glucose absorption (using ^{13}C-6-glucose). Notably, T1R2-KO mice had reduced rates of glucose absorption independent of the dietary intervention, likely due to reduced transport and/or enhanced glucose oxidation in enterocytes. High dietary sugar consumption, frequently seen in obesity and linked to metabolic diseases, can regulate intestinal STR transcripts leading to alterations in glucose absorption. These data suggest that STRs function to coordinate adaptive responses of the intestine to changes in nutrient availability regulating energy absorption. Understanding the mechanisms of these regulatory pathways may assist in the development of dietary interventions that prevent or delay metabolic dysfunction. Acknowledgements: Institute funds. FCOI Disclosure: None.

#153 **POSTER SESSION IV**

Does sweetener synergy depend on multiple T1R2/T1R3 receptor binding sites?

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Sweet taste is primarily transduced by the T1R2/T1R3 heterodimer in humans. To date, 4 distinct binding sites have been identified on this dimer, with differing specificity for various ligands that are perceived as sweet. Currently, we are testing whether a biologically informed model can predict pairs of sweeteners that elicit synergistic (hyperadditive) responses: we hypothesize ligands which bind to different sites will show synergy, while those that act upon a common binding site will not. While the literature contains many reports of sweetener synergy, others have noted much of this work is critically flawed as simple additive models fail to account for nonlinear dose response functions (e.g., Lawless 1998; Suhnel 1993). Accordingly, here we apply the isobole method from pharmacology to avoid this problem when defining synergy. In two preliminary studies, aspartame/acesulfame potassium (AceK) and fructose/acesulfame potassium blends of varying concentrations were tested in water. In each, ~100 participants rated sweetness and bitterness using a general Label Magnitude Scale (gLMS). As expected, AceK/Aspartame blends show evidence of synergy: when using a 3D isobologram that takes the different dose-response functions of the individual sweeteners into account, we find the blends are ~60-70% sweeter than would be otherwise expected. In contrast with previously published work, we also find evidence that AceK/fructose blends synergize, with observed values ~50-55% sweeter than the values predicted from a response surface based on the individual dose response functions. As the isobole method appears to be successful for assessing synergy, we are currently testing additional pairs of sweeteners to explore the binding site hypothesis further.

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#154 **POSTER SESSION IV**

Further Psychophysical Evidence that the Taste of Glucose Polymers is Transduced via a T1R2+T1R3-Independent Mechanism in Mice

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In mammals, the T1R2+T1R3 heterodimeric taste receptor binds with sweeteners such as glucose. However, glucose polymer mixtures, e.g. Polycose and Maltrin 580, have been suggested to activate a T1R2+T1R3-independent receptor. We have previously reported that T1R2+T1R3 knockout (KO) mice and wild-type controls (WT) can detect Polycose using a two-response operant taste detection procedure although a bimodal distribution was observed in the KO mice. Here we trained these same water-deprived mice (WT: N=11, KO: N=12) to discriminate sucrose from water to earn a water reinforcer. WT mice performed with high accuracy across all tested suprathreshold concentrations ($p \leq 0.01$). Five KO mice were unable to learn the discrimination and the remaining 7 showed severely impaired performance. However, these 7 KO mice could discriminate at least the highest sucrose concentration above chance levels (1.0 M: all $p \leq 0.04$). To determine if these 7 KO mice were attending to viscosity to discriminate sucrose from water, we trained all mice to discriminate the putatively tasteless viscous agent xanthan gum from water. The resulting response profiles in the KO mice across stimuli indicated that viscosity likely enabled sucrose detection, but may have had only a minor role in Polycose detection. Therefore, we pitted Maltrin (4, 18, and 28%) against sucrose (0.25, 0.85, 1.25 M), matching the concentrations for viscosity to render it an irrelevant cue. Overall, the KO mice were able to discriminate Maltrin and sucrose comparable to WT mice (KO: 78.1%, WT: 85.3%; $p=0.13$), with performance in the KO mice likely being driven by a Maltrin and not sucrose taste cue. Collectively, these data support the presence of a glucose polymer taste receptor, at least in mice, as originally hypothesized by Tony Sclafani over two decades ago. Acknowledgements: NIH R01-DC004574 to ACS and NSF GRFP & NIH T32-DC000044-20 to KRS. FCOI Disclosure: None.

#155 POSTER SESSION IV

Genome-wide analysis of quantitative trait loci for behavioral and neural taste responses to sweeteners in F2 hybrids between C57BL/6ByJ and 129P3/J mice

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Inbred mouse strains differ in taste responses to sweeteners. Mice from the C57BL/6ByJ (B6) strain have stronger behavioral and neural responses to sweeteners than mice from the 129P3/J (129) strain. To conduct genetic analyses of sweet taste responses, we produced hybrids of the second filial generation (F_2) between the B6 and 129 strains, and measured their sweetener consumption in two-bottle preference tests and integrated responses of the chorda tympani gustatory nerve to lingual application of sweeteners. We determined genotypes of the F_2 mice for chromosomal markers distributed across the whole genome and analyzed their associations with sweet taste phenotypes. The resulting genome scan identified several quantitative trait loci (QTLs) with unique patterns of effects on phenotypes of behavioral and neural responses to sweeteners. We have confirmed our previous finding that polymorphisms of the *Tas1r3* gene (encoding the T1R3 taste receptor protein) underlie a QTL on distal chromosome 4 that has a pleiotropic effect on behavioral and neural responses to multiple sweeteners. This was a major QTL influencing chorda tympani responses to sweeteners: although we have detected several additional QTLs for neural responses, all of them were suggestive ($P < 0.63$). Another QTL on chromosome 2 influenced consumption of several nutritive and non-nutritive sweeteners (sucrose, saccharin, D-phenylalanine, glycine) and may be involved in central mechanisms of sweet taste. Several additional QTLs influenced consumption of sucrose but not other sweeteners; their effect may be mediated by postingestive effects of sucrose. These results illustrate complex genetic architecture of consummatory responses to sweeteners and set a stage for identification of corresponding genes. Acknowledgements: Supported by NIH R01DC00882 (AAB and GKB), and NIH P30DC011735 (Robert F. Margolskee). FCOI Disclosure: None.

#156 POSTER SESSION IV

Preference for sweeteners mixed with compounds preferred or avoided by golden hamsters (Mesocricetus auratus)

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Hamsters prefer sucrose and ethanol, sweet tastes with a reward hierarchy of sucrose > ethanol. Chorda tympani (CT) mixture responses to caloric sucrose (or non-caloric sucralose) + ethanol are enhanced above component response additivity (Browne et al, 2012). Mixed with 500 mM sucrose, aversions to natural bitter stimuli such as salicin and quinine were reduced; but avoidance of 30mM caffeine and 30μM cycloheximide remained unchanged (Hettinger et al, 2007). The bitters show a harm hierarchy of cycloheximide > quinine > salicin > caffeine. The most averse cycloheximide and least averse caffeine are ineffective CT stimuli (Frank et al, 2004). We performed 2-bottle, solution vs. water, intake tests to determine whether [1] preference behavior to 30 mM or 100 mM sucrose + 5% or + 10% ethanol were enhanced and whether [2] binary mixtures with 500 mM sucrose ameliorated aversions to much lower concentrations of caffeine (1, 3, 5mM) and cycloheximide (1, 3, 5μM) that lacked tastes. The hamsters chose to drink sucrose components (95±1%) and binary sucrose + ethanol mixtures (94±%) with preferences over water ($p < 0.01$) approaching 100%. Sweet preference behavior appeared to be predictable from the reward of the elicited taste quality. Caffeine was not, but cycloheximide at 3μM ($p = 0.04$) and 5μM ($p = 0.03$) was aversive. At these lower concentrations, adding sucrose increased intake of both caffeine ($p = 0.02$) and cycloheximide ($p = 0.00004$), converting the average 46±5% intake of caffeine and 35±3% cycloheximide aversion to 65±4% preferences. Caffeine and cycloheximide retained residual aversions after adding the 85±4% preferred 500 mM sucrose. Thus, 48-hr preferences may reflect reward-harm hierarchies rather than the size of taste-nerve responses. Acknowledgements: Supported by School of Dental Medicine Alumni Research Fellowships, the UCONN Foundation and NIH R01 DC004099. FCOI Disclosure: None.

#157 POSTER SESSION IV

Mice selected for high and low saccharin intake differ in consumption of appetitive taste solutions regardless of their taste quality

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In mice, variation in sweet taste is influenced by polymorphisms of the *Tas1r3* gene. However, there are *Tas1r3*-independent genetic factors that influence variation in sweet taste responses as well. To examine *Tas1r3*-independent sweet taste mechanisms,

we selectively bred two strains of mice that share a common *Tas1r3* allele, yet differ in saccharin intake: the Sac-H and Sac-L strains, with high and low saccharin intakes, respectively. Electrophysiological recordings of taste-evoked responses in the chorda tympani nerve showed that these strains had similar peripheral taste responsiveness. These results suggest that strain differences in saccharin intake may be caused by strain differences in central taste processing. To distinguish whether these central mechanisms are specific to sweet taste or are more general and apply to other appetitive taste stimuli, we examined preferences for sweet (saccharin, sucralose, and sucrose) and non-sweet (oil, maltodextrin, MSG, and IMP) palatable solutions using two-bottle choice tests. We found that regardless of taste quality, when preference scores exceeded 80% in both strains, Sac-H mice consumed significantly more taste solution than Sac-L mice. Interestingly, although mice preferred MSG, preference scores did not exceed 80% at any concentration tested, and MSG intakes did not differ between strains either. Given that MSG and IMP have the same taste quality (i.e., umami), these results support the conclusion that differential consumption of taste solutions by Sac-H and Sac-L mice is driven by the hedonic value of a taste solution, not taste quality. Our data suggest that mice selected for saccharin intake are a valuable model for analyzing mechanisms of reward because taste stimuli must evoke a strong appetitive response to trigger differential intake. Acknowledgements: Supported by NIH R01DC00882 (AAB), and NIH P30DC011735 (Robert F. Margolskee). FCOI Disclosure: None.

#158 **POSTER SESSION IV**

Reciprocal crosses between C57BL/6J and DBA/2J reveal chromosomal and epigenetic taste-preference signatures in laboratory mice

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Reciprocal crosses between inbred strains provide evidence related to maternal (pre/post-natal), mitochondrial, sex chromosomal and genomic imprinting factors in biobehavioral phenotypes. C57BL/6J and DBA/2J mouse strains, which differ markedly in gustatory phenotypes (D2 avoids sucrose octacetate or SOA but B6 does not; B6 more averse to quinine and stronger preference for sucrose), were reciprocally crossed (B6/D2, D2/B6; female parent first in pairings) and 20 mice in each cross/sex sub-group given 2-bottle preference tests (% solution/total intake) for bitter (SOA, Quinine and Nicotine), salty (NaCl) and sweet (Sucrose) solutions. Patterns of preference inheritance among males and females of the 2 crosses were consistent with 4 hypotheses. (1) The 1mM SOA aversion is an example of sex-specific imprinting, with D2 allele silent in D2/B6 males (preference 50%) yet contributing to aversion in the 3 other sex X cross-combinations (D2/B6 females, 35%; B6/D2 males, 40%, females 36%; all $p < .01$). (2) The 0.2M NaCl aversion suggests imprinting because regardless of sex, D2/B6 is less averse than B6/D2; this pattern is inconsistent with sex chromosome and maternal influences. (3) The 0.1 and 0.3mM Quinine and 0.15 and 0.46mM Nicotine aversions suggest sex differences with females more averse than males but no difference between crosses. (4) The 0.03 and 0.1M Sucrose preferences show no epigenetic or sex chromosomal influences. Our data provide new perspectives on sex differences and epigenetics in aversions to SOA (Harder et al, Chem Senses 17: 391, 1992), the bitter taste modality, and hypertonic NaCl ingestion (Curtis & Contreras, Behav Neurosc 120: 917, 2006). The role of imprinting in peripheral taste receptors requires examination of methylation patterns at genomic locations of taste genes. Acknowledgements: Supported by NIH R01 DC004099, the UCONN Foundation and the PSU Dept of Biobehavioral Health. FCOI Disclosure: None.

#159 **POSTER SESSION IV**

Genetic analysis of mouse strains selectively bred for high and low saccharin intake

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Inbred mouse strains differ in their sweet taste responses. Although allelic variation of the *Tas1r3* gene is partially responsible for these differences, our genetic analyses using C57BL/6ByJ (B6) and 129P3/J (129) strains suggest that other quantitative trait loci (QTLs) are also involved. To confirm and identify these QTLs, we produced an F_2 intercross between B6 and 129.B6-*Tas1r3* congenic mice. Despite the genetic identity at the *Tas1r3* locus, F_2 mice varied in consumption of 20 mM saccharin. Therefore, starting from the F_2 generation, we conducted selective breeding of strains with high (Sac-H) and low (Sac-L) saccharin intakes. The phenotype-based selection resulted in dramatic divergence between these strains, which confirms presence of sweetener intake QTLs other than *Tas1r3*. To refine chromosomal positions of these QTLs, we have genotyped mice from the 4th, 8th and 18th generations of selective breeding with markers on chromosomes (Chr), which were previously linked to saccharin intake in segregating crosses between the progenitor strains. For all chromosomes, there was a significant divergence of frequencies of alleles in these regions, which gradually increased in subsequent generations of selective breeding. The Sac-H strain accumulated B6 alleles in Chr1, 3 and 13, and 129 alleles in Chr2 and 7. The Sac-L strain accumulated alleles from the opposite progenitor strain at these locations. Our results show that saccharin intake is a trait with a complex genetic architecture, and that its regulation may involve different physiological mechanisms. Using our approach of selective phenotype-based breeding coupled with genotyping, we have detected several new QTLs for sweet taste. Acknowledgements: Supported by NIH R01DC00882 (AAB and GKB), and NIH P30DC011735 (Robert F. Margolskee). FCOI Disclosure: None.

#160 POSTER SESSION IV

Gustatory Stimulation with Sucralose Results in Differential Patterns of Fos-like Immunoreactivity in the Rostral Nucleus of the Solitary Tract of Sucralose-Preferring and –Avoiding Rats

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Psychophysical analyses of the salient taste quality of the sweetener sucralose reveal that 25% of rats generalize the taste of concentrated sucralose solely to that of sucrose (sucralose preferrers; SP), while the remaining 75% of rats detect a quinine (QHCl)-like quality at identical concentrations (sucralose avoiders; SA). 'Bitter' and 'sweet' taste qualities are known to evoke starkly differing patterns of neural activity. While 'bitter' QHCl activates a population of cells clustered in the dorsal medial (DM) area of the rostral nucleus of the solitary tract (rNTS), 'sweet' sucrose evokes a more diffuse pattern with greater activity in the dorsal central (DC) area, relative to QHCl. Based on these findings, we reasoned that sucralose would elicit differing patterns of neuronal activity in SP and SA. To test this hypothesis, Fos-like immunoreactivity (FLI) was quantified in the rNTS (bisected into dorsal/ventral halves with each subdivided into medial/central/lateral thirds) after oral infusion of QHCl, sucrose and sucralose in SA and SP. Neuronal representation of the taste of sucralose differed in SP/SA in a manner consistent with their preference/avoidance profile ($F(5,70)=3.46$, $P< 0.01$). In SA, sucralose elicited FLI primarily in the DM rNTS, a pattern that was indistinguishable from QHCl. Sucralose elicited similar amounts of FLI in the DM rNTS of SP as SA ($P=0.56$), but FLI was increased in the DC area in SP, relative to SA ($P< 0.01$), similar to the pattern of FLI elicited by sucrose. These data are the first to examine the neural responses to sucralose among SA and SP. They provide evidence that the differing SP/SA phenotype is the result of an increased sweet-like signal generated by sucralose in SP, and suggest a potential polymorphism in one of the T1R receptor proteins inherent to rats. Acknowledgements: T32 DC-000044.
FCOI Disclosure: None.

#161 POSTER SESSION IV

Not all artificial sweeteners are created equal

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Artificial sweeteners bind to the canonical sweet taste receptor, T1r2/T1r3. Because T1r2/T1r3 is expressed on taste cells in the mouth, enteroendocrine cells in the intestine and beta cells in the pancreas, it has been hypothesized that artificial sweeteners may alter insulin release and glucose tolerance. While some studies provide support for this hypothesis, others provide contrary evidence. One source of confusion may be that investigators often use different artificial sweeteners. To test this possibility, we asked whether the ingestion of two commonly used artificial sweeteners (sucralose and saccharin) produces similar changes in plasma insulin and blood glucose levels in C57BL/6 (B6) mice. We used concentrations of sucralose (20 mM) and saccharin (38 mM) that elicit vigorous licking in B6 mice; we also included glucose (1 M) as a positive control. We took tail blood samples both before and after each mouse had completed a specified number of licks (i.e., 4.3 licks/gram mouse) from a sweetener solution. As expected, ingestion of the glucose solution increased plasma insulin and blood sugar levels. Unexpectedly, ingestion of the sucralose solution *decreased* plasma insulin and slightly increased blood sugar levels. Ingestion of saccharin had no systematic effect on plasma insulin or blood sugar levels. Our findings indicate that not all artificial sweeteners are created equal—some alter blood sugar homeostasis, while others have no measurable impact. Acknowledgements: Support provided by Barnard College. FCOI Disclosure: None.

#162 POSTER SESSION IV

Maltodextrin acceptance and preference in eight strains of mice

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We used brief-access tests and 48-h two-bottle choice tests to survey the avidity for maltodextrin in mice from 8 inbred strains (A/J, CAST/EiJ, C57BL/6J, NOD/ShiLTJ, NZO/JILtJ, PWK/PhJ, WSB/EiJ, and 129S1/SvImJ). These strains, which are founders of the Collaborative Cross, capture ~90% of the genetic variation in laboratory mice; they thus provide a useful initial screen to assess genetic variation in taste responses. To characterize their taste acceptance, we gave 5-s brief access tests with 0, 2, 4, 6, 8 or 16% maltodextrin or sucrose. Each carbohydrate was given in 3 sessions on alternating days and each concentration was presented ~9 times in pseudo-random order over 15 min. The CAST and PWK strains licked more sucrose than maltodextrin at every concentration tested. The B6 and NOD strains licked more 16% sucrose than 16% maltodextrin only; lick rates for other concentrations did not differ. The other 4 strains did not differ in lick rates for any concentration of maltodextrin and sucrose. To examine strain differences in taste preferences, the mice received two 48-h tests with continuous access to two bottles, one containing either 4% maltodextrin or 4% sucrose and the other containing water. Consistent with the results of the brief-access tests, the CAST and PWK strains had higher preference scores for sucrose than for maltodextrin; the other strains preferred the two solutions equally. These results show that the Collaborative Cross founder strains capture variation in maltodextrin perception that is distinct from the variation in sucrose perception. This extends previous research suggesting the avidity for maltodextrin and for sucrose involve distinct genetic mechanisms. The phenotypes characterized here will aid in identifying the genes responsible for maltodextrin acceptance. Acknowledgements: Supported by DC10149 and DC10393. FCOI Disclosure: None.

#163 **POSTER SESSION IV**

Dietary Sugar Levels Affect Sweet Taste Intensity

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Various health organizations have recommend reductions in the intake of added sugars. Replacing simple sugars with non-nutritive sweeteners can help, but non-nutritive sweeteners are not acceptable to all consumers. The literature on salt taste suggests another potential approach: After at least several weeks on a lowered sodium diet, subjects may perceive a given concentration of salt in food as more intense or their optimal (preferred) level of salt shifts to lower concentrations. Might sweet perception adjust in a similar fashion with reduced sugar intake? Healthy adults participated over several months. All followed their usual diet for the first (baseline) month. During the next three months, half (control group) were randomly assigned to follow their normal diet, whereas the other half (low sugar group) were instructed to reduce their intake of simple sugars by 40% (calories were replaced with fats, protein, and complex carbohydrates). Each month, subjects rated the sweet intensity and pleasantness of vanilla puddings and raspberry beverages that varied widely in sucrose concentration. There were no systematic differences in rated sweetness intensity between the groups during the baseline month or the first diet month. However, the low-sugar group rated pudding samples with low levels of sucrose as more intense than did the control group during the second diet month, and rated a broad range of the pudding sugar levels as about 40% more intense during the third diet month. A weaker intensity effect was obtained for the model beverages. Despite the intensity shifts, diet had no measurable effect on pleasantness ratings. Thus, dietary sugar levels may influence sweet taste intensity, though further work is needed to determine the underlying mechanisms and significance for eating behavior. Acknowledgements: PepsiCo funded the study. The views expressed in this research report are those of the authors and do not necessarily reflect the position or policy of PepsiCo, Inc. FCOI Disclosure: LJF and LN are employees of PepsiCo Inc.

#164 **POSTER SESSION IV**

Short-term Exposure to Sucralose, but not Sucrose, Increases Sweet Taste Preference in Adults

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Non-nutritive sweeteners (NNS) provide sweet taste without calories, yet increases in consumption have been linked to weight gain. Several explanations for this association have been proposed. Preliminary data from our lab show a 2-fold decrease in sweet taste sensitivity and 3-fold decrease in umami sensitivity following consumption of a Splenda-sweetened beverage for 10 days. Since effects were observed for sweet and umami, we suggested that the effect is produced by down regulation or desensitization of the T1R3 subunit, common to both taste receptors. Here we tested whether exposure to NNS alters sweet taste preference using the Monell 2-series, forced-choice tracking method. At a pre-test, after a 1 hour fast, 15 healthy weight adults tasted pairs of solutions (10mL each) that differed in sucrose concentration (3%, 6%, 12%, 24%, 36%). Preference was established after participants selected the same concentration of sucrose 2 consecutive times. Participants were then randomized to consume an equisweet beverage (355 ml) containing either sucrose (n = 8, 30.38 g) or sucralose (n = 6, 59.99 mg) on 6 separate days over 2 weeks. Sucrose preference was assessed following the 6 exposure sessions at a post-test. Groups were matched for age, sex, BMI, percent body fat and NNS usage. Scores on the sucrose preference test increased by 57% in the sucralose-exposed group ($r = 0.924$, $p = 0.008$), but not in the sucrose-exposed group ($r = 0.465$, $p = 0.216$). Percent body fat was also correlated with post-exposure sucrose preference scores in the sucralose group only ($r = 0.827$, $p = 0.042$). These preliminary findings suggest that short-term exposure to sucralose, but not sucrose, increases sweet taste preference in adults, particularly in those with greater body fat. Acknowledgements: (Funded by R01DC006706).
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#165 **POSTER SESSION IV**

Cocaine Does not Alter Sweet Taste Sensitivity

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In rodents, saccharin consumption decreases when an animal is taught that this sweet taste will precede a moderate dose of cocaine. This has been interpreted a number of different ways by behavioral scientists through the lens of motivation, reward and large-scale behavioral responses to drugs. We considered an alternative explanation; that cocaine exposure alters the afferent taste response, decreasing the animal's sensitivity to sweet taste. The goal of this study was to apply a model commonly used in taste studies, but rarely used in drug studies, to evaluate whether depressed saccharin consumption after saccharin-cocaine pairing is due to an alteration of sweet taste sensitivity. To do this, a Davis rig lickometer was used to briefly present multiple concentrations of saccharin in a single session to mice. We administered this brief-access test both before and after a standardized saccharin-cocaine pairing (pre-test vs. post-test, respectively). Our results indicate that the detection threshold (~3mM) and EC_{50} (~10-15 mM) for saccharin are unaltered by saccharin-cocaine pairing, suggesting that sensitivity is unaltered by cocaine exposure. In contrast, the avidity of licking saccharin,

an indicator of motivation, was depressed by ~69% for the highest concentration of saccharin and the latency to first-lick, a negative indicator of motivation, increased seven fold. Thus, our findings indicate that saccharin taste sensitivity is unaltered by moderate doses of cocaine and validates the previously held interpretation that a rodent's decreased consumption of a natural reward following cocaine pairing is due to changes in higher-order reward circuitry. Acknowledgements: NIH grant R01 DC006308. FCOI Disclosure: None.

#166 **POSTER SESSION IV**

Children's liking for the taste of nutritive and nonnutritive sweeteners

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In nature, carbohydrates are a source of energy often equated with sweetness, the detection of which is associated with powerful hedonic appeal, especially among children. Intakes of both nutritive sweeteners which contain carbohydrates and energy, and nonnutritive sweeteners (NNS) which provide sweetness with few or no calories, have risen consistently over the past two decades. Despite their prevalence in the food supply, there is a paucity of pediatric research on the behavioral and psychophysical evaluation of these ingredients. To this end, we used a variety of methods (e.g. 3-point face scale, 5-point face scales, and the general labeled magnitude scale (gLMS)) to assess liking of varying concentrations of nutritive (sucrose) and nonnutritive (sucralose, aspartame, acesulfame potassium, and stevia) sweeteners among 6- to 14-year-old children (N=48) and their mothers, all from whom saliva was collected for future genotyping. To allow for age-related comparisons, methods were identical for children and adults. Subjects tasted each solution individually and in randomized order, indicated their hedonic rating, and rinsed their mouth twice with water and waited 1 minute before tasting the next sample. For both 3- and 5-point face scales, subjects selected the face that best represented their liking for the stimulus; for the gLMS, subjects were asked to indicate if the solution had a 'good taste' or a 'bad taste', and to rate the intensity of the good or bad taste on the gLMS. Preliminary analyses revealed that while children tended to prefer higher concentrations of sucrose and stevia, they were similar to adults in their pattern of preferences for the sweeteners. Regardless of psychophysical method used, sweetener type was a better predictor of hedonic rating than was subject age. Acknowledgements: This project was funded by R01DC011287 from the National Institute on Deafness and Other Communication Disorders and NIH postdoctoral training grant T32-DC0014. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIDCD or the National Institutes of Health. FCOI Disclosure: None.

#167 **POSTER SESSION IV**

Laboratory demonstration of volatile-enhanced-sweetness

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Volatile organic compounds can enhance sweetness. However, in over 25 years few such volatiles have been identified. Recently we discovered that volatile-enhanced-sweetness is far more common than previously suspected and makes a substantial contribution to the sweetness of fruit (e.g., fruit sweetness can double with appropriate volatiles). The purpose here was to quantify this effect in the laboratory. Sweet-enhancing volatiles previously identified in tomato and strawberry (Tieman et al, 2012; Bartoshuk and Klee, 2013; Schweiterman et al, 2014) and 2% sucrose were combined to make 4 solutions: sucrose, sucrose with 11 volatiles from tomatoes, sucrose with 24 volatiles from strawberries and sucrose with all volatiles. Subjects used a global intensity scale (variant of the gLMS with intermediate descriptors deleted). Subjects held their noses closed, swished the solution, swallowed, opened their noses and rated the sweetness perceived before and after opening their noses; additionally flavor and quality were rated. This technique was devised to emphasize the taste/flavor distinction (Snyder et al, AChemS, 2014). Repeated measures ANOVA (all 38 subjects) showed a significant before-after effect ($F(1, 108)=11.987$, $p=.001$). T-tests showed no sweet intensification for sucrose alone (control), but significant sweet intensification for all 3 mixtures containing volatiles. The intensification for the mixture with all volatiles was significantly greater than for either tomato or strawberry volatiles alone; volatiles intensified sweet by a factor of 1.7. Not all subjects reported sweetness intensification. For those that did (N=23), the factor was 2.3. Conclusions: Volatiles previously identified as sweet enhancers in fruit enhanced 2% sucrose solution; sweet enhancing volatiles from different fruits added. Acknowledgements: This work was supported by R21 DC013751. FCOI Disclosure: None.

#168 POSTER SESSION IV

Heightened preferences for sweetness in patients with Wolfram Syndrome

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Objective: Wolfram syndrome (WFS) is a rare (1 in 770,000) autosomal recessive genetic disease with clinical signs apparent in early childhood. It is characterized by insulin-dependent diabetes, optic nerve atrophy, vision loss, hearing loss, olfactory loss, and neurodegeneration. The aim of this study was to test the hypothesis that taste function is altered in WFS. Design and Methods: 23 subjects with WFS (16.2 ±1.2 years old, range: 5-28 y) and 40 age equivalent controls (20 healthy controls (HC; 14.7 ±1.2 years old) and 20 individuals with type 1 diabetes (T1DM; 14.1 ±1.2 years old) participated in the study. We assessed: 1) sucrose preferences (Monell forced-choice paired-comparison tracking procedure) and 2) regional and whole mouth taste perception (NIH toolbox Taste Intensity Test) of sucrose (90, 350 and 1050 mM), sodium chloride (100, 320 and 1000 mM) and quinine (1mM), counterbalanced in presentation order. Results: There were no significant differences on sweetness, saltiness and bitterness perception in the regional or whole-mouth taste test among control subjects and subjects with WFS (all P' values >0.10). However subjects with WFS preferred higher sucrose concentrations than age matched controls (WFS=24 ±6 % w/v, HC=17±8 % w/v and T1DM=1717±8 % w/v; P=0.04). Conclusion: Subjects with WFS prefer significantly sweeter solutions than age matched controls, independent of diabetes status. The heightened preference for sweetness in WFS is not due to reduced perception of sweetness intensity. Unlike that observed for the other senses, taste function is remarkably well conserved in the majority of subjects with WFS. Acknowledgements: FUNDING ACKNOWLEDGMENTS This project was supported by the McDonnell Center for Systems Neuroscience (to MYP) and National Institutes of Health (NIH) grants P30 DK05634 (NIH DK 56341) (Nutrition Obesity Research Center) (to S. Klein) (Nutrition and Obesity Research Center) and HD070855 (to TH). FCOI Disclosure: None.

#169 POSTER SESSION IV

Epstein Barr Virus Induced Dysosmia, Hyposmia, Dysgeusia and Hypogeusia in the Absence of an Upper Respiratory Infection

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Objective: To describe a case of Epstein Barr induced chemosensory dysfunction with the absence of upper respiratory symptoms. Method: Case Report: 50 year old male presented 1 year ago with fatigue, paresthesias, myalgias, tinnitus, ear fullness, impaired hearing, headaches, lymphadenopathy, elevated Liver function tests, hyponatremia, with a positive EB viral titer. Coincident were persistent complains of a 70% reduction in smell and taste, dysosmia and dysgeusia. He denied phantosmia and phantogeusia. Unresponsive to steroid nasal sprays and Betahistine. Results: Paranasal Sinus CT, MRI of Head, and Fiberoptic Endoscopy –normal; Audiogram – abnormal bilaterally. Exam: bilateral palmar erythema, bilateral Hoffman reflexes. Olfactory Test: Anosmia: ETOH Sniff - 7 cm; Sniffin Sticks (SST) Threshold – Left (L) < 1 Right (R) < 1 Dirhinis < 1; SST Discrimination Dirhinis – 6; UPSIT - L 13 R 15; Olfactometer Threshold - L 4. Hyposmia: Quick Smell Identification Test - 2/3; Odor Memory - 2 at 10, 4 at 30, 2 at 60 sec total 8/12; SST Identification Dirhinis – 9; Sniff Magnitude: 0.76; Olfactometer Identification - L 12 R 15. Normosmia: Pocket Smell Identification Test- 3/3; Suprathreshold Amyl Acetate Odor Intensity: normal pattern; Olfactometer threshold - R 8. Jelly bean Retronasal Smell Test– 5. Gustatory: Hypogeusia to HCl, Normogeusia to NaCl, sucrose, urea, PTC and PROP disc; Quadrant Testing: Decreased posteriorly. Other: Beck Depression Inventory II – 38 (depression); Zung Anxiety Scale – 39 (anxiety). Saxon test – normal, Electrogustometry Tongue: Posterior - L 14 R 8, Anterior - L 6 R 6, Palate - L & R > 34. Discussion: The lack of upper respiratory symptoms suggests a hematogenous or central dissemination possibly through the olfactory epithelial pathway (perineural) or paracellular transport. Acknowledgements: Smell & Taste Treatment and Research Foundation. FCOI Disclosure: ARH: has ownership in the Smell & Taste Treatment and Research Foundation and consults with multiple companies and health care facilities.

#170 POSTER SESSION IV

Smell and Taste Disorders – Current Care and Treatment in the Dutch Health Care System

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Smell and taste disorders affect 5-20% of the general population and can have profound effects on the quality of life. However, at present, not much attention is given to these patients in the regular health care system, and diagnosis and treatment are limited. In order to improve this, we decided to set up a Smell and Taste clinic in the Netherlands, as collaboration between ENT physicians and sensory scientists. We designed a questionnaire to explore the current care and treatment that patients receive in the Dutch health care system, and how they perceive this, to investigate what can be improved. The questionnaire was sent out to members of the Dutch anosmia patient organization. Data of 83 respondents so far (26 male) show that, while 36% report an olfactory disorder and 65% a combined smell- and taste disorder, 25% of these patients have not been formally diagnosed, even though 90% of them have visited one or more physicians (general practitioners,

ENT specialists or other), with over 10% having visited 4 or more physicians. More than half (56%) of the respondents have not had any smell or taste tests to objectify their complaints, and only 26% has received medication as possible treatment. Perhaps surprisingly, 43% of respondents report to be (very) satisfied with the received care and treatment so far by the physicians, while 17% feel (very) unsatisfied. More data will be collected and analyzed. These data give insight into the current care and treatment of smell and taste disorders in the Dutch health care system, and show that this can be clearly improved, especially given the impact these disorders can have on daily life. Opening a Smell and Taste clinic, as a one-stop-shop for diagnosis, treatment and information, will prove to be valuable for these patients. Acknowledgements: Supported by University Funds, as well as by the Nutrional Alliance Gelderse Vallei. FCOI Disclosure: None.

#171 **POSTER SESSION IV**

Pyrethroid Insecticide Induced Anosmia and Hypogeusia

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Objective: To highlight effects of Pyrethroid Insecticide on objectively measured chemosensation. Case study: A 55 year old female was exposed to Lambda-Cyhalothrin, with gradual loss of smell and taste with partial temporary response to steroids. Initially dirty-musty phantosmia were present, which resolved within a year. Results: Neurological examination: Facilitatory paratonia, Bilateral finger to nose dysmetria. Low amplitude, high frequency tremor of both upper extremities on extension; diffuse hyperreflexia, positive jaw jerk. Bilateral Hoffman reflexes. Tests : Cognitive: Normal. Psychological: Normal. Chemosensory: Olfactory: Anosmia on: Pocket Smell Identification: 0/3, Quick Smell Identification: 0/3, Odor Memory: 0/4 at 10 sec, 1/4 at 30 and 1/4 at 30 sec, Total 2/12, Alcohol Sniff: 0 cm, Suprathreshold Amyl Acetate Odor Hedonics and Intensity: Straight Line, Sniff Magnitude: 1.11, Olfactometer Butanol Threshold: L-1.0, R-1.0, Olfactometer Identification: L-5, R-6, Sniff and Sticks: Threshold-L < 1, R < 1, Dirhinous < 1; Discrimination Dirhinous 6, Identification Dirhinous 4. Retronasal smell: Jelly Bean Difference 0/10 (Absent). Gustatory: Normogeusia to HCl, PTC, mild hypogeusia to NaCl, moderate to severe hypogeusia to sucrose and urea, Taste Quadrant: Normal except decreased salt in palate, Propylthiouracil Disc: 8/10 (Normal), Electrogustometry-Palate: L-34, R-34; Anterior Tongue Lt-14, Rt-26; Posterior Tongue L-12, R-28. Other: Fungiform Papillae: L-32, R-20, Saxon: 2 gm, Piesesthesiometry: Normal, Culture: Positive for C. Albicans, MRI without contrast: Mucosal thickening with fluid filling bilateral frontal sinuses, L maxillary sinuses and anterior ethmoidal air cells. Conclusion: Partial response to steroids suggests this treatment for pyrethroid induced chemosensory dysfunction. Acknowledgements: Perraju Dinavahi: Smell and Taste Treatment and Research Foundation. FCOI Disclosure: Perraju Dinavahi is the student of Dr. Alan R Hirsch. Dr. Hirsch is the owner of Smell and Taste Treatment and Research Foundation and consultant for multiple companies and healthcare facilities. Student gets financial support from Smell and Taste Treatment and Research Foundation.

#172 **POSTER SESSION IV**

Chronic Cigarette Exposure Associates with Self-reported Smell Alterations: Findings from the U.S. National Health and Nutrition Examination Survey (NHANES) 2011-2012

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Background: Research on the effects of cigarette smoking on smell function is inconclusive. Some studies suggest smoking decreases function due to neurotoxic effects on olfactory receptors. Other studies report no effects or protective effects. Here, we examined associations between smoking exposure and self-reported function in a nationally-representative sample of adults. Methods: 3603 adults ages ≥40 years (mean=60±12) answered questions on smell-related problems and related health or demographic risks. Three questions defined self-rated alterations: smell problems in the past year, worse ability since age 25, and phantom odor sensations. Smoking exposure was described (non, former, current) and quantified in pack years (PY, packs of cigarettes smoked/day times years smoked). Results: Prevalence estimates of smell alterations were 23%, showing age-related increases but no sex differences. Of the sample, 53% were non-smokers, 29% former smokers, and 18% current smokers. Non-smokers were least likely to report smell declines with age; former smokers were most likely to report improvements. For quantity smoked, 26% of the sample smoked ≥10 PY in their life—they were more likely than non-smokers to report smell alterations, including phantom sensations, and risks of olfactory dysfunction (frequent nasal congestion in past year, persistent cold or flu in past year, history of head/face injury, low income-to-poverty ratio, IPR). In models controlling for demographic (age, sex, ethnicity and IPR) and risks of olfactory dysfunction, ≥10 PY smokers (vs. non-smokers) were 50% more likely to perceive smell alterations (OR=1.50; 95% CI: 1.04, 2.16). Conclusion: These data indicate that chronic tobacco exposure increases risk of smell alterations, either directly or via risk factors for smell alterations. Acknowledgements: NIDCD/NIH. FCOI Disclosure: None.

#173 POSTER SESSION IV

Cannabis Responsive Chemosensory Dysfunction

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Objective: Chemosensory dysfunction responsive to cannabis has not heretofore been described. Such a case is presented. Methods: Case Study: 52 year old female with past history of hypothyroidism, and treated stage III breast carcinoma in remission. Two years prior to presentation developed a severe URI which left her with unrelenting chemosensory complaints, including anosmia. Evaluation at that time included an UPSIT of 20/40 (severe microsmia), a negative MRI, sinus CT, and fiberoptic endoscopy. She was unresponsive to 2 courses of prednisone, and 3 courses of triamcinolone. Two weeks prior to presentation she smoked marijuana and consumed a marijuana cupcake followed by reduction of her symptoms within a day, most returning over 6 weeks (see chart below). Results: 2 wks after cannabis: Quick Smell Identification Test (QSIT) 3/3; Pocket Smell Test (PST): 2/3; Olfactometer Butanol Threshold (OBT): left 1 right 0.0; Brief Smell Identification Test (BSIT): 9/12. Retronasal Smell Jelly Bean Difference (JBD): -3. 6 wks after cannabis: QSIT 2/3; PSIT 1/3; JBD -1. ETOH Sniff: 2. UPSIT 24; BSIT: 8/12; OBT: left 1.0 right 0.0. Discussion: Possible mechanisms for cannabis' effect include activation of cannabis CB1 receptors, impact of endocannabinoids on olfactory neurons, or through central (hippocampal) discharge. Expectation effect and drug seeking are also considerations. Acknowledgements: Smell & Taste Treatment and Research Foundation. FCOI Disclosure: ARH: has ownership in the Smell & Taste Treatment and Research Foundation and consults with multiple companies and health care facilities.

	Subjective Smell	Olfactory windows per day	Foul rotten Dysosmia	Subjective Taste	Sweet Dysgeusia	Sweet Phantogeusia
Prior to Cannabis	0 %	0	10-20 times per day	40%	3-4 days per week	All day 3-4 days/ week Intensity 7/10
1 day after Cannabis	80%	20-40	Resolved	80%	Resolved	Resolved
2 weeks after Cannabis	40 %	3-5	Resolved	85%	Resolved	Resolved
6 weeks after Cannabis	20%	0	10-20 times per day	50%	3-4 days per week	Resolved

#174 POSTER SESSION IV

Do Sjogren's Syndrome affect odor identification abilities?

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Sjogren's syndrome (SS) is an autoimmune disease affecting multiple organ systems [1-3]. Previous studies have shown change in odor identification ability and smell threshold in SS patients. [2, 4]. This change is thought to relate to changes within the nose [5-6]. In contrast, burning mouth syndrome (BMS) is an idiopathic pain syndrome with normal findings and smell loss is not a typical complaint in these patients. [7] Therefore, BMS patients were chosen as a control group. Using Sniffin' Sticks, SS patients and BMS patients were tested. A total of 47 female patients were recruited. The SS group consisted of 15 SS patients between the age of 31 and 75. The BMS group consisted of 32 diagnosed BMS patients between the ages of 37 and 90. Patients identified odors by selecting one odor name from a list of four descriptors each. Scores were compared to normative values. There were no significant differences between the number of correctly identified odors (SS = 10.7± 3.5, BMS = 11.8 ± 2.6, p=0.21) between the two groups. In general, no SS patient scored perfect on the odor identification test and only 2 BMS patients had perfect scores. Our BMS and SS subjects who scored below normal presented with proportion similar to those reported for SS patients by Waiffenbach, which may indicate that some smell identification impairment also exist in BMS patients as well as in SS patients. In summary, SS patients had lower odor scores than BMS patients; however, the difference was not significant. Although high proportion of SS patients scored lower than normative values [9], there was no significant difference between BMS and SS patients, suggesting that BMS patient may have reduction in their odor identification ability and are different than normal age matched controls. Acknowledgements: Self-funded. FCOI Disclosure: None.

#175 POSTER SESSION IV

Hyposmia alters olfactory memory performance

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In order to memorize a stimulus of any kind, conscious perception of the same stimulus to a previous time point is mandatory. Recently, Arshamian et al. (2011) have shown that olfactory awareness significantly influences the performance in olfactory memory tests. In this study we aimed to investigate odor memory abilities in subjects with a reduced sense of smell. Sixteen hyposmic subjects (8f/8m, mean age 52.68, age range 25-67, TDI mean 24.12, TDI range 17.25 – 30.5) participated in the study. Besides olfactory testing using the Sniffin' Sticks test battery a sociodemographic questionnaire was assessed and subjects were asked to rate their ability to smell on a 9-point Likert-scale. Further testing included two olfactory memory tests (OM1; Arshamian et al. 2011, OM2; Codhury et al 2003) and a visual memory test (Amthauer et al 2000), including a verbal and a figural task. A significant correlation between TDI and both olfactory memory tests was obtained (OM1: r=.501, p=.048; OM2: r=.556, p=.025), whereas no significant correlation was obtained for the subjective ratings. The results from OM2 were also found to be significantly correlated to verbal memory abilities (r=.513, p=.042). However, both olfactory memory tests were not related to educational background of the subjects. Due to the results of this study we can suggest that the conscious

perception of odorants triggers the ability to memorize odors. Furthermore, we were able to show that olfactory memory performance may be confounded by verbal memory abilities. Thus, the selection of the olfactory memory test is of great importance. Acknowledgements: Austrian Science Fund (P23205-B09). FCOI Disclosure: None.

#176 **POSTER SESSION IV**

Projected Olfactory Reference Syndrome: A Case Report

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Introduction: A filial variant of olfactory reference syndrome with perceived odor originating from a child and spreading to the mother has never been described. Case Description: A 35 year old divorced woman, meeting criteria for depression and generalized anxiety disorder, reports that for one year her 13 year old daughter constantly smelled putrid, despite increasingly intense cleaning. One month after onset, the mother noted the same unremitting malodor on her own skin. The daughter and others deny any malodor, but the patient remains persuaded nonverbal signals from others indicate revulsion. Invasive, preoccupying thoughts are accompanied by compulsive washing. Symptoms are exacerbated by heat, stress and consumption of select substances including coffee and cheese. Intensity of perception is not related to menses. Nor did it markedly diminish with changes in soap or perfume, or after deliberately initiating smoking to camouflage the odor. Patient has also initiated courses of select pharmaceuticals without symptom relief. She endorses persistent low mood in association to preoccupation with smell along with intermittent tearfulness and feelings of unreality. No odor was detected by multiple examiners before and after one cup of coffee. Smell testing confirmed intact olfaction, and physical exam was unremarkable. Discussion: Extension of the malodor from the daughter to the mother indicates a maternal delusional disorder. Psychodynamically, merging of mother and daughter causes the perception that the malodor emanates from both. Acknowledgements: Smell and Taste Research and Treatment Foundation. FCOI Disclosure: FCOI: Dr. Alan Hirsch has ownership in Smell and Taste Research Foundation and consults with multiple companies and healthcare facilties. Dr Alfred Goldyne has no FCOI to declare, Ayham Alagha has no FCOI to declare.

#177 **POSTER SESSION IV**

Comparative analysis of morphological changes in human nasal biopsies across ages and pre-existing medical conditions

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Human olfactory epithelium (OE) undergoes constant regeneration throughout the life time of an individual. Unlike the uniform fetal OE, the adult OE may be patchy and its morphology and function may be affected by various factors such as toxin and chemical exposure, head injuries and age. Substantive insights into the underlying cellular and molecular mechanisms are limited. This study aims to investigate the changes in OE morphology with respect to stem cells, in human biopsy specimens across ages and medical/treatment history. The surgical biopsies were harvested from the lateronasal wall of the middle turbinate from younger patients undergoing nasal septoplasty and older patients undergoing dacryocystorhinostomy, with or without pre-existing medical conditions such as radioactive iodide (RAI) treatment for thyroid cancers or hyperthyroidism and chemotherapy. A total of 20 biopsies were analyzed for this study, four of which from patients with a history of RAI treatment. None of these 4 patients had a distinct OE-like structure based on immunohistochemical analysis indicating a possible change in OE morphology with this treatment. Interestingly, one of the biopsies from a chemotherapy patient showed very few neurosphere cultures indicating a possible loss of OE. Also, patients who were < 30 years old showed a more intact OE with uniform basal keratin 5-expressing cells than patients who were >75 years. Additionally, patients >75 years showed mostly respiratory epithelium (RE) in immunohistochemical analysis as well as in *in vitro* neurosphere cultures. Taken together, these preliminary results indicate that age and medical/treatment history may affect the overall structure and possibly the function of the OE. Acknowledgements: Monell Chemical Senses:Anosmia Research Fund. FCOI Disclosure: None.

#178 **POSTER SESSION IV**

Solitary Chemosensory Cells trigger avoidance behavior to inhaled irritants

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The nasal epithelium houses a population of solitary chemosensory cells (SCCs) that express T2R (bitter) taste receptors and the transduction channel TrpM5. These cells are innervated by trigeminal polymodal nociceptive nerve fibers. Nasal SCCs respond to bitter compounds including bacterially-produced molecules, to evoke protective respiratory reflexes and neurogenic inflammation (Tizzano 2010, Saunders 2014). Here we test whether SCCs trigger avoidance to their specific inhaled irritants. We developed an apparatus that consists of two identical circular chambers connected by a short tunnel. Each chamber is filled with a mist of either water or an irritant in water. When denatonium benzoate (2-5-10mM) is nebulized in one of the two chambers, wildtype mice avoid the irritant mist chamber. To test the involvement of SCCs in the avoidance behavior we used TrpM5-KO (bitter signaling cascade disrupted) and Skn-1a deficient mice (taste receptor cells and SCCs missing). These mice show no aversion for the irritant mist and prefer denatonium at higher concentrations. To show that mice use SCCs but not the taste system to avoid the irritant chamber,

we used the P2X2/P2X3 double knockout mouse lacking ATP receptors required for taste transmission. Like wild type mice, P2X double KO mice avoid denatonium, reinforcing the finding that SCCs are triggering the avoidance behavior. Our results demonstrate that activation of the SCCs can lead to rapid avoidance responses. This avoidance behavior and the inflammatory responses triggered by trigeminally innervated SCCs in response to an inhaled irritant, represent an important defense mechanism against respiratory epithelial assault by noxious chemicals. Acknowledgements: Supported by NIDCD R03 DC012413 (M.T.), R01 DC009820 (to T.E. Finger), and P30 DC04657 (to D. Restrepo). FCOI Disclosure: None.

#179 **POSTER SESSION IV**

TRPM5-expressing Microvillous Cells are Involved in Modulation of Olfactory Function after Irritant Exposure

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The main olfactory epithelium (MOE) in the nasal cavity detects inhaled odorants with high sensitivity. During inhalation, harmful microorganisms, pollutants, and irritants may also be introduced into the MOE. Mechanisms for detecting these substances in the MOE and regulating olfactory activity are poorly understood. We previously demonstrated that a set of microvillous cells (MCs) in the MOE expressing the transient receptor potential channel M5 (TRPM5) (Lin et al 2008) are responsive to various harmful substances. Furthermore, these cells are capable of releasing acetylcholine (ACh), which alters intracellular calcium levels in neighboring supporting cells and olfactory sensory neurons (Ogura et al 2011). We hypothesize that cholinergic TRPM5-MCs play a role in the detection of foreign substances in the MOE, and subsequently modulate olfactory activity. To investigate this, we utilized Skn-1a knockout mice (Skn-1a$^{-/-}$), which lack TRPM5-MCs in the MOE (Yamaguchi et al 2014) and assessed olfactory function using behavioral assays. Under normal housing conditions, both Skn-1a$^{-/-}$ and wild type mice perform similarly in finding buried food. After two-week continuous exposure to odorous irritants, irritant-exposed Skn-1a$^{-/-}$ mice take significantly longer to locate buried food than vehicle-exposed Skn-1a$^{-/-}$ mice, whereas the performance of wild type mice is unaltered by the irritant exposure. Moreover, olfactory preference tests indicate that wild type mice avoid high concentration odorants to a greater extent after irritant exposure, while the tendency to avoid these stimuli is unchanged by irritant exposure in Skn-1a$^{-/-}$ mice. These behavioral alterations suggest that TRPM5-MCs contribute to the modulation of olfactory function in response to inhaled irritants. Acknowledgements: This work was supported by research grants NIH/NIDCD 012831 to W. Lin and the UMBC Meyerhoff Graduate Fellowship and LSAMP Bridge to Doctorate Fellowship to K.Lemons. FCOI Disclosure: None.

#180 **POSTER SESSION IV**

Treatment of neural anosmia by topical application of bFGF-gelatin hydrogel in the nasal cavity: an experimental study in mice

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The aim of this study was to investigate the effects of bFGF-gelatin hydrogel on recovery of neural anosmia that was induced in mice by intraperitoneal injection of 3-methylindole (3-MI, 200 mg/kg). One week later, the animals were subjected to one of the following three procedures bilaterally: 1) group A: single-shot intranasal drip infusion of PBS, 2) group B: single-shot intranasal drip infusion of bFGF, and 3) group C: placement of bFGF-hydrogel in the nasal cavity. The olfactory function of the animal was evaluated by the odor-detection test (ODT) 2 and 4 weeks later. Following the testing, the animal was killed, the thickness of the olfactory epithelium was measured, and the number of olfactory marker protein (OMP)-positive cells was counted. The ODT proved that neural anosmia recovered in group C but not in groups A and B. Histologically, olfactory epithelium became thicker and the number of OMP-positive cells increased in group C, while such functional and histological recovery was poor in groups A and B. These findings suggested that placement of bFGF-hydrogel in the nasal cavity was an efficient way to facilitate recovery of neural anosmia. As a gelatin hydrogel degrades slowly in the body, bFGF is gradually released around the site of the lesion; thus, it constantly exerts its effects on neural regeneration. Acknowledgements: Supported by Ehime University Funds. FCOI Disclosure: None.

#181 **POSTER SESSION IV**

Numbers Matter: a Mouse Model with Reduced Mitral Cells and Olfactory System Dysfunction

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The olfactory system constitutes a sensory system of major importance for mammals and a valuable tool for studying neuronal connections in the central nervous system. Much knowledge has been obtained in recent years on the organization of the odorant receptor map, while little is known about the corresponding mitral cell map, located in the mitral cell layer, consisting of the main projection neurons of the olfactory bulb (OB). Our work aims to shed light on the organization and function of mitral cells. Our experimental approaches include

immunohistochemistry and in situ hybridization, transplants, neuronal activation experiments and behavioral analysis. We show that the absence of the cell adhesion molecule Transient Axonal Glycoprotein-1/Contactin 2 (TAG-1/CNTN2), results in a significant and specific defect in the number of mitral cells inside the main OB in mice. This defect occurs as a consequence of impaired migration of a subpopulation of neurons born at embryonic day 11.5. We report on the developmental series of events that occur before the final positioning of projection neurons into the mature mitral cell layer. We show that TAG-1/CNTN2 is expressed developmentally in the olfactory epithelium, while its absence doesn't affect its gross organization. Moreover, the organization of the accessory olfactory bulb, as well as other neuronal populations in the main OB are unaffected. Our study reveals that the detected alterations in the number of mitral cells are reflected in an aberrant neuronal activation profile as well as disturbed olfactory behavior, with significant impairment in odor discrimination. Our results propose that TAG-1/CNTN2 function is crucial for the organization of projection neurons in the main OB, a prerequisite for its proper function and circuitry formation. Acknowledgements: FP7-funded project 'TransPOT', Translational Research Potential in Human Diseases, IMBB intramural grants, Manasaki Fellowship and Medical School Fellowship of the University of Crete, FP7- funded project "ARISTEIA". FCOI Disclosure: None.

#182 **POSTER SESSION IV**

Resilient Properties of Olfactory Ensheathing Cells after Neuronal Loss

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Olfactory ensheathing cells (OECs) are specialized glial cells found exclusively in the olfactory system. The remarkable ability of the sensory neurons (OSN) to continually regenerate makes this tissue an excellent model for studying neurogenesis and neuron-glial interactions. In our study, we focused on the interaction between OSNs and the OECs that ensheath the OSN axon bundles projecting from the OE to the bulb. Due to their close association, we hypothesized that OECs play a major role in OSN regeneration. The response of OECs to OSN loss and regeneration *in vivo* remains largely unexplored. Therefore, the main objective of this study was to elucidate the molecular and cellular characteristics of OECs in adult mice following acute OSN degeneration and during regeneration. We examined OECs in their normal state and ascertained their morphology, proliferative state and changes in gene expression in normal epithelium and at several points during sensory neuron regeneration and axon extension. RNA-seq was performed on nasal OECs isolated directly from mice at various time points during methyl bromide-induced OSN regeneration. Our RNA-seq results showed that surprisingly few genes expressed in OECs were significantly up-/down-regulated during the lesion time-course. We also sparsely-labeled OECs using cre-mediated recombination and confocal imaging at various time points. Similarly, our morphological analysis of OECs indicated a remarkable stability in several parameters of OEC morphology. Finally, MeBr lesions did not induce significant turnover of OECs. Overall, our results indicate that OECs remain largely unaffected by the loss and regeneration of OSN. We conclude that the role for OECs in adult mice is to maintain an overall stable environment for continuous regeneration of olfactory neurons. Acknowledgements: This work was supported by Ruth L. Kirschstein National Research Service Award (F31DC012477) by NIDCD to T.S. FCOI Disclosure: None.

#183 **POSTER SESSION IV**

Comparison olfactory event-related potentials (OERP) with fMRI in patients with olfactory dysfunction

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Olfactory disorders after head injury account for about 15% of the olfactory disorder and mainly caused by mechanisms such as olfactory bulb contusion or frontal lobe damage. In some cases olfactory dysfunction after head injury are naturally recovered But permanent disability can occur. Currently, a psychophysical test such as CCSIT are used for assessing the smell function. But there is lacks of tools for objectifying the patient's symptoms. Therefore, olfactory event-related potentials and a fMRI were performed at the same time to compare each other for complementing the objectivity.15 patients have olfactory dysfunction after head trauma which take place from October 2013 to August 2014 and visit outpatient department of ORL were chosen to CCSIT test and OERP, fMRI. A BOLD signal was detected by calculating the difference of signal recorded between the olfactory stimulation given to fMRI. We get evoked potentials of the patients by giving repetitive olfactory stimulation. And use them to extract the figure of latency and amplitude of the signal from OERP. Among 15 patients 12 were men and 3 were women. The average age of them was 45 years (range 27-63). Psychophysical test showed all of 15 people lost sense of smell. Four patients were observed the positive results in both fMRI and OERP, but only 3 patients were not observed any positive finding in both fMRI and OERP. 6 patients were observed in fMRI not OERP, otherwise 2 patients were observed in OERP not fMRI. There was no significant correlation with one another. Patients who complain olfactory dysfunction after head trauma, even if they showed the olfactory loss in psycophysical test, had various results in OERP and fRMI. Therefore, a need for the objective test of olfactory disorders is emphasized as well as psycophysical test Acknowledgements: by Kunkuk university fund. FCOI Disclosure: None.

#184 POSTER SESSION IV

Experience-dependent Axon Targeting and Guidance Molecule Expression in the Mouse Olfactory System

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Olfactory sensory neurons (OSNs) that express the same olfactory receptors (ORs) are scattered in the olfactory epithelium, yet their axons coalesce into just a few bundles of axons, or glomeruli, on each olfactory bulb, a process that is still not fully understood. Previous work indicates that both activity-dependent and –independent mechanisms are involved in OSN axon fasciculation and targeting, but whether olfactory experience plays a role is unclear. This study aimed to determine the effect of early postnatal odor experience on expression of axon guidance molecules and subsequent OSN axon targeting, focusing on two related ORs, M71 and M72, that normally converge into nearby yet separate glomeruli on the olfactory bulb. First, mice were exposed to acetophenone, a cognate ligand for both receptors, for 16-hours per day from the day of birth until the mice were 21 days old. Strikingly, stimulation affected M71- and M72- OSNs so that the axons of these OSNs coalesced onto the same glomerulus. Double-label fluorescent in situ hybridization was then used to compare the expression levels of known axon guidance molecules, including Kirrel2, Kirrel3, EphA5, ephrinA5, and BIG-2, in OSNs. The data suggest postnatal stimulation with acetophenone influences expression of certain axon guidance genes involved in OSN targeting: in mice stimulated with acetophenone, M71-OSNs exhibited significant downregulation of Kirrel3 expression, while in M72-OSNs, Kirrel2, ephrinA5, and BIG-2 expression were significantly downregulated. Together, our study reveals an experience-dependent component involved in OSN targeting in the olfactory bulb. Acknowledgements: NIH R01 DC012095. FCOI Disclosure: None.

#185 POSTER SESSION IV

Olfactory sensory deprivation as a model for homeostatic plasticity

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Sensory deprivation is a model for driving neural plasticity and induces dramatic changes in olfactory bulb (OB) circuitry. These changes appear to be compensatory and are therefore not driven by Hebbian, but instead are driven by homeostatic plasticity. The behavioral consequences specific to homeostatic plasticity in the adult OB are unclear because the methods that induce the plasticity either kill the sensory neurons or block airflow in the nasal cavity inducing a slew of compensatory mechanisms. To avoid these issues we present an initial study into the consequences of homeostatic plasticity in the adult OB following chronic sensory deprivation induced with nasal irrigation of a dilute detergent (0.1% triton; 6 µL; 3x, every other day for 5 days). Gross olfactory ability was then tested with an olfactory habituation/dishabituation protocol (i.e. on day 6). Habituation was not altered by treatment as most animals (15/16) habituated to the 'blank' odor (mineral oil). Detergent-treated mice failed to dishabituate the 'test' odor (acetophenone; p=0.3, N=6), in contrast to sham (p=0.018, N=6) and untreated mice (p=0.04, N=4), indicating that the detergent-treated mice were unable to detect the new odor. We next measured OB dopamine levels in detergent- and sham-treated mice as chronic sensory deprivation is known to halt dopamine production. Detergent-treated mice had 20% less dopamine compared to sham mice (p=0.029, N=7). These effects, both behavioral and biochemical, are likely due to a chronic loss of olfactory cilia and not sensory neurons because all mice were treated with detergent irrigation on day one. Detergent-induced chronic cilia destruction offers a new method for studying the post-synaptic changes during and following homeostatic plasticity in awake, behaving mice. Acknowledgements: Eastern Michigan University: start-up funds (TGM) and an undergraduate research fellowship (JR). FCOI Disclosure: None.

#186 POSTER SESSION IV

Wide-spread disruption of brain networks by chronic peripheral sensory loss

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Neuroplasticity effects have already been shown for sensory loss, e.g. for blindness. At the cerebral level the sense of smell stands out of all other senses, as peripheral input can be processed directly, without thalamic projections. Further, the olfactory sense projects ipsilaterally into the brain in contrast to all other senses, and the spatial organization of olfactory processing areas is much more dispersed compared to other sensory systems. In addition, the olfactory system holds the unique ability to be activated by the sensorimotor act of sniffing, without the presentation of an odor. Thus, the main aim of the study was to investigate neuroplasticity effects in patients with chronic smell loss in a sniffing paradigm. In a functional MRI (fMRI) study eleven anosmic patients (8f, 3m) and 14 healthy controls (7f, 7m) underwent the same sniffing paradigm. Functional imaging data was analyzed by two different methods: the data-driven independent component analysis (ICA) and the hypothesis-

driven functional connectivity analysis (FCA). The results of the ICA revealed an olfactory network that did not differ in anosmic patients and healthy controls. In the subsequently performed FCA significant differences between the subject groups were obtained. In healthy controls a range of olfactory-related brain areas show an increased number of functional connections during the sniffing paradigm. These findings indicate that absent peripheral input may cause alterations of functional networks. Although no differences were detected in the spatial distribution of neural activation, a decrease in functional connectivity in olfactory-related networks was determined. Acknowledgements: Austrian Science Fund (P23205-B09). FCOI Disclosure: None.

#187 **POSTER SESSION IV**

WITHDRAWN

#188 **POSTER SESSION IV**

The effect of intensive training on olfactory performance

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Olfactory specialists such as perfumers and sommeliers undergo smell training for years, which may explain their superior olfactory abilities. In addition, several studies suggest that even a short smell training can improve olfactory performance, but most of these studies have been carried out in patients with olfactory dysfunction. The underlying physiological mechanisms are not yet clear. In this pilot study we aimed to develop an intensive olfactory training paradigm which could be used for future studies. A total of 16 individuals participated. The training group consisted of 8 participants, whereas the remainder served as controls. For six consecutive weeks, the training group returned to the lab on each weekday to perform a 20 minutes intensive olfactory training paradigm. It consisted of an odor intensity classification task, a binary odor mixture sorting task, and a target odor detection task. For the weekends we gave them odorant containing bottles to be smelled at home. The control group did not participate in any training. Both, the training and the control group participated in three evaluation sessions: before (SESSION I), directly after (session 2) and two weeks after (session 3) the six weeks training period. Here we evaluated odor thresholds (for phenyl ethanol and for butanol) and assessed their odor identification abilities by means of the UPSIT. While we did not notice any changes for the UPSIT scores from SESSION I to 3, we observed significantly increased sensitivity in the detection scores from SESSION I to 2, which then remained stable. This effect was somewhat stronger for the training group, although the differences between groups were not significant.
An intensive olfactory training paradigm as the one we used may therefore be used for future studies. Acknowledgements: Startup grant, Sacre-Coeur Hospital Montreal, Startup grant, UQTR. FCOI Disclosure: None.

#189 **POSTER SESSION IV**

Long Term Recovery of Olfactory Function Following Bulbectomy and Olfactory Nerve Transection in Adult Mice

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Previous studies of olfactory nerve injury have shown that following complete transection, olfactory nerves have the capacity to regenerate and restore functional connections with the olfactory bulb. However, complete removal of the olfactory bulb (bulbectomy) has been thought to induce permanent anosmia in adult mice. In this study, we examined whether olfactory function improves after an extended (>1 year) recovery period following injury. Transgenic mice (P2-IRES-tau-LacZ) underwent a bulbectomy or olfactory nerve transection (NTX) procedure, and olfactory function was assessed via a behavioral assay at 2 week and 1 year recovery periods. Whole mount staining of the P2 axons was used to examine axonal organization. All bulbectomized mice (N=8) were found to be anosmic following the 2 week recovery period, however 1 mouse showed recovery of olfactory function following the 1 year recovery period. All NTX mice (N=3) showed behavioral recovery after 2 weeks, with no significant improvement in performance following the 1 year recovery period. However, morphological analysis showed some pruning in axonal connections to the olfactory bulb following the extended recovery period. These results suggest that in adult mice, functional recovery is rare following bulbectomy and requires an extended recovery period, while functional recovery from nerve transection occurs within 2 weeks. Acknowledgements: Supported in part by a grant from the MEDARVA foundation. FCOI Disclosure: None.

#190 **POSTER SESSION IV**

The Pig Olfactory System

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Pigs (sus scrofa) and people have lived together for about 15,000 years. While both species are omnivores, have evolved complex social interactions, and are very intelligent, pigs have much more sophisticated olfactory abilities. Both wild and domestic animals use odors for the recognition of ingroup/outgroup differences, status, sexual receptivity and to keep roving bands together. Pigs have evolved 9 separate glands for the production of odors (digital, preputial, vulvar, anal, mental, salivary, buccal, preorbital and Harderian glands), and they have one of the largest olfactory receptors repertoires with 1,113 functional OR genes and 188 pseudogenes (Nguyen et al., BMC Genomics 2012). While a few studies have examined the pig vomeronasal system, not much is known about the main olfactory pathways. A species with such varied and differentiated

olfactory capacities deserves rigorous study. The present work is an examination of the pig brain with Nissl, Golgi, and fluorescence-immunostained sections. Adult brains are large, measuring almost 10 cm from rostral tip to the back of the cerebellum, and weigh about 90 gm. The olfactory bulb, over a centimeter long itself, encircles a large olfactory ventricle. The rhinal fissure extends for over 5 cm, delineating the extensive olfactory cortex. Golgi stains allow the visualization of most major cell classes in the bulb, peduncle and anterior piriform cortex. The neurons are much larger than those in lab rodents. For example, pyramidal cells in pars principalis of the anterior olfactory nucleus have 50% more dendritic branches and twice the total dendritic length as those in the rat or mouse. The work provides a detailed examination of a member of the Suidae family and therefore is a step towards an understanding of the phylogeny of olfaction. Acknowledgements: Supported by Grant DC000338 from NIH (NIDCD). FCOI Disclosure: None.

#191 **POSTER SESSION IV**

The Diffuse Chemosensory System in Lampreys

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In addition to the olfactory and gustatory systems, vertebrates possess another chemosensory system, the diffuse chemosensory system. Receptors in this system are specialized epithelial cells associated with a nerve fiber called "solitary chemosensory cells" (SCCs). SCCs have been reported in all vertebrate lineages examined so far. However, their localization varies widely among species. For instance, in fish, SCCs are present in the oropharynx, gills and skin, whereas they are restricted to the airways in mammals. Physiological studies pointed out that their function differs as well. In fish, SSCs serve as food or predator detectors, whereas in mammals SCCs appear to act as toxin detectors. This study deals with the diffuse chemosensory system in lampreys, a basal vertebrate lineage, in the hope of shedding light on the function of this system. Putative SCCs have been reported on the body surface and on cutaneous finger-like extensions named "papillae" of brook lampreys (Whitear and Lane, 1983, J Zool Lond). Our investigation, in the sea lamprey, also revealed the presence of papillae. Examination of their epidermal surface under SEM revealed the presence of microvillar-like protrusions. Histochemistry experiments confirmed that these protrusions are microvilli. Immunofluorescence experiments showed the presence of nerve fibers in the central portion of the papillae. Extracellular recordings from papillae showed multiunitary action potentials in response to chemical stimulation, confirming their chemosensory role. Anatomical and physiological experiments allowed us to identify the nerves supplying innervation to these papillae as well as their central targets in the brainstem. Our study constitutes the first step in the characterization of the diffuse chemosensory system in lampreys. Acknowledgements: GLFC 8400272, CIHR 15129, NSERC 217435, FRSQ 5249. FCOI Disclosure: None.

#192 **POSTER SESSION IV**

Solitary Chemosensory Cells During the Sea Lamprey Life Cycle

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The sea lamprey, *Petromyzon marinus*, is an invasive vertebrate species in the Great Lakes. The larval stage occurs in streams or rivers, the transformed parastic stage feeds on prey fish in the lakes and the spawning stage migrates up streams and rivers, where spawning occurs during the sexually mature phase. While the olfactory system plays an important role in motor responses, the diffuse chemosensory system may also activate locomotion in lampreys. These microvillar "oligovillous" solitary chemosensory cells were seen on papillae protruding along the oral disc, gill pores and tail fins of the brook lamprey (Whitear and Lane, 1983 J Zool 199). It is still unknown whether these oligovillous cells are involved in feeding or migration and spawning behaviors. We utilized scanning electron microscopy (SEM) to visualize oligovillous cell surface morphology to assess abundance across life stages, and immunolabeling to identify the oligovillous cells in sectioned tissue. By SEM, the oligovillous cells were recognized by the microvillar tufts on the surface of oral, gill and tail papillae. The cytoplasm was calretinin-immunoreactive in sectioned preparations. Acetylated tubulin and serotonin immunoreactive fibers were seen adjacent to these cells. The oligovillous cells were most abundant in the spawning stage, compared to the less mature transformer and parasitic stages. Spawners were then further subdivided and preliminary counts indicate that the oligovillous cells were more abundant in sexually mature spermiated/ovulated spawning lampreys than in sexually immature migrating lampreys, suggesting a role during migration and reproduction. This study helps in understanding the function of this extra-nasal chemosensory system. Acknowledgements: GLFC8400272, NSERC 03916. FCOI Disclosure: None.

#193 POSTER SESSION IV

Identification and Taste Sensory Evaluation of Naturally Occurring Taste Modifier Octenyl sulfate from Marine Organisms

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Various naturally occurring taste modifiers have been identified, and almost all of them are phyto-derived compounds. Some marine organisms, hermit crab and sea squirt, are known to evoke a sweet taste of water after ingestion, but their chemical analyses are not fully understood. These situations prompted us to identify the components responsible for the taste modification, and isolation of octenyl sulfate sodium salt (OS-Na) guided by tasting was succeeded. In addition, using a synthesized authentic standard, it was confirmed that OS-Na has the taste modifying activity, and hardly has any taste of its own. OS-Na has a simple molecular structure like detergents. It was therefore assumed that some related congeners had also sweet after tastes, but some others linear alkenyl or alkyl sulfates did not make water taste sweet. We have evaluated the sweetness characteristics of OS-Na. When the water was continuously taken in 30s interval after exposure of OS-Na, the sweetness of water was retained for few minutes. However, when OS-Na was mixed with sweeteners, the sweetness intensity was dramatically decreased. On the other hand, after pre-treated with OS-Na, the sweetness intensity of sweeteners was significantly enhanced. These characteristics of OS-Na were similar to that of lactisole, which is considered to be an inverse agonist of the sweet receptor, and OS-Na may share the same mode of action (Schiffman et al., 1999; Galindo-Cusperino et al., 2006). Other taste sensory tests mixed with Na-glutamate or NaCl were also performed. The umami taste, as expected, was partially suppressed, but, surprisingly, salty taste was also decreased. These results show that OS-Na alters various taste perceptions and elicits the sweet after-taste, which may be useful for flavor or masking unpleasant tastes. Acknowledgements: Kao Corporation. FCOI Disclosure: The authors are employees of kao corporation.

#193.5 POSTER SESSION V

Permissive Binding Pocket and Low Activation Threshold Underlie Exceptionally Broad Responsiveness of Some G-Protein Coupled Odorant Receptors

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Mammals detect and discriminate numerous odors via a large family of G protein coupled odorant receptors (ORs). While most ORs respond to structurally similar odorants, some ORs show broad responsiveness to diverse odorants. Using site-directed mutagenesis and molecular dynamics modeling, we investigated the molecular mechanisms underlying tuning breadth of ORs sharing high sequence homology. The broadly-tuned receptor MOR256-3 (also known as SR1) has a permissive binding cavity constituted by amino acid residues that directly interacts with diverse odorants. Single mutations at a few residues lead to drastic reduction in the responses to all selected odorants. However, these residues are not directly involved in ligand binding. Remarkably, single swapping at the same residues is sufficient to confer broad responsiveness to the narrowly-tuned receptor MOR256-8, suggesting their potential involvement in conformational switch for receptor activation. Furthermore, the overall responsiveness of an OR is positively correlated with its basal activity. These results suggest that in addition to the binding cavity, the receptor activation threshold shapes the OR tuning profile. FUNDING ACKNOWLEDGMENTS: Supported by grants from the National Institute on Deafness and Other Communication Disorders, National Institute of Health (DC011554 and DC006213 to M.M., and DC005782 and DC012095 to H.M.) and APEX Region PACA to J. G. FCOI DECLARATIONS: none

#194 POSTER SESSION V

G protein-dependent activation of PI signaling by a mammalian olfactory receptor

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It is increasingly understood that many G protein-coupled receptors (GPCRs) can induce a network of signaling pathways through both G protein-dependent and –indepedent mechanisms, rather than just a sole second messenger pathway. The hierarchy of signaling is thought to be imposed in part by the conformation of the ligand-GPCR complex such that different ligands shift the balance of the network towards different pathways, a process known as ligand-induced selective signaling (LiSS). Building on evidence that odorants can inhibit olfactory receptor neurons (ORNs) by activation of phosphoinositide (PI) signaling through the same olfactory receptor (OR) that excites the cell in a ligand-specific manner, and that activation of PI signaling is mediated by the Gβγ subunit, we co-expressed a mouse OR in HEK293T cells with a panel of Gα subunits previously detected in olfactory cilia and tested for odorant-evoked phosphoinositide-3-kinase (PI3K) activity by measuring the production of phosphatidylinositol 3,4,5-trisphosphate. Only Gαo co-expression increased PI3K activation by the heterologously expressed OR. The OR could also activate signaling through a chimeric Gα15 in which the c-terminal amino acids were

replaced with those of Gαo. Virally expressed G□ o can be detected in the cilia of native ORNs, indicating that it is not excluded from the site of signal transduction, even though endogenous protein levels appear to be too low to detect with traditional immunocytochemistry. These findings, together with previously published evidence that Gαo transcripts are present in most, if not all, mammalian ORNs, implicates, although does not prove, the involvement of a second heterotrimeric G protein complex as a substrate for LiSS by mammalian ORs. Acknowledgements: This work was supported by the National Institute on Deafness and Other Communication Disorders though award DC005995 to BWA. FCOI Disclosure: None.

#195 **POSTER SESSION V**

The Role of Van Gogh in the Rotation of Olfactory Dendrites in Drosophila melanogaster

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Diseases like malaria cause immense human suffering, and insect olfaction plays a key role in the spread of the diseases. Our goal is to elucidate the mechanisms of insect olfactory circuit development using the *Drosophila* antennal lobe (AL) as a model. In *Drosophila*, targeting of the dendrites of Projection Neurons (PNs) pioneers the olfactory circuit. We discovered that the PN dendrites undergo dramatic circular movement of ~30˚ around each other to attain the final glomerular positions. We showed that this movement is regulated by the non-canonical Wnt5 protein. Although non-canonical Wnts play powerful roles in cell migration and cancer, the mechanisms by which they direct cell movements are obscure. Our data showed that Wnt5 acts as a repulsive cue to guide the targeting of the PN dendrites. We further show that the Derailed/Ryk transmembrane protein acts in the PN dendrites to repress Wnt5 signaling. To isolate molecules acting downstream of Wnt5, we screened signaling mutants for defects that mimic that of the *wnt5* mutant. We found that the *Van Gogh* (*Vang*) mutant shows AL defects that bear strong resemble to that of *wnt5*. Vang, a four-pass transmembrane protein, is a component of the core planar cell polarity (PCP) pathway, which plays essential roles in cell movements. We show that the PN dendrites failed to undergo rotational movements in the *Vang* mutant as in the *wnt5* mutant. We also show that the *wnt5*; vang double-homozygote exhibited a wnt5-like phenotype. Collectively, our data support a model in which *Vang* acts downstream of *wnt5* to guide the novel rotation of the PN dendrites during the development of the fly olfactory circuit. Our work shows that development of the brain employs a conserved signaling mechanism that also regulates the rotation of cells in epithelial tissues. Acknowledgements: NIH R15 DC010916-02. FCOI Disclosure: None.

#196 **POSTER SESSION V**

Loss of Odor-Evoked Electro-Olfactogram in Mice with Genetic Ablation of Heterotrimeric G-protein β1 Subunit

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Heterotrimeric G-protein, in particular, the Gα subunits are known for their essential roles in olfactory signaling transduction and proper axon targeting. However our knowledge about the functions of G-protein βγ subunits is limited. We previously performed a comprehensive gene-transcript expression analysis of all known Gβ and Gγ subunits in the main olfactory epithelium (MOE) and found strong expression of $G\beta_1$ and $G\gamma_{13}$ subunits in olfactory sensory neurons (OSNs; Sathyanesan et al., 2013). Here, we examined embryonic expression of $G\beta_1$ subunit and olfactory deficits in mice with genetic ablation of $G\beta_1$. Our RNA in situ hybridization experiments revealed that $G\beta_1$ transcript is expressed as early as E15 and is present in both mature and immature OSNs of wild type mice. In neonatal P0 $G\beta_1^{-/-}$ mice, $G\beta_1$ transcript expression was not found in MOE, however, transcript expression of Gαolf and $G\gamma_{13}$ was unaffected. In electroolfactogram (EOG) recordings, the MOE of $G\beta_1^{-/-}$ mice did not show any odorant-evoked response to a battery of odorants and pheromones. Using immunohistochemistry of P0 MOE, we found similar expression and localization of cAMP signal transduction proteins in both P0 $G\beta_1^{-/-}$ mice and wild type littermates. In contrast, $G\gamma_{13}$ protein expression was apparently reduced. Together, our results indicate potential importance of $G\beta_1$ in OSN development and its critical role in MOE responsiveness to odor molecules. Acknowledgements: Supported by NIH/NIDCD grants DC009269 and DC012831 to WL. FCOI Disclosure: None.

#197 **POSTER SESSION V**

Olfactory marker protein is an indicator of olfactory receptor-associated events in non-olfactory tissues

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Olfactory receptor (OR)-associated event is mediated by well-conserved components such as OR as a starting G-protein coupled receptor (GPCR), olfactory G-protein (G_{olf}), adenylate cyclase III (ACIII), and olfactory marker protein (OMP) in the olfactory epithelium. Additionally, the ectopic ORs monitoring external chemical cues in the non-olfactory tissues are recently extended significantly. However, large number OR genes and their structural similarities make a demand to find the effective and useful way to detect ectopic OR-associated event and the identification of additional expression profiles and physiological functions of ORs in non-olfactory tissues remains to be totally unknown. In this study, to overcome the limitations of using the

OR as a target protein, we have used OMP with G_{olf} and ACIII as targets for screening the potential OR-mediated sensing system in non-olfactory tissues. Here, we show that OR and OR-associated proteins are expressed together in non-olfactory tissues by systematically using western blotting, real-time RT-PCR, and single/double immunodetection procedure. Immunohistochemical analysis (IHC) found OMP (+) cells in the bladder, thymus, thyroid, testis and heart. All ORs tested in this study were expressed in the OMP (+) cells and another ectopic expression of ORs in OMP (+) tissues was analyzed by refined microarray, real-time RT-PCR, and co-immunostaining method. These results suggest that OMP is involved in potential ectopic OR-mediated signal transduction cascade with olfactory canonical signaling components between nervous and the endocrine systems. Taken together, OMP IHC is a useful tool to identify ectopic expression of ORs, suggesting OMP expression as an indicator of potential ectopic OR-mediated chemoreception in non-olfactory systems. Acknowledgements: This research was supported by National Research Foundation of Korea (2013R1A1A2009145) & Korean Health Technology R&D Project, Ministry for Health, Welfare & Family Affairs (HI13C0423). FCOI Disclosure: None.

#198 **POSTER SESSION V**

Functional characterization of heterologously expressed Codling Moth Olfactory Receptors

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Olfaction plays a major role in host-selection behavior in the Codling Moth (*Cydia pomonella* L.), one of the most notorious pest species threatening apple orchards worldwide. Functional expression of C. pomonella olfactory receptors (CpomORs) not only allows investigating mechanisms of insect OR functioning but also developing novel means to intervene and control the pest behavior. Earlier, based on an antennal transcriptome analysis, we identified a number of critical elements of the moth olfactory system including the olfactory co-receptor (CpomORco) and the potential pheromone receptors CpomOR1, CpomOR3, CpomOR4, CpomOR5, and CpomOR6 (Bengtsson et al 2012). To date, we have described CpomOR3 using heterologous expression in Drosophila T1 trichoid and ab3A basiconic sensilla (Bengtsson et al 2014). We are now extensively characterizing other codling moth ORs transiently expressed in HEK cells. Using calcium imaging and whole-cell and outside-out patch clamp recordings, we demonstrated that both homomeric CpomORco channel forming subunit and heteromeric CpomOR complexes can be activated by ORco agonists VUAA1 and VUAA3, as expected for insect ORs. Different OR complexes were characterized by both different sensitivity to the agonists and different activation/inactivation kinetics. Both homo- and heteromeric OR complexes were also susceptible to inhibition by amiloride and amiloride derivatives when activated by agonists. The HEK cells thus represent an adequate expression system for CpomORs and their functional characterization. Acknowledgements: - Italian Ministry of Education, Universities and Research (MIUR) [project 'Analisi integrate dei processi molecolari e cellulari responsabili dell'elaborazione di segnali sensoriali in condizioni normali e patologiche' (no. 2010599KBR_005, PRIN 2011)] - Università degli Studi di Milano, Department of Food, Nutritional and Environmental Sciences (DeFENS), PhD Course of Chemistry, Biochemistry and Ecology of Pesticides (CBEA) - Fondazione Edmund Mach, Research and Innovation Centre. FCOI Disclosure: None.

#199 **POSTER SESSION V**

Hyperpolarization-activated currents in granule cells of the olfactory bulb

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In sensory systems, neural circuits exhibit oscillatory dynamics driven by sensory input, extrinsic regulation, and intrinsic circuit properties. Activation and inactivation of currents, such as hyperpolarization-activated currents (Ih), are involved in generating membrane potential oscillations in neurons and contribute to network oscillatory dynamics. In the olfactory system, initial processing of odor signals occurs in the olfactory bulb (OB) and an important aspect of this processing, network synchrony, arises from the activity of reciprocal synapses between output neurons, the mitral and tufted cells (M/TCs), and inhibitory intrinsic neurons, the granule cells (GCs). Interestingly, M/TCs exhibit varying expression of Ih, suggesting a contribution of this current to the fidelity of information coding by M/TCs. In contrast, the presence of Ih in the GC population is poorly characterized. Here, using patch-clamp recordings, we show that GCs in the main OB (MOB) and accessory OB (AOB) exhibit I_h. Although maximal current for I_h varied across cells, voltage dependency was consistent in both regions (MOB: V_{half} = 109.6 ± 4.2 mV, n = 5; AOB: V_{half} = 109.8 ± 2.5 mV, n = 6). In addition, I_h in AOB and MOB GCs showed a similar sensitivity to changes in extracellular K+ concentration, and blockage by ZD-7288 (30µM). However, loading the intracellular pipette solution with cAMP (500µM) shifted the activation curve of I_h to less negative potentials in GCs in the AOB, but not the MOB (MOB: V_{half} = 109.0 ± 4.9 mV, n = 7; AOB: V_{half} = 98.4 ± 3.1 mV, n = 5). These results suggest the

expression of I_h across different cell types, including GCs and M/TCs, may impart unique features to odor processing in the OB and facilitate oscillatory dynamics in both the main olfactory and Vomeronasal systems Acknowledgements: This work was supported by the MBL Neurobiology Course and a NIDCD RO1-DC-009817 to R.C.A. FCOI Disclosure: None.

#200 **POSTER SESSION V**

Lateral inhibition differences between mitral and tufted cells of the mammalian olfactory bulb

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Mitral cells (MCs) and tufted cells (TCs) are the principal output neurons of the olfactory bulb. Although recent studies suggest that MCs and TCs encode distinct features of olfactory information, the circuit mechanisms leading to these functional differences are unknown. We hypothesize that these differences are mediated in part by differences in lateral inhibition (LI), the circuit motif that allows M/TCs to indirectly inhibit one another and that enhances contrast between similar odors. Here we show that LI received by MCs and TC differs both in its strength and in the range of firing rates over which it is effective. We performed whole-cell *in vitro* recordings from olfactory bulb slices in M72-ChR2-EYFP mice that express ChR2 in a single type of olfactory sensory neuron. Photoactivation of the M72 glomerulus evoked LI onto M/TCs projecting to glomeruli adjacent to the M72. We measured the strength of LI by recording IPSCs evoked by stimulating the M72 glomerulus with 10ms light pulses. We found that LI onto MCs (41±18 pA, N=8) was significantly larger than LI onto TCs (12±4 pA, N=8). To assess the impact of LI on MC and TC spiking, we constructed FI curves for each M/TC via somatic current injections of increasing amplitudes. At each current step, we recorded the number of action potentials evoked with and without M72 photostimulation (8 x 10ms light pulses at 15 Hz). We found that LI affects MCs at intermediate firing rates (25–75 Hz) and TCs at low firing rates (5-40 Hz) although the magnitude of the maximal reduction was similar. Together, these findings indicate that LI impacts MCs and TCs differently and may underlie the functional differences between them. Future work will build on these findings to better understand the types of olfactory tasks that each cell is best suited to perform. Acknowledgements: NIDCD R01DC011184. FCOI Disclosure: None.

#201 **POSTER SESSION V**

Deep Short-Axon Cells Mediate Interglomerular Disinhibition in the Mammalian Main Olfactory Bulb

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Olfactory stimuli are first encoded by the activation pattern of multiple glomeruli across the surface of the main olfactory bulb. Within each glomerulus, periglomerular cells (PGCs) regulate the strength and duration of sensory input by inhibiting presynaptic sensory axons and postsynaptic principal neuron dendrites. Currently, there are no known circuits that coordinate PGC activity across multiple glomeruli. Here, we provide the first functional evidence for a novel olfactory bulb circuit capable of tuning glomerular activation patterns by regulating interglomerular PGC activity. Specifically, we have identified the first selective molecular marker (nicotinic acetylcholine receptor subunit α2; *chrna2*) of a class of glomerular layer-projecting deep short-axon cells (GL-dSACs) that synaptically inhibit PGCs across multiple glomeruli. Using Chrna2-Cre mice, we find GL-dSACs to have large cell bodies, extend 2-5 sparsely branched aspiny dendrites within the internal plexiform layer, and project axons directly across the external plexiform layer to arborize profusely across multiple glomeruli. In acute slices, GL-dSACs are bombarded with spontaneous EPSCs (11.6±9.6 Hz, μ±σ, n=16), which drive tonic theta frequency firing (7.4±6.0 Hz, n=16). Sensory axon stimulation evokes rapid (< 10 ms) and highly reliable GL-dSAC firing at stimulation intensities subthreshold for evoking glomerulus-wide long-lasting depolarizations, suggesting that GL-dSACs receive strong excitation from external tufted cell axons. Currently, we are using optogenetics to both characterize the GL-dSAC-PGC synapse and to explore GL-dSAC-mediated glomerular disinhibition. In the future, we will examine the impact of chemogenetic GL-dSAC perturbation on olfactory perception. Acknowledgements: Supported by NIDCD grants F31DC013490 (S.D.B.), R01DC005798 (N.N.U.), and R01DC011184 (N.N.U.). FCOI Disclosure: None.

#202 **POSTER SESSION V**

Pilot exploration of the functionality of the human olfactory bulb using high-resolution fMRI

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In the last decades, functional brain imaging (fMRI) studies revealed many of the critical structures involved in human olfaction, from early processing in anterior piriform cortex to high order olfactory areas. Nevertheless, the functionality of the

olfactory bulb (OB), the first relay of olfactory information processing, has not been studied in detail. Whereas the anatomy of this structure has been widely explored in humans, its small size limits any functional exploration. In this study, we set out an fMRI exploration of the OB using a 3 Tesla MR-scanner and a newly developed prototype of receiving superficial coil (Siemens, Germany). Eight subjects were tested (age range: 24-28 y; 3 w). The study comprised 2 sessions, one for each nostril with 3 odorants intermixed with a no-odor condition in pseudo-random paradigm: Butanol (3% in propylene glycol – PG, CID 263, rancid-sweet like odor), 2-phenylethyl alcohol (100%, CID 6054, rose-like odor), p-menth-8-en-3-ol (75% in DPG, CID 24585, minty-like odor). Participants' tasks included ratings of odor intensity, pleasantness, familiarity, irritation, cooling and qualitative description of odors. fMRI data were collected with an EPI sequence (15 slices; TR: 1860ms; voxel size: 2x0.82x0.82mm). A high-resolution T2-weighted image was acquired (voxel size: 2x0.47x0.47mm) and used to draw individual regions of interest in the OB. Preliminary multivariate analyses comparing odor vs. no odor conditions revealed heterogeneous individual classification patterns from 50% ($p>.05$, binomial) to 64% ($p< .05$) above chance that differed with time (first 3 blocs: 48% to 66% of classification; last 3 blocs: 52% to 62%). A detailed analysis comparing odor conditions in the left/right nostril in the ipsilateral OB in a larger sample of individuals will be presented. Acknowledgements: This study was partly supported by a grant from the ANR to MB (EMCO program, ICEO Project). FCOI Disclosure: None.

#203 **POSTER SESSION V**

The role of distributed and segregated synaptic clusters in the olfactory bulb

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The specific spatial organization of olfactory bulb network elements and their synaptic connectivity has evolved in such a way to subserve special computational functions needed for odor detection and recognition, but which are still largely unknown. A particularly intriguing example of this organization is the spatially distributed and segregated synaptic clusters, experimentally observed in the olfactory bulb, that often take the form of cellular columns with preferential connectivity in a spatially restricted region below a glomerulus. Understanding the neural basis of odor processing therefore requires understanding the computational functions and role of glomerular units, and how they interact with each other to modulate the input/output (I/O) processing occurring in the olfactory bulb. To address these problems, we used a model with a realistic three dimensional (3D) representation of overlapping and interacting dendrites of mitral and granule cells. With this model, we have been able to obtain new findings that are not obtainable with experiments or simpler artificial models, and make several experimentally testable predictions. In particular, we have found that column formation is an activity-dependent process, whose main functional role is to regulate the spread of activity out of a glomerulus; that columns interact, positively or negatively, in a distance-dependent manner; and that sequential odor exposure produces non-commutative processing. Finally, we introduce the concept of "odor operator", defined as the overall interaction between mitral cells belonging to the same or different glomerular units, and show how it can be developed into a promising theoretical framework for insight into the neural basis of olfactory processing. Acknowledgements: We are grateful for support from the SenseLab Project DC 00997701 (National Institute of Deafness and Other Communication Disorders) and grant NS11613 from NINDS which supports NEURON development, and CINECA (Bologna, Italy) for granting access to their IBM BG/Q supercomputer system.
FCOI Disclosure: None.

#204 **POSTER SESSION V**

Suppression of odor processing by another odorant in mouse main olfactory bulb

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It has been shown that perception of certain odorants can be distorted by other odorants in humans and rodents, which is known as "masking". However, the physiological mechanisms underlying this phenomenon remain unclear. Here we studied if interactions among odorants can be detected in olfactory bulbs in vivo using OMP-spH mice. We delivered several combinations of odorants to anesthetized OMP-spH heterozygous mice whose skull was exposed and thinned to allow imaging of stimulus-dependent changes in spH fluorescence. Such signals reflect changing presynaptic activity of olfactory sensory neurons. We found that some glomeruli activated by acetaldehyde displayed suppressed responses to a mixture of acetaldehyde and methyl benzoate. Moreover, basal spH fluorescence was suppressed by methylbenzoate alone. Our results show that complex interactions between odorants can be detected physiologically at the level of olfactory bulb glomeruli. These may correlate with changes in the intensity and/or quality in odor perception. Acknowledgements: Japan Tobacco INC.
FCOI Disclosure: None.

#205 **POSTER SESSION V**

Entrained Oscillatory Discharge in an Accessory Olfactory Bulb Microcircuit

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The accessory olfactory system is a key component in rodent conspecific chemical communication. However, coding of socially relevant chemosignals within the involved brain areas - the accessory olfactory bulb (AOB), the 'vomeronasal' amygdala and the hypothalamus - is poorly understood. In the AOB, the first stage of information processing in the mouse vomeronasal pathway, the main projection neurons - mitral cells (MCs) - receive sensory input from vomeronasal sensory neurons. The AOB network transforms this sensory input and MCs then transfer this information to third-order nuclei. A subpopulation of MCs exhibits slow oscillatory discharge that persists upon pharmacological inhibition of fast synaptic transmission. Here, we identify an excitatory circuit within the AOB network that entrains oscillatory activity in a second MC subpopulation. Using a battery of physiological approaches, we investigate the underlying mechanisms in acute AOB tissue slices. Entrained MCs display periodically increased excitatory synaptic input that correlates with their respective rhythmic discharge patterns. Block of fast glutamatergic synaptic transmission reveals that entrainment depends on an intact glutamatergic network. By contrast, block of fast GABAergic transmission shows that tonic GABAergic inhibition frequently masks entrained slow MC oscillations. Ongoing experiments aim to identify the detailed mechanisms of MC entrainment and the role of slow rhythmic activity in AOB information processing. Acknowledgements: This work is funded by the VolkswagenFoundation (83533) and the Deutsche Forschungsgemeinschaft (SP724/9-1). FCOI Disclosure: None.

#206 **POSTER SESSION V**

A Shared Molecular Mechanism Regulates Transcription of Tyrosine Hydroxylase and Glutamate Decarboxylase 1 in Olfactory Bulb Interneurons

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A subset of GABAergic olfactory bulb (OB) interneurons contain dopamine and preferentially co-express *Tyrosine hydroxylase* (*Th*) with *Glutamic acid decarboxylase1* (*Gad1*), rather than *Gad2*. The molecular mechanisms responsible for this preferential co-expression are poorly understood. We recently showed that *Th* transcription in OB dopaminergic interneurons is regulated, in part, by a mechanism mediated by highly conserved G:C-rich regions within the proximal promoter that both recruit heterogeneous nuclear ribonucleoprotein (hnRNP) K and form single-stranded DNA secondary structures. In this study, we show that highly conserved G:C-rich regions in the *Gad1* proximal promoter are also critical for regulating transcription in OB interneurons. Protein pull-down assays demonstrate that hnRNP K preferentially binds the C-rich single strand sequences within the G:C-rich regions. hnRNP K over-expression in NT-2 cells up-regulates *Gad1* promoter activity. Circular dichroism analyses establish that single strands of the *Gad1* promoter G:C-rich regions can also form either G-quadruplex or i-motif secondary structures, which are stabilized by small molecule like TMPyP4. Chromatin immunoprecipitation studies with mouse OB tissue confirm that these secondary structures are present on the *Gad1* promoter *in vivo*. We also describe studies testing whether methylation of *Gad1* promoter alter either secondary structure formation or the hnRNP K binding. Together, these findings show that highly conserved G:C-rich regions in *Th* and *Gad1* proximal promoters mediate similar transcription regulatory functions, which provides novel insight into the mechanisms underlying the preferential co-expression of *Th* and *Gad1* in OB interneurons. Acknowledgements: NIH DC008955 Burke Medical Research Institute. FCOI Disclosure: None.

#207 **POSTER SESSION V**

The spatiotemporal input-output function of the olfactory bulb is modulated by respiratory cycle activity

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Spontaneous breath-evoked activity modulates mitral and tufted cell (MTC) odorant responses. Odorants activate unique patterns of glomeruli. The responses reaching the MTC somas backpropagate into the lateral dendrites, and are balanced by feedback and lateral inhibition through granule cells. How temporal and spatial glomerular input patterns influence the firing of a single MTC, the main output of the olfactory bulb, is not currently understood, although distinct patterns of lateral inhibition are hypothesized to shape MTC firing. Utilizing optogenetic stimulation techniques, we systematically analyze how specific temporal patterns of ORN glomerular input, which include combinations of primary and/or non-primary glomeruli, influence MTC output activity. The spatial pattern, duration, frequency, and intensity of dorsal glomerular stimuli are manipulated optically across all breath phases using a custom-built Digital Micro-mirror Device, while *in vivo* extracellular recordings from single MTCs are obtained in anesthetized OMP-ChR2 mice. Our data highlight the importance of temporal coding by revealing how spontaneous activity associated with particular phases of respiration influences the efficacy of glomerular input patterns. Consistent with previous studies examining MTC responses to odor stimuli across the respiratory cycle, we find unique respiratory cycle phase shifts in peak MTC activity following optical stimulation in the glomerular layer. Experiments here probe all dorsal olfactory

bulb glomerular inputs to map MTC receptive fields and associated lateral inhibitory connections across the respiratory cycle. Acknowledgements: NIH grants R01DC011286, R01DC009994, R01DC009977, T15LM007056, and T32NS007224.FCOI. FCOI Disclosure: None.

#208 **POSTER SESSION V**

Morphological analysis of mitral cell populations in the mouse accessory olfactory bulb

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In most mammals, the vomeronasal/accessory olfactory system is crucial for inter- and intraspecific communication. The accessory olfactory bulb (AOB) represents the first stage of information processing within the vomeronasal pathway. Mitral cells (MCs) are the only excitatory AOB projection neurons. Two subpopulations of MCs display strikingly different spontaneous discharge patterns. Members of one population fire action potentials in a seemingly random fashion, while others intrinsically exhibit slow oscillatory discharge with subthreshold membrane potential fluctuations and superimposed trains of action potentials. Here, we comparatively investigate the morphology of both MC subpopulations using an immunohistochemical approach. During patch-clamp experiments in acute mouse AOB slices, MCs were diffusion-loaded with biocytin for *post-hoc* staining and 3D reconstruction. Surprisingly, the morphological properties of both populations are strikingly similar. However, we observe one notable exception: primary dendrites of intrinsically oscillating MCs terminate in significantly larger glomeruli. On-going multi-photon imaging experiments now aim to provide a more detailed characterization of the glomerular organization in the AOB, specifically focusing on correlations between MC physiology and glomerular morphology. Acknowledgements: This work was supported by the Deutsche Forschungsgemeinschaft SPP 1392: "Integrative Analysis of Olfaction" and the Volkswagen Foundation (83533). FCOI Disclosure: None.

#209 **POSTER SESSION V**

Reduction in Absolute Volume of Olfactory Bulb Layers in Juvenile Male American Minks (Neovison vison var. atratus)

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The olfactory bulb is a dynamic structure with continuous replacement and addition of neurons and neuronal elements: sensory axons from the periphery and neuronal migration via the rostral migratory stream from central areas. The neurons are arranged in specific layers producing the typical structural appearance of the bulb. We were interested, if all layers increase proportionally when the olfactory bulb grows, or if specific layers preferentially develop during certain phases. Therefore we analyzed the absolute volume of each layer in a total of 36 male minks at different ages, from newborns (postnatal day 0: P0) to 7 months using histological sections evaluated morphometrically. Unexpectedly, although the olfactory bulb continuously increases in size, the layers not only show different growth rates but several layers even exhibit phases of reduction in absolute volume. The mitral cell layer increases from birth (P0: 0.30±0.01 mm^3) to a high at P90 (4.91±0.14 mm^3), followed by a significant reduction during juvenile age, by 16.9% to P120 (4.08±0.65 mm^3), and by another 19.6% to P150 (3.28±0.64 mm^3). Afterwards, the mitral cell layer expands again significantly (P180: 5.59±0.75 mm^3; P210: 7.91±1.75 mm^3). A significant absolute reduction in layer volume was also observed in the stratum album between P60 (13.41±0.58 mm^3) and P90 (10.14±1.25 mm^3) as well as in the subependymal layer (P60: 4.90±0.06 mm3; P90: 3.12±0.07 mm^3) decreasing continuously more than 70% to P210 (1.39±0.02 mm^3). In contrast, information processing layers, such as the granule cell and external plexiform layer, show a continuous increase in volume after P60. These results indicate a rearrangement of neurons and underlying networks, probably due to functional changes during biological phases (nutritional and social changes). Acknowledgements: Supported by University Ulm Neurobiology Institutional Students Training Award (2014/2015) to WB and by FORUM 208/00M122/13 to EW. FCOI Disclosure: None.

#210 **POSTER SESSION V**

Sniffing Out Ovarian Cancer; An Interdisciplinary Approach to Early Detection

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Ovarian carcinoma is the most lethal of the gynecological malignancies and the fourth leading cause of cancer death in women. The high mortality rate is often due to the late stage of detection. Approximately 75% of ovarian cancers are diagnosed when therapeutic strategies are limited and morbidity and mortality is high. Diagnosis of ovarian cancer is severely hindered by the lack of reliable early-stage diagnostic tools, despite its importance for successful treatment. Based on prior studies involving trained dogs, we hypothesized that endogenous volatile metabolites emanating from ovarian tumors can provide a reliable, early-stage, detectable signal of cancer's presence. To test this hypothesis, we have employed a multidisciplinary approach using 1) trained canines to demonstrate the presence of volatile organic compounds (VOCs) indicative of the disease,

2) analytical chemistry techniques (SPME; GC/MS) to identify the volatile biomarkers and 3) the development of a nanotechnology-enabled E-nose to detect the VOCs and serve as a screening device. Results to date demonstrate that canines trained on biopsied tissue or plasma can recognize the odor of ovarian carcinoma from blood samples collected from patients prior to surgery. The canines are able to distinguish blood samples from ovarian cancer patients vs. benign ovarian tumors and healthy controls with > 95% sensitivity and specificity. Results obtained using both analytical techniques and nano-sensor devices suggest the existence of reliable quantitative differences in VOCs emanating from pooled blood samples collected from healthy controls, patients with benign growths and patients with various forms of primary ovarian cancer. Recent attempts to identify the nature and abundance of the compounds distinguishing the samples will be discussed. Acknowledgements: This work was supported by funds from the Kaleidoscope of Hope Foundation as well as funds donated by Ms. Bonnie Hunt (to G.P.) in memory of her parents, Ida and Percy Hunt and the NIH Training Grant #2T32DC000014-32A1 (to K.A.P.).
FCOI Disclosure: None.

#211 **POSTER SESSION V**

Animals biosensors detect odor signatures of hepatocellular carcinoma in urine of mice with experimental tumors

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Animals release a variety of chemical signals, particularly through urine, which mediate social interactions and endocrine function. Studies have been conducted to investigate the presence of urinary chemosignals from cancer tumor in mice. Behavioral responses of dog and mice to urine samples of mice with experimental tumors of different duration of time have been examined. In this study, we monitored changes in odor signatures of hepatocellular carcinoma released from mouse urine by sensitivity animals sensors, which followed the development of the tumor. To create tumors, hepatocellular carcinoma H33 tissue was inoculated subcutaneously (n=79). As a control, we used the urine samples collected from healthy mice after treatment with saline (n=91) or normal liver tissue (n=80). We used a match-to-sample-like protocol of scent identification lineups by dogs (n=4). The scent lineup consisted of 10 urine samples from healthy mice, and one sample with urine collected from a cancer mouse. The accuracy of dogs' indication of 1-, 2-, 3-, 6- and 9-day tumor are 67%; 54,5%; 79%; 92% and 80% respectively. Thus, the specificity are from 94% to 98%. We have conducted experiments with mice with modified "habituation-dishabituation" test. At the first, second and fourth presentation a sensor mouse sniffed the urine sample of the same healthy mouse donor after saline/normal liver tissue injection. At the third presentation the urine sample of the same donor 24 hours after tumor inoculation (n=20) was provided. The sensor mice reliably distinguished the urine after the hepatocellular carcinoma tissue inoculation. Our studies showed that dogs and mice can have diagnostic success not only in detecting hepatocarcinoma signatures in urine, but also in discriminating between cancer and inflammation. Acknowledgements: Acknowledgements: Supported in part by CITiS grant 14-04-01150 A (01201450553).
FCOI Disclosure: None.

#212 **POSTER SESSION V**

The Pairing of Lavender and the Voluntary Lowering of Heart Rate

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The results from previous studies have shown that with biofeedback people can learn to voluntarily raise or lower their heart rates. The purpose of the present study was to pair learned cardiac deceleration with the smell of lavender. Specifically, when, in the presence of lavender, individuals voluntarily learn to reduce their heart rates using biofeedback, it was hypothesized that smelling the odorant by itself would decrease the heart rate. Pre-training heart rates were measured when the subjects were exposed to lavender, distilled water and nothing. The subjects then underwent biofeedback training in which, while watching their heart rates continuously displayed on a computer screen, they attempted to decrease their heart rates while smelling lavender, distilled water or nothing. Following 10 five minute training sessions, subjects had their heart rates monitored as they were exposed to lavender, distilled water and nothing. During the training, only subjects in the lavender group were consistently able to reduce their heart rates. Pre-training, exposure to lavender decreased heart rate by about 2% in all three groups whereas the water and nothing had no effect. Post training, the lavender exposure produced the same decrease in heart rate seen pre-exposure in the water and nothing groups. However for the group trained with lavender, post-training exposure to lavender decreased heart rate by 3.4%. Surprisingly, the water control also showed a cardiac deceleration (about 2%) in the lavender group. Perhaps lavender and biofeedback can have a synergistic effect. In any event, the data is consistent with the hypothesis that when lavender is present during biofeedback training, a post training exposure may trigger the learned response. Acknowledgements: St. Lawrence University funds. FCOI Disclosure: None.

#213 POSTER SESSION V

The Effects of Peppermint Scent Administration on Augmenting Driving Performance During a Distracted Driving Scenario

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Past research has shown that there is a relationship between scent and cognitive ability, scent and physiological responses, and scent and physical activities. The current study assessed the effects of a peppermint scent presented, while driving and being distracted with tasks and questions. Fifty-one undergraduate volunteers (18 males and 33 females) participated in the study. The participant was in either the control room with no scent or a room scented with pharmaceutical grade peppermint oil. Participants then "drove" using the driVR system for 15 minutes. Researchers asked questions at one-minute intervals from a list of distractor tasks. After completion of the driving course, participants completed the NASA-TLX (to assess workload) and POMS (to assess mood) surveys. Significance was found for mental demand, such that completing the driving course was less mentally demanding with peppermint scent administration, $F(1,41)=4.494$, $p=.040$. Trends were also noted with the physical demand sub-scale of the NASA-TLX [$F(1,41)=3.523$, $p=.068$] and the anxiety sub-scale of the POMS [$F(1,41)=3.263$, $p=.078$], with both dimensions decreased in the peppermint scent condition. These results provide support for peppermint scent use as a non-pharmacological stimulant to promote driving alertness while distracted. Acknowledgements: Supported by University funds. FCOI Disclosure: None.

#214 POSTER SESSION V

Effects of Peppermit Scent Administration on Augmenting Swimming Performance: Challenges Related to Orthonasal vs. Retronasal Scent Administration

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Past research indicates the positive effects of peppermint scent administration on athletic performance in a variety of sports (ie. soccer, basketball, golf) and exercise (ie., push-ups, hand-grip, running speed) domains. The present study assessed the effects of peppermint scent administration on augmenting swimming performance. In Study 1, Division II swimmers completed both a 50m and a 200m race in both a orthonasal peppermint scent administration condition and a non-scented control condition. In addition, questionnaires related to mood (POMS) and workload (NASA-TLX) were completed. Controlling for sex, BMI and years of competition, there was a trend for the peppermint scented condition to decrease the 50m time by 3.3% and the 200m time by 0.7%, $F(1, 15)=3.55$, $p=.08$. Since this effect is lower than that noted in other sports, it was hypothesized that the sport of swimming presents a unique challenge, since swimmers often breathe through their mouth, thus diminishing the effects of orthonasal scent administration. In Study 2, swimmers completed the same races, but with the administration of an orally inhaled peppermint scented oxygen, with the hypothesis that this retronasal administration may be better suited to swimmers who breathe through their mouth. No significant oxygenated peppermint effect was found, $F(1,9) = 0.94$, $p=.36$. The results of these two studies indicate that some effect can be found through orthonasal scent administration for swimmers; however, the addition of a retronasal scent administration produces no tangible augmentation of swimming performance. With swimming competitions typically being won or lost by mere hundredths of a second, future studies should address additional ways of overcoming this scent administration challenge. Acknowledgements: This research was funded by a West Virginia Space Grant to Dr. Bryan Raudenbush. FCOI Disclosure: None.

#215 POSTER SESSION V

Subliminal Smells Modulate Audiovisual Speech Perception

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Speech perception is multisensory. A canonical example is the McGurk effect in which disparate audio and visual speech produces the illusory auditory percept. What influences the binding of the different sensory inputs is not well understood. We previously introduced the seemingly unrelated sense of olfaction into the McGurk paradigm, choosing the smell to be subliminal and its valence to match that of either the spoken word or the fused audiovisual percept. We demonstrated that subjects were more likely to perceive a pleasant word in the presence of a pleasant smell and an unpleasant word in the presence of an unpleasant smell. Nevertheless, our finding could be due to semantic bias. Here we present findings of two followup experiments to rule out alternative explanations. In one experiment, subjects were exposed to purified air and told that the air contained a low concentration of either a pleasant or an unpleasant smell. We showed that while semantic bias altered subjects' perception of the pleasantness of air, it did not alter audiovisual speech integration. In another experiment, subjects performed the tasks in the presence of suprathreshold smells. The results were similar to those observed with subliminal smells, albeit with a smaller effect size. Together, our findings strengthen the evidence that olfaction modulates audio-visual binding in speech perception, and yield the surprising conclusion that speech is perceived not only from what we hear and see, but also from what we smell. Acknowledgements: Baylor College of Medicine Internal Funding. FCOI Disclosure: None.

#216 POSTER SESSION V

Effects of Male Income and Male Pheromone Scent Administration on Ratings of Online Dating Profiles of Males Made by Female Participants

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Past research has demonstrated a relationship between subjective ratings of physical attractiveness and reported income. For example, Townsend and Levy (1990) had participants rate a dating profile and found females were more likely to choose a relationship with a partner who had a higher socioeconomic status. Similarly, Roszell, Kennedy and Grabb (1989) reported the highest ratings of attractiveness were found for individuals with larger incomes. The present study further investigates this relationship while also administering the scent of androstadienone within the testing room. Lundstrom and Olsson (2005) note this chemical compound significantly alters the mood of women, which, it was hypothesized, may also influence their ratings of dating profiles. A dating profile was created, and the salary of the portrayed male was manipulated into a low ($50,000), medium ($75,000) or high ($150,000) condition. 41 females participants were assigned to one of the salary conditions and completed a 10 question evaluation of the profile (ex. "How intelligent/trustworthy/attractive/etc. is this person?") in either a non-scented room or a room administered androstadienone via an oxygen concentrator at 3mL/min. Confirming past research, as salary increased attractiveness ratings increased, $F(2,34)=2.68$, $p=.08$. Data were also analyzed using a 2 (scent condition) x 3 (salary condition) independent groups MANOVA among the 10 profile ratings and a significant scent condition effect was found, $F(10, 25)=3.10$, $p=.01$. In the androstadienone condition females rated the profile individual as being more successful and were more willing to have sex with them than the non-scented condition. Results are discussed in terms of social and cultural anthropology. Acknowledgements: University Funding. FCOI Disclosure: None.

#217 POSTER SESSION V

Can learned responses to body odor affect human social interactions?

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Body odor conveys a great deal of information about an individual, and is important in social evaluations and bonding. Given the communicative value of body odor and the increasing interest in the effects of perfume on that value, we investigated a) whether an aversive conditioning paradigm could engender learned responses to individual body odors; b) whether this effect might be enhanced by the addition of perfume worn by the odor donor; and c) whether this conditioned response could affect the interpretation of visual social information. 46 females underwent a classical conditioning training period where they were exposed to perfumed or unperfumed body odors obtained from two different female donors, one of which was paired with an aversive shock on 50% of trials. During training, we monitored galvanic skin response (GSR), and participants rated the pleasantness and intensity of odors following each trial. After conditioning, subjects participated in two rounds of an ostensibly unrelated task where they were asked to rate the emotions of a set of neutral faces. Unbeknownst to subjects, conditioned (CS) or unconditioned body odor samples were embedded in a lab coat they wore during each of the two rating rounds. Though we observed no changes in GSR during conditioning, conditioned odors were rated as less pleasant for perfumed trials ($p< .05$). In the presence of the CS, subjects were slower at rating amount of fear shown ($p>.05$), and perceived faces as more surprised ($p< .01$). Though perfume may have some effect on the signaling value of body odor, human social signals are rarely uni-modal or olfactory alone. In order to investigate the effects of olfactory information on social behavior, the most effective tools may be multimodal stimuli that include olfactory as well as visual and auditory cues. Acknowledgements: Sage Fellowship, Cornell University. FCOI Disclosure: None.

#218 POSTER SESSION V

Effects of L-Tryptophan Consumption on Attitudes Towards Community Service

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Past research indicates the consumption of L-Tryptophan (TRP), the biochemical precursor of 5-HT, can produce significant changes in mood, decision-making behaviors, and interpersonal trust. In particular, participants who consumed TRP prior to a mutual trust game transferred significantly more money to their partners in the game. The present study assessed the consumption of TRP on attitudes towards community service. Participants (N=88, 32 men, 56 women, age range = 16 to 24 years) were administered either 200 mL of orange juice (the control condition) or 200mL of orange juice to which 0.8 g of TRP had been added. Both prior to and after consumption, participants completed a variety of questionnaires over the course of one hour, such as the Profile of Mood States (to assess mood) and a general health questionnaire, and had their physiological responses (e.g., heart rate, blood pressure) tracked. After one hour, participants completed a survey related to community service attitudes. Participants receiving TRP reported significantly fewer negative impacts of community service than the control group, $t(85)=-2.15$, $p=0.034$. The TRP group also reported significantly more positive impacts of community service than the control group $t(85)= 2.91$, $p= 0.01$. Thus, the administration of TRP can have a marked effect on promoting positive attitudes towards community service, thereby building a

better community. Future studies should assess how such an effect then equates to actually performing community service. Acknowledgements: Supported by University funds.
FCOI Disclosure: None.

#219 **POSTER SESSION V**

Aroma Music Concert for Improving Mental Health

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The majority of U.S. adults struggle to manage their psychological stress and mental disorder with daily activities. Listening to music is the most common activity for coping with psychological stress, and similarly, aromas have been used for the purpose of enhancing psychological well-being. In this way, can the coupling of aroma and music exert a synergistic effect in improving mental health? Our previous clinical study demonstrated that listening to music accompanied by inhalation of well-selected aroma could enhance a stress-relieving effect. This study was designed to demonstrate a practical application of aroma and music as an effort to improve mental health. A concert composed of two sessions (for 25 min. per session) was used to present emotionally congruent pairs of aroma and musical stimuli. More specifically, during the concert, calming (or exciting) musical pieces were presented with lavender (or citrus) aroma. Participants' emotional state, impression to the concert, and willingness to revisit were surveyed before and after the concert. Participants' positive feeling was significantly increased during the concert. In addition, participants were satisfied with the concert to a high degree and wanted to re-attend the concert. In conclusion, our findings demonstrate that an "Aroma Music Concert" (or a combination of aroma and music) can be used as an alternative method to improving mental health in everyday life. Acknowledgements: This study was supported by 1) the Office of the Vice Provost for Research and Economic Development and 2) the Office of the Provost and Vice Chancellor for Academic Affairs at the University of Arkansas. FCOI Disclosure: None.

#220 **POSTER SESSION V**

Prognostic value of olfactory nerve assessment with olfacto-scintigraphy in patients with olfactory disorders

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Objectives: To show the prognostic value of nasally administered thallium-201 transport to olfactory bulb in patients with olfactory disorders. Methods: 27 patients with olfactory disorders were enrolled in the study (13 women and 14 men; 23–71 years old). The causes of olfactory dysfunction in the patients were head trauma (n = 6), upper respiratory tract infection (n = 4), chronic rhinosinusitis (n = 7), and idiopathic (n = 10). Thallium-201 was administered unilaterally to the olfactory cleft, and SPECT-CT was conducted 24 h later. Separate MRI images were merged with the SPECT images. The improvement was judged according to the criteria of the Japanese Rhinologic Society. Log-rank tests and Cox-proportion hazard tests were performed for the statistical analysis. Results: The period to the improvement was significantly shorter in the patients with high nasal thallium-201 transport to the olfactory bulb than in the patients with low thallium-201 transport to the olfactory bulb. The prognostic value of nasal thallium-201 transport to the olfactory bulb was also significant in the multivariable analysis. Conclusions: Assessment of olfactory nerve damage with nasal thallium-201 transport to olfactory bulb was useful to predict the prognosis of olfactory-impaired patients. Acknowledgements: This research was supported in part by a Grant-in-Aid for Scientic Research from the Ministry of Education, Science and Culture of Japan (C25462670 to H.S.). FCOI Disclosure: None.

#221 **POSTER SESSION V**

Normal Flavor Despite Orthonasal and Retronasal Anosmia; A Case Report

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Title: Normal Flavor Despite Orthonasal and Retronasal Anosmia; A Case Report Objective: To report a patient with anosmia without a loss in perceived flavor. Background: Since olfaction contributes up to 80% of flavor, smell loss should reduce flavor. Methods: Case report: A 20 year old female was nasute until one year prior to presentation when she developed a mild cold and gradual loss of smell over a few days, without improvement since then, despite a three week trial of prednisone. She can detect no odors including irritant vapors, cleaning fluids, and ammonia. She denies olfactory windows, dysosmias, phantosmia, and pallinosmia. She feels her sense of taste is

normal and can easily detect subtle flavors. This has not affected her preferred foods, appetite, or weight. Results: General physical and neurological exams; normal, Neuropsychiatric exam - normal: Semantic fluency test: 37. CT of the paranasal sinuses: normal. ENT exam: mild left deviation of the superior septum. Olfactory tests - anosmia for: UPSIT: 9/40, Quick Smell Identification Test 0/3, Pocket Smell Identification Test; 0/3, Brief Smell Identification Test; 6/12, Olfactometer Threshold; 1.00 right, 1.00 left, Alcohol Sniff Test 0 cm, Odor Memory Test; 1 at 10 sec, 1 at 30 sec, 0 at 60 sec, total: 2/12. Retronasal Smell Test: Jelly Bean Difference Test: 0 (absent retronasal smell) Taste tests – Normogeusic to PROP DISC (9/10), HCL, and PTC; mild hypogeusia to NaCl, sucrose, and urea. Conclusion: Possible mechanisms for her findings include: odor taste linkage with expectation effect, cortical plasticity with secondary compensation, multisensory input induced illusion (law of closure), and narcissistic loss with unconscious denial. Further investigation into the dissociation between anosmia and normal flavor is warranted. Acknowledgements: FCOI: Dr. Alan Hirsch has ownership at the Smell and Taste Treatment and Research Foundation, and consults with multiple companies and health care facilities. Alexander Roussos: none Source of Funding: Smell and Taste Treatment and Research Foundation. FCOI Disclosure: None.

#222 **POSTER SESSION V**

Gravidity Responsive Phantosmia and Phantoguesia

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Objective: Resolution of phantoms with pregnancy has not previously been reported. Methods: Case study: 25 year old female complains of phantosmia and phantogeusia 2 months status post head trauma, over 5 years became constant and severe. The smell and the taste are similar in nature and parallel each other, are of rotten fruit, fecal, or burnt, and overcome any true odors or tastes. They are worse with spicy foods, mastication, odors, and valsava and are better with breath holding, nostril occlusion, snorting salt water, and exercise. She notes reduced ability to smell and taste, depression and lost 20 Ibs. No response to nasal sprays, antibiotics, antidepressants and anticonvulsants. During the 2nd and 3rd trimester the phantoms resolved with recrudescence 5 days postpartum. Exam: scalloped tongue. Olfactory Testing: Anosmia: Alcohol Sniff Test – 1 cm; Sniffin' Sticks Threshold (SST), Left (L)< 1, Right (R)< 1, dirhinis < 1. Hyposmia: Olfactometer: Threshold - L 6.5, R 3.5, Identification test (ID)- L 16, R 16, UPSIT - L 31, R 29; SST ID L 10, R 12; Odor Memory – 3 at 10, 3 at 30, 3 at 60 secs, total 9/12. Normosmia: Suprathreshold Amyl Acetate Intensity: Quick Smell Identification Test- 3/3; Pocket Smell Identification Test - 3/3; SST Discrimination; L11, R10; Sniff Magnitude: 6.68. Retronasal smell: Jelly Bean Difference - 0. Gustatory: Threshold: NaCl, HCl, and PTC- Normal; Sucrose and urea - hypogeusia; PROP Disc- Normal; Quadrants to NaCl, sucrose, citric acid, quinine hydrochloride – Normal; Electrogustometry: Tongue: Anterior –L 4, R 4, Posterior - R 24, L 18, Palate - L 10, R 20; Saxon test - 6: normal. Fungiform Papillae – R 8, L 12; Candida culture: normal; Saline in Moffat's position: Resolution for > 1 hr. Discussion: Gravidarium inhibition suggests possible hormonal treatment of phantoms. Acknowledgements: Smell and Taste Treatment and Research Foundation. FCOI Disclosure: FCOI: Authors; JSM- none, AKA- None, ARH- Dr.Alan hirsch has ownership in smell and taste research foundation and consults with multiple companies and healthcare facilities.

#223 **POSTER SESSION V**

Olfactory Reference Syndrome (ORS) with Mysophobia

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Introduction: ORS (olfactory monosymptomatic delusional disorder) has not heretofore been reported to occur with mysophobia. Methods: Case report: A 54 year old divorced African immigrant, for 10 years noted ambient malodors attach to her (fecal, urine, rotten food, body, and animal odor) and then emanate from her body. To try to rid herself of this, throughout the day she recurrently washes her clothes and hair, brushes her teeth, gargles with mouthwash and takes multi-hour showers. She avoids socialization, panicking if she must closely interact with others, describes how people back away from her because of her smell, but when confronted they deny she smells bad. This has been unresponsive to nasal cauterization, paroxetine and gabapentin. The malodors overcome any odor actually present, reducing her ability to smell. She admits depression, crying spells, sadness, trouble with memory and thinking, and decreased energy level and sex drive. She affirms worrying most of the time, compulsion of door knob checking and hand washing, and phobias to dirt, germs and rust. She washes her hands 50 to 100 times per day, and spends many hours in the bathroom washing herself. She cleans the bathroom multiple times a day. Results: Abnormalities on physical exam: General: scalloped tongue. Neuro: Mental Status Exam: Digits: 6 forwards, 3 backwards. Recent recall: 0/4 in 3 minutes, 1/4 with reinforcement. Cranial nerve III; IV; VI: bilateral reverse ptosis. Motor: drift testing: bilateral abductor digiti minimi sign. Gait: unstable tandem. Reflexes: absent both lower extremities. Chemosensory: olfactory testing for threshold and identification werenormal. Retronasal smell: Jelly Bean Difference: 0. Discussion: Along with social phobias, mysophobia should be queried in those with ORS. Acknowledgements: Smell & Taste Treatment and Research Foundation. FCOI Disclosure: ARH: has ownership in the Smell & Taste Treatment and Research Foundation and consults with multiple companies and health care facilities.

#224 POSTER SESSION V

Losing the Match: Chemosensation vs. Inflatable Sumo Wrestling Suits

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Introduction: The inflatable sumo wrestling costume has only been recently popularized in the US. Two cases of head injury induced chemosensory dysfunction with use of these suits are described. Case Study 1: 24 year old female while posing for a sumo wrestler station photograph, was knocked over backwards, striking her head on the floor with loss of conscious, sustaining a subarachnoid hemorrhage, basilar skull fracture, and a right frontal epidural hematoma. Within a month, everything possessed the same garbage-like smell and taste. Evaluation included negative ENT exam, sinus CT, and sinus MRI. Physical exam revealed hyperreflexia throughout, and bilateral Hoffman reflexes. Chemosensory: Olfactory testing including identification, threshold, and sniff magnitude testing demonstrated hyposmia. Taste Tests: Accusens T and Quadrant Test: Hypogeusia to HCL, decreased anteriorly sucrose and HCl. Piesesthesiometry, Electrogustometry, Fungiform papillae, and Candidiasis culture – normal. Case Study 2: 17 year old female wearing sumo wrestling costume was knocked off the mat, striking her head on the floor with loss of consciousness, with occipital skull fracture, bifrontal subdural hematoma, and bifrontal intracerebral hemorrhages. Three weeks later noted no smell or taste, followed one week later by everything smelling and tasting like fecal or rotten food. Abnormalites in neurologic exam: cranial nerve: III, IV, VI: left Lateral Rectus weakness, Motor: Drift Testing: left Abductor digiti minimi sign. Gait: Decreased left arm swing. Reflexes: hyporeflexia throughout. olfactory tests demonstrate anosmia. Taste tests suggest hypogeusia to NaCl and ageusia to sucrose. Decreased quinine posteriorly. Conclusion: Use of inflatable sumo wrestling suit includes risk of head trauma with chemosensory dysfunction. Acknowledgements: Smell & Taste Treatment and Research Foundation. FCOI Disclosure: ARH: has ownership in the Smell & Taste Treatment and Research Foundation and consults with multiple companies and health care facilities.

#225 POSTER SESSION V

Olfactory bulb volume predicts therapeutic outcome in major depression disorder

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The volume of the olfactory bulb is strongly reduced in patients with major depressive disorder. This group of patients also exhibits a markedly decreased olfactory function. It has been suggested, that olfactory input is important for maintaining balance in limbic neurocircuits. Aim of the study was to investigate whether a reduced olfactory bulb volume is associated with emotion regulation deficits and predicts the response to therapy in major depressive disorder. 24 inpatients (all women, 21-49 years, mean 37.75 years +/- 9.6 SD) with acute major depressive disorder were scanned and the olfactory bulb volume was compared between responders and non-responders to psychotherapy. Retest of olfactory bulb volume was performed about half a year after the end of therapy in nine of the patients. Therapeutic non-responders exhibited an olfactory bulb volume, that was 23% smaller on average compared to the responders ($p=0.0011$). Furthermore, olfactory bulb volume was significantly correlated to change of depression severity ($r=.46$, $p=0.024$). Volume of the olfactory bulb did not change in the course of therapy. The olfactory bulb volume may be a biological vulnerability factor for the occurrence and of maintenance of depression. Acknowledgements: This work was supported by a grant from the "Roland Ernst Stiftung" to Thomas Hummel and grant from the DFG (Deutsche Forschungsgemeinschaft) to Ilona Croy. FCOI Disclosure: None.

#226 POSTER SESSION V

Chronic Piscine Diet Induced Metallic Phantogeusia, Dysgeusia, & Hypogeusia

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Objective: Chemosensory dysfunction as the primary complaint after arsenic and mercury exposure through ingestion of seafood has not heretofore been described. Methods: Case Study: 54 year old male with hypothyroidism, for one year had adhered to a fish only diet. He presented with 2.5 months of yellow tongue, unrelenting metallic sensation on the posterior tongue (severity 3/10), and bad tasting excess saliva. Carrot soup with ginger intensifies the taste; blueberries and tongue scraping reduce it. All foods including water take on the same metallic taste. He denies any other chemosensory or nervous system dysfunction. Results: Abnormalities in Physical exam: General: 1 + bilateral pedal edema, scalloped tongue, decreased blink frequency. Neuro: Cranial nerves: IX, X: Uvula deviated to R. XII: tongue deviated to R on protrusion. Motor: Drift testing; L abductor digiti minimi sign and L cerebellar spooning. Gait: Decreased arm swing. Reflexes: 2+ upper extremities, 3+ lower extremities with pendular quadriceps femoris. Chemosensory Testing: Quick-Smell Identification Test: 2/3, Brief Smell Identification Test: 10/12. Retro-nasal: Jelly bean difference test: 5/9. Taste testing: Propylthiouracil Disc: 10/10: Normogeusia to salt, HCl, and PTC; Ageusia to sucrose and urea. Urine: inorganic arsenic: < 15mcg/L, Organic arsenic: 39 mcg/L, predominately arsenobetaine (found in seafood). Mercury: normal. Vanadium: normal Blood: Arsenic 21 ng/mL (Normal < 12) Mercury:

15 ng/mL (normal < 9). Vanadium: normal Discussion: The findings may be due to concentration of metals in saliva, which acts to disrupt taste transduction, reception, or central pathways. While arsenobetaine and organic mercury are considered relatively benign, these findings suggest further investigation for the effects on chemosensation. Acknowledgements: Smell and Taste Treatment and Research Foundation. FCOI Disclosure: Saul Bello Rojas: None Dr. Jerrold B. Leiken: None Dr. Alan R. Hirsch: He has ownership of The Smell and Taste Research and Treatment Foundation and consults with multiple companies and healthcare facilities.

#227 **POSTER SESSION V**

Chemosensory Dysfunction as an Enantiopathy for Gustatory Rhinitis

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Introduction: Resolution of gustatory rhinitis due to chemosensory loss has not been described. Methods: Case Report: An 82 yo F with a PH of hypothyroidism, Raynaud's syndrome, 3 nasal traumas and 5 rhinoplasties, describes a lifetime phenomenon whereby smelling or eating hot solid foods induces clear rhinorrhea. This does not occur with liquids or cold food and are unrelated to the food's hedonics or her hunger. This would only occurs at the start of the meal. About 1½ yrs before presentation she developed an URI, associated with unremitting loss of smell and taste and a chemical dysgeusia. Her taste is limited to salt, sugar, ginger and vinegar. She disaffirms olfactory windows, dysosmia, phantosmia, palinosmia and flavorful eructations. She no longer enjoys food, has lost 70 lbs. and admits to depression. No FH of chemosensory problems or gustatory rhinitis. Results: Abnormalities on PE: General: 2+ BLE. Neuro: CN: II: Pupils 1-2mm, optic atrophy OU; III, IV, VI: R Ptosis; VIII: Hearing intact to CALFRAST, Strong 2, absent to Ambassador Hear Pen AU; Gait: Unstable heel, toe, tandem. Cerebellar: BUE low amplitude high frequency tremor and finger to nose dysmetria; Sensory: Vibration Rydel-Seiffer: 4/8 BLE. Reflexes: 1+ BLE. Chemosensory Tests: Gustatory: PROP Disc: 10; Taste Quadrant: Normal to quinine and citric acid, decrease palate to NaCl and sucrose; Taste Threshold: Normogeusia: HCl, urea, PTC - Hypogeusia: NaCl and sucrose; Normal Trigeminal stimuli including ETOH and carbonated H_2O. Olfactory: BSIT 8/12, QSIT 1/3; ETOH Sniff: 3. Retronasal Smell: Jelly Bean Difference: 6. other: Fiberoptic Endoscopy: WNL; MRI of brain: Moderate atrophy. Discussion: Chemosensory loss inhibition of gustatory rhinitis casts doubt on a primary allergenic, immunologic or trigeminal mechanism of this disorder. Acknowledgements: Smell and Taste Treatment and Research Foundation. FCOI Disclosure: Dr. Alan Hirsch has ownership in The Smell and Taste Treatment and Research Foundation and consults with multiple companies and health care facilities.

#228 **POSTER SESSION V**

Nothing But Aftertaste

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Introduction: Perceptually correct aftertaste in the absence of taste is described. Method: Case Study: 55 year old male 2 years ago was exposed to Scott's Crabgrass Preventer herbicide and 1 week later lost taste and noted dysgeusia. For the last year, he has intermittent salty phantageusia. Six months ago, despite no taste on consumption, he noted aftertaste of the last food eaten hours earlier, the way it should taste, but of 50% intensity. He complain of mild dysphagia, halitosis, omeprazole responsive reflux. Since onset, he has lost 60 pounds, and admits to sadness and paranoid ideation. Results: Results: Exam: decreased blink frequency, bilateral palmar erythema. Motor: Right (R) pronator drift. Reflexes: absent. Chemosensory: Olfactory Threshold, Identification, and Sniff Magnitude Tests consistent with hyposmia. Jelly Bean Retronasal Smell: 2. Taste Threshold: normogeusia to sucrose, and ageusia to NaCl, HCl, urea, and PTC. Taste quadrant: weakness to all modalities. Prop disc: absent. Electrogustometry: palate: Left (L) – 24, Right (R) – 28, and L and R anterior and posterior tongue all > 34. Fungiform papillae 8 R, 10 L. Saxon test, Candidiasis culture, Piesesthesiometry, Beck Depression Inventory & Zung Anxiety Scale were all normal. Discussion: differential diagnosis includes: Zenker's diverticulum, esophageal dysmotility, GE reflux, Pendimethalin toxicity, prolonged chemosensory deprivation, autosuggestion, somatoform delusion, somatic representation of depression or psychosis. Patient's strong desire to taste the aftertaste may induce an illusion of taste due to autosuggestion. Prolonged chemosensory deprivation may have induced phantageusia and palinageusia. (chemosensory equivalent of Phantom Eye syndrome) Distortions in the aftertaste warrant further exploration and research. Acknowledgements: Konstantin Gaftanyuk: none Dr. Alan Hirsch has ownership in The Smell and Taste Treatment and Research Foundation and consults with multiple companies and health care facilities. FCOI Disclosure: Dr. Alan Hirsch has ownership in The Smell and Taste Treatment and Research Foundation and consults with multiple companies and health care facilities.

#229 POSTER SESSION V

Oral Sensory Pathology Moderates the Relationship Between Fungiform Papilla Density and Taste Intensity

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Data from multiple laboratories show that taste perception rises with fungiform papilla (FP) density, yet three recent studies report no such association (Fisher et al., 2013; Feeney & Hayes, 2014; Garneau et al., 2014). Nerve damage can alter taste sensation without affecting tongue anatomy, so reports that fail to assess it neglect a key source of oral sensory variation. To illustrate, the present study shows a relationship between FP density and taste intensity in individuals with healthy oral sensation, but not in those with damage. Participants (N=591) provided health information relevant to taste damage (i.e., otitis media, tonsillectomy, head trauma, taste phantoms), took a spatial taste test, and sampled a filter paper infused with 6-n-propylthiouracil (PROP, 1.6 mg). A global intensity scale (i.e., modified gLMS) was used for all ratings. FP density was measured in a circular area (6 mm diameter) adjacent to the tongue tip and midline. We found a significant correlation between FP density and the intensity of anterior taste cues and PROP paper, but only in participants without health conditions related to taste damage; those with taste-related pathology showed no such effect. We also computed a ratio of anterior to whole-mouth bitter taste for each subject, as oral sensory damage preferentially afflicts the chorda tympani (due to its meandering path) and bitter taste fibers (which are small and unmyelinated). Participants with ratios above the median (i.e., healthy anterior taste function) showed a significant correlation between FP density and anterior taste intensity, but those with ratios below the median (i.e., anterior taste loss) showed no such association. We conclude that oral sensory nerve damage can obscure the relationship between FP density and taste perception. Acknowledgements: NIH DC00283. FCOI Disclosure: None.

#230 POSTER SESSION V

Chemosensory Dysfunction as an Enantiopathy for Gustatory Rhinitis

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Introduction: Resolution of gustatory rhinitis due to chemosensory loss has not been described. Case Report: An 82 year old female with a past history of hypothyroidism, Raynaud's syndrome, 3 nasal traumas and 5 rhinoplasties, describes a lifetime phenomena whereby smelling or eating hot solid foods induces clear rhinorrhea. This does not occur with liquids or cold food and is unrelated to food hedonics or hunger. This would only occur at the start of the meal. About 1½ yrs before presentation she developed an upper respiratory infection, associated with unremitting loss of smell and taste and a chemical dysgeusia. She disaffirms olfactory windows, dysosmia, phantosmia, palinosmia and flavorful eructations. She no longer enjoys food, has lost 70 lbs. and admits to depression. No family history of chemosensory problems or gustatory rhinitis. Abnormalities on neurologic exam: Cranial nerve: II: bilateral optic atrophy; VIII: Hearing absent to high frequency; Gait: Unstable. Cerebellar: bilateral low amplitude high frequency tremor and finger to nose dysmetria; Sensory: decreased vibration in both legs. Reflexes: 1+ bilateral lower extremities. Results: Chemosensory Tests: Gustatory: PROP Disc: 10; Taste Quadrant: Normal to quinine and citric acid, decrease on palate to NaCl and sucrose; Taste Threshold: Normogeusia: HCl, urea, PTC - Hypogeusia: NaCl and sucrose; Normal Trigeminal stimuli including alcohol and carbonated H_2O. Olfactory: Brief Smell Identification Test 8/12, Quick Smell Identification Test 1/3; Alcohol Sniff Test: 3. Retronasal Smell: Jelly Bean Difference: 6. Other: Fiberoptic Endoscopy: normal; MRI of brain: Moderate atrophy. Discussion: Chemosensory loss inhibition of gustatory rhinitis casts doubt on a primary allergenic, immunologic or trigeminal mechanism of this disorder. Acknowledgements: Smell & Taste Treatment and Research Foundation. FCOI Disclosure: ARH: has ownership in the Smell & Taste Treatment and Research Foundation and consults with multiple companies and health care facilities.

#231 POSTER SESSION V

Prevalence and Factors Associated with Reported Taste Loss and Distortions (Dysgeusia) during the Past 12 Months in Adults Aged 40+ Years: The U.S. National Health and Nutrition Examination Survey (NHANES), 2011-2012

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Most studies of taste involve patients at specialty clinics. The NHANES 2011-2012 Chemosensory Component conducted interviews and exams on a nationally-representative sample of non-institutionalized U.S. adults. Two questions answered by 3,603 adults (mean [+SD] age=60+12) were: "Have you had a problem with your ability to taste?"; "Have you had a taste or other sensation in your mouth that does not go away?" The interview asked 12-month Hx of persistent head cold or flu, dry mouth, or nasal congestion; ever had tonsils/wisdom teeth removed, head injury/loss of consciousness, broken nose/facial injury, or 2+ sinus infections. Adults reported discussing taste problems with health providers and reduced quality of life (QoL) due to taste (or smell) problems. Odds ratios (OR) and 95% confidence intervals (CI) were calculated using multivariable

logistic regression models. For adults >40 years, 5.28% (7.5 million [M]) had a taste problem and 5.12% (7.3 M) a persistent taste (dysgeusia) in the past 12 months; overlap was 28% (n=2.1 M). Age-specific prevalence was 4.5%, 5.4%, and 7.0% for taste problems, 4.5%, 5.8%, and 6.0% for a persistent taste/dysgeusia, in adults 40-54, 55-69, and 70+ years, respectively. After multivariate adjustment, taste problems were associated with: wisdom teeth removal OR=1.7, CI: 1.1–2.8; dry mouth OR=2.0, CI: 1.4–2.8; broken nose/facial injury OR=2.2, CI 1.1–4.5; discussed with health provider OR=4.5, CI: 2.3–8.8; reduced QoL OR=10.1, CI 4.6–22.2. Associations for a persistent taste were: female OR=1.5, CI: 1.04–2.1; broken nose/facial injury OR=1.8, CI: 1.1–3.0; discussed with health provider OR=5.1, CI: 2.1–12.3; dry mouth OR=6.5, CI 3.7–11.4. Prevalence above 5%, increased healthcare burden, and reduced QoL reveal unmet needs for prevention/treatment of taste problems. Acknowledgements: Support for the NHANES 2011-2012 Chemosensory Component was provided by NIDCD/NIH research contract funds via Interagency Agreement with the National Center for Health Statistics (NCHS), Centers for Disease Control and Prevention (CDC). FCOI Disclosure: None.

#232 **POSTER SESSION V**

Opiorphin Levels in Fluids of Burning Mouth Syndrome Patients. A Case Control Study

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Burning mouth syndrome (BMS) is a poorly understood condition affecting mainly women at menopause with anxiodepressive background. Oral pain is the main symptom although taste and salivary disturbances are often experienced, suggesting involvement of local factors. Opiorphin mainly produced by submandibulary glands is a natural inhibitor of the enkephalin inactivating ectopeptidases that has demonstrated analgesic properties. The main objective of this study was to test the hypothesis of a decrease of the opiorphin levels in the saliva of BMS patients compared to control subjects. The main judgment criteria was the level of salivary opiorphin in ng/mL. Secondary judgement criteria were blood and urinary opiorphin levels, pain assessed on visual analogic scale (VAS); anxiety/depression assessed with HAD scale and salivary flow in basal and stimulated conditions (mL/min). 21 BMS patients and 21 matched (sex age, hormonal status) controls subjects were included in a simple blinded case control study conducted at the GHPS hospital in Paris between 2011 and 2012. Results are expressed as mean values ± SD and compared using the Wilcoxon Signed Rank test. Score of Pain (VAS) was 32.2 ±19.5 for patients. Anxiety and depression scores were significantly higher in patients vs controls (p=0.0034 and p=0.0002) with an OR of 2.15 correlating anxiety and BMS. Opiorphin levels in patients vs controls were: stimulated saliva: 28.76 ±25.33 vs 31.09± 29.06 (NS, p=0.5034); basal saliva: 37.8±42.5 vs 26.55±17.07 (NS, p=0.7255); blood: 4.62±5.42 vs 1.99±1.37 (p=0.046 *); urine 68.47±259.82 vs 8.93±6.18 (NS 0.1029). In conclusion, opiorphin does not seem to play a local role in the etiopathogeny of BMS but was found in "significant" higher quantity in the blood of BMS patients. Acknowledgements: OPIODYN, PHRC P081106. FCOI Disclosure: None.

#233 **POSTER SESSION V**

Characterization of nestin-expressing cells in the circumvallate papilla of mice

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Nestin is a class VI intermediate filament protein, which was originally described as a neuronal stem cell marker during central nervous system development. Nestin is also expressed in many non-neuronal progenitor cells of adult tissues. Recently, nestin-expressing mesenchymal cells were identified around peripheral sensory nerves within the core of fungiform taste papillae (FFP) (Mii. et al., 2014; J. Cell. Biocehm. 115:1070). However, nestin expression in the posterior circumvallate taste papilla (CVP) has not been characterized. Here we use transgenic mice in which expression of GFP is driven by the promoter and the 2nd intron of the nestin gene (Mignone et al., 2004; J. Comp. Neurol. 469:311), to carefully define nestin-GFP+ cell populations in the CVP. As in the FFP, nestin-GFP+ cells are common in the subepithelial mesenchyme of the CVP. Additionally, nestin-GFP+ cells are abundant in the CVP epithelium, both perigemally and intragemally. Nestin-GFP+ cells in the CVP epithelium are cytokeratin (K)14+, and are actively proliferating (Ki67+ and rapidly incorporate EdU), indicating that nestin-GFP is expressed by taste bud progenitor cells (Okubo et al., 2009 Stem Cell 27:442). In addition, CVP taste buds house nestin-GFP+ cells. Using immunostaining for taste cell type-specific markers of tissue sections and dispersed taste bud preparations, we show that nestin-GFP+ taste cells are exclusively Type I glial like cells and not Type II or III taste cells. Finally, using EdU birthdating, we find that newly formed cells inside CVP taste buds are also nestin-GFP+. These results suggest the hypothesis that nestin-GFP+ cells play a role in taste receptor cell renewal and cell fate determination. Acknowledgements: NIH Grants: DC012383, DC012675. FCOI Disclosure: None.

#234 POSTER SESSION V

Characterization of Keratin 14 Progenitors that Give Rise to Taste Cells

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Our previous studies have shown that Glial-like (Type I) and Receptor (Type II) cells of the taste bud are replaced by the progeny of basal Keratin 14+ cells. The dynamics of this replacement suggested that these cell types come from separate pools of K14 progenitors. To further examine these separate lineages, we used 4-color fluorescent reporter "Confetti" mice, sparsely induced via tamoxifen (tam), to generate well-separated clones. In 46 taste buds (10 mice, 30-45 days post-tam), we found that cells of a given clone differentiated into only one taste cell type: either Type I or Type II, confirming our earlier observation that such progenitors are lineage restricted. We further asked how long K14 progenitors are able to produce keratinocytes and taste cells by examining K14-Cre; YFP mice for months after induction with a high dose of tam. In the non-taste epithelium, the pattern of YFP expression across 9 months post-tam suggests that K14 is expressed both in early (stem-like) progenitors and late (amplifying) progenitors. 30 days post-tam, >70% of taste buds contained YFP+ cells. A minority of buds included YFP+ cells at 270 days, and surprisingly, each bud contained as many as 17 YFP+ cells. This suggests that as with keratinocytes, long-lasting YFP label may come from induction of K14+ early progenitors. Recently, in non-taste epithelium, Bmi1 was shown to mark slow-cycling stem cells which also express K14. Using Bmi1-Cre; YFP mice, we found no clonal expansion of YFP+ cells in the taste bud up to 30 days post-tam, suggesting that Bmi1 is not a marker of late progenitors for taste cells. In contrast, 100 days post-tam, occasional taste buds were detected that contained many YFP+ cells. This suggests the possibility of common features between stem cells for keratinocytes and taste buds. Acknowledgements: NIH grant R01 DC006308 to NC. FCOI Disclosure: None.

#235 POSTER SESSION V

Continuous Requirement for GLI-mediated Signal Transduction in Taste Organ Maintenance

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The secreted Hedgehog (HH) ligand, Sonic hedgehog (SHH), regulates fungiform papilla and taste bud maintenance, but the downstream transcriptional effectors of HH signaling in taste organ maintenance are largely unexplored. Previous work demonstrated that GLI1 and GLI2 are expressed in HH-responsive cells in the lingual epithelium and underlying connective tissue. To disrupt GLI function, we used mice with a doxycycline-inducible *Gli2deltaC4* transgene, which dominantly represses GLI target gene expression, in K5 expressing cells (designated K5-rtTA) or K5 expressing cells and their progeny (designated K5-Cre). We also used a $Gli1^{lacZ}$ allele to examine HH pathway activity. *Gli2deltaC4* expression was induced in adult mice for 1-36 days. With changes detected as early as 5 days, typical papillae and intact taste buds were progressively reduced (~30% of control, K5-rtTA) or eliminated (K5-Cre), and HH-responding cells were no longer detected in the epithelium with transgene activation. Remaining papillae had thick keratinized cell layers on apical spine-like protrusions. Although Ki67+ and p63+ cells remained in papilla and lingual epithelia, there was an increase in TUNEL+ cells in abnormal fungiform and in filiform papillae. In taste bud remnants a few K8+ cells were observed and large, ovoid SHH-expressing cells were detected at the base of these remnants. Within the stroma of dysmorphic papillae there was robust innervation that penetrated the apical epithelium, shown with neurofilament and P2X3 antibodies. Papilla and taste bud disruption or loss also was observed in *Gli2* conditional knockout mice after 2-3 weeks of gene deletion in lingual epithelium. Together these data strongly suggest that continuous HH/GLI signaling is required for maintaining both fungiform papilla and taste bud integrity. Acknowledgements: NIH Grants NIDCD DC000456 (CMM) NIAMS AR045973 (AAD) U-M Organogenesis Project Grant (BLA, AAD, CMM). FCOI Disclosure: None.

#236 POSTER SESSION V

An analysis of sour and salt responses in clonal cell lines derived from murine taste buds

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We have recently established clonal cell lines (TBD cells) from taste buds of a p53-deficient mouse. Immunocytochemical analysis demonstrated that the cell lines are positive to NTPDase2 as Type I cell marker, gustducin and T1R3 as Type II cell markers or NCAM as Type III marker. In addition, it was also demonstrated by RT-PCR that TB cell lines express several receptor molecules for tastants including sour, salty, sweet and bitter tastes such as T1Rs, T2Rs and PKD2L1, transduction-related molecules such as PLCß2, SNAP25, HCN4, ion channels including epithelial Na^{2+} channel (ENAC), voltage-dependent Na^{2+} channel (VNaC) and voltage-dependent Ca^{2+} channel (VCaC). However, it was revealed that TBD cells has responded only to sour and salt tastes, which are thought to be mediated by ionotropic type of transduction mechanism,

but not to sweet, bitter and umami taste, which are mediated by metabotropic transduction mechanism using Ca^{2+}-imaging. A transient increase of $[Ca^{2+}]i$ was elicited by sour stimulation in TBD cells and the magnitude of responses to organic acid were much larger than that of inorganic acid over the range of pH tested as reported in vivo. Removal of extracellular Ca^{2+} and perfusion with a nonspecific VCaC blocker, verapamil suppressed the citric acid response of TBD cells, indicating that Ca^{2+}-responses to sour stimulation of TBD cells are mediated by influx of extracellular Ca^{2+} through VCaCs as shown in previous studies. Stimulation of 135 mM NaCl elicited $[Ca^{2+}]i$ elevation in 95.5 % of TBD cells tested but only 7.9% of TBD cells responded to 90 mM KCl, These results suggest that TBD cells is useful tool as *in vitro* model for study of sour and salty transduction. Acknowledgements: Supported in part by the Research funds from Japan Women's University Bio-imaging Center and The Salt Science Research Foundation 1442.
FCOI Disclosure: None.

#237 **POSTER SESSION V**

Neural Crest Derived Cells are Distributed in Mature Taste Buds in Adult Mice

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We have previously demonstrated that P0-Cre labeled neural crest (NC) derived cells are abundantly distributed in early taste buds in newborn and 2-4 week old mice. The objective of this study was to evaluate the contribution of NC derived cells to taste buds in adult mice. Using two independent mouse models for NC derivation assay, P0-Cre and Dermo1-Cre, we examined the distributions of NC derived cells in mature taste buds and types I and II taste cells in 8- and 16-week-old mice. We found that both P0-Cre and Dermo1-Cre labeled cells were abundantly distributed in K8-positive taste buds resided within all three types of taste papillae (fungiform, circumvallate and foliate) and soft palate, in addition to the extensive distribution in the mesenchyme of the lamina propria. Over 80% of fungiform taste buds were labeled. P0-Cre labeled NC derived cells are comprised of at least 20% of mature fungiform taste bud cells at each stage. With the exception of taste buds, P0-Cre and ¬Dermo1-Cre brightly labeled cells were not observed in the papilla epithelial wall or inter-papilla tongue epithelium. A significant population of the P0-Cre labeled mature taste buds is co-localized with type I (NTPDase II) or type II (alpha-Gustducin and PLC-beta2) taste cell markers, respectively. Taken together, our findings suggest a significant population of mature taste bud cells have the same origin as the underlying mesenchymal cells that are derived from NC. Distribution of NC derived cells in early immature and adult mature taste buds indicate the contribution of NC derived cells to both the formation and maintenance of taste buds. Acknowledgements: NIDCD, NIH Grant R01DC012308 to HXL.
FCOI Disclosure: None.

#238 **POSTER SESSION VI**

An Olfactory Cilia Pattern in the Mammalian Nose Ensures High Sensitivity to Odors

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In sensory organs such as the retina and skin, specialized receptors are strategically arranged to enhance detection sensitivity and acuity. However, it remains unclear whether a similar scheme is used by the olfactory system to optimize odor detection. Here, we report a novel spatial organization in the mouse nose using immunohistochemistry, computational modeling, and electrophysiological analyses. Olfactory sensory neurons (OSNs) situated in highly stimulated regions, including the anterior septum and dorsal recess, have much longer cilia and are more sensitive to odorants than those in the weakly stimulated posterior septum. Surprisingly, sensory experience and neuronal activity are not required for the formation and maintenance of the cilia length pattern, which may be determined by intrinsic mechanisms. Furthermore, genetic ablation of type III adenylyl cyclase (ACIII), a key olfactory signaling molecule and ubiquitous marker for primary cilia, significantly alters the cilia pattern. These findings reveal a previously unrecognized mechanism to ensure high sensitivity to odors and offer new insights into the structural and functional modulation of sensory cilia. Acknowledgements: NIDCD, NIH (R01DC011554 and R01DC006213 to M.M., R03DC008187 to K.Z., and R21DC013177 to L.H). FCOI Disclosure: None.

#239 POSTER SESSION VI

Arl13b Localization and Function in Cilia in the Olfactory Epithelium

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Cilia are evolutionarily conserved microtubule-based organelles found in most organ systems, often regulating cellular signaling and sensory perception. In primary cilia, the small GTPase Arl13b (ADP-ribosylation factor-like protein 13b) is an important regulator of ciliogenesis, sensory signaling, and trafficking of proteins in the cilium. Mutations in Arl13b disrupt cilia function and underlie the ciliopathy, Joubert Syndrome. While cilia are crucial in regulating olfactory function, the role of Arl13b in olfactory cilia function is poorly understood. Work from our lab has shown antibodies to Arl13b label primary cilia on horizontal basal cells (HBCs) and the apical surface of the olfactory epithelium. When ectopically expressed in olfactory sensory neurons (OSNs), Arl13b is capable of bi-directional movement via intraflagellar transport. Therefore, our objective is to elucidate the function of Arl13b in cilia from both OSNs and HBCs. Using a transgenic mouse model expressing Arl13b:eGFP we analyzed localization in the OE. Live *en face* confocal imaging as well as imaging in coronal sections of fixed tissue in the OE of Arl13b:eGFP mice revealed intense GFP signal in a subset of OSN cilia, consistent with antibody labeling of Arl13b. In contrast, cilia on HBCs were clearly and consistently labeled in coronal sections of OE. To analyze a potential role for Arl13b in olfactory cilia, Arl13b floxed mice were crossed with OMP:Cre mice to generate olfactory specific Arl13b null mice. Surprisingly, Arl13b immunostaining was observed in a subset of OSN cilia in both wildtype and null mice. These data indicate that Arl13b is absent in OMP positive neurons, however, may be involved in ciliogenesis in immature OSNs or in signaling in non-canonical olfactory neurons. Acknowledgements: NIH R01 DC009606-06 (JRM), NIH K99 DC013555-01 (JCM), NIH F31 DC013496-02 (AMJ) . FCOI Disclosure: None.

#240 POSTER SESSION VI

Zinc Sulfate Affects Ciliated Olfactory Sensory Neurons More Than Microvillous Olfactory Sensory Neurons in the Adult Zebrafish

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Our aim was to examine the effects of zinc sulfate on olfactory sensory neuron (OSN) subtypes in the adult zebrafish (*Danio rerio*). Fish were anesthetized and 1M $ZnSO_4$ was infused into the right olfactory organ, while the left served as an internal control. Hematoxylin and eosin stain showed severe morphological disruptions 2 d after exposure: the olfactory organ was highly inflamed and lamellae appeared fused. After 5 d, inflammation had subsided but the olfactory epithelium appeared thinner than controls. By 10 d, the olfactory organ appeared recovered. Optical density was used to quantify anti-calretinin labeling of mature OSNs. There was a significant decrease in the treated side compared to control side 2 d after exposure. Labeling was not different from control at 10 d. Scanning electron microscopy was used to examine the ultrastructure of the olfactory organ. The surface of unlesioned organs appeared densely packed with ciliated OSNs and longer non-sensory cilia. At 2 d, the organ appeared absent of ciliated OSNs though non-sensory cilia were still present. At 5 d, areas of ciliated OSNs were observed, and at 10 d the sensory area of the organ surface resembled controls. Olfactory-mediated behavior was assayed to determine function of the lesioned organs. Control fish and treatment groups were exposed to an amino acid or bile salt mixture, and the number of turns fish made pre- and post-odor exposure was counted. At 2 d, fish could not detect either odor mixture. Given 10 d to recover, the ability to perceive amino acids was regained, but it was not until 14 d that the ability to detect bile salts recovered. Thus, structure of the olfactory organ returns prior to function, and microvillous OSNs recover before ciliated OSNs showing differential effects of this chemical on neuron subtypes. Acknowledgements: Supported by NIH-NIDCD 011137 (CBJ), NSF-REU DBI-1062883 to WMU (JTH), WMU College of Arts & Sciences (JTH), and WMU OVPR (JTH).
FCOI Disclosure: None.

#241 POSTER SESSION VI

Primary Cilia on Olfactory Horizontal Basal Cells Regulate OE Regeneration

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The olfactory epithelium (OE) is one of the few tissues to undergo constitutive neurogenesis throughout the lifespan of an organism. It is comprised of various cell types including olfactory sensory neurons (OSNs), which are readily replaced by two populations of basal stem cells, frequently dividing globose basal cells (GBCs) and quiescent horizontal basal cells (HBCs). The precise mechanisms regulating OE regeneration are unclear. Here, we show, using immunohistochemical (IHC) analysis with cell specific markers, that HBCs in the mouse OE possess primary cilia. Due to the growing evidence that cilia play a role in stem cell function, we hypothesized that cilia on HBCs regulate proliferation and/or differentiation. To test this hypothesis, we generated an inducible, cell-type specific knockout mouse (*K5rtTA;tetOCre;Ift88$^{fl/fl}$*) that upon administration of doxycycline (dox) shows a significant loss of HBC cilia. Initially, we tested if the loss of cilia affected regeneration of the OE following insult. At 3 weeks post-recovery, there was a significant reduction (~53%) in the number

of mature OSNs in conditional null mice that was more pronounced (~75%) by 8 weeks of recovery. These data indicate that loss of cilia affects OE regeneration after insult. We then tested if the loss of cilia affected OE neurogenesis during development. IHC analysis of the OE revealed a region-specific reduction (~38%) of OSNs in anterodorsal regions of the conditional null mouse. Together, these data suggest that cilia may contribute to both HBC neurogenesis and regeneration in the OE, which should be considered in assessing olfactory phenotypes in ciliopathy patients. Acknowledgements: F31DC013496 to AMJ R01DC009606 to JRM.
FCOI Disclosure: None.

#242 **POSTER SESSION VI**

Perinatal and Adult-Born Granule Cell Connectivity in the Mouse Olfactory Bulb

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In the olfactory bulb (OB) of mammals, two different types of principal cells exist, mitral and tufted cells (MCs and TCs). MCs and TCs are functionally distinct and process different aspects of olfactory information, forming two different sub-circuits. Their activity is modulated by inhibitory GABAergic interneurons, including granule cells (GCs), which are produced from birth to adulthood. However, whether the time of birth of these interneurons contributes differently to the inhibition of MC and TC output neurons is unknown. The aim of this work is to investigate whether adult-born and perinatal-born GCs have different functions in M/TC sub-circuits. Injecting adeno-associated viruses (AAV) encoding different fluorescent proteins (GFP or RFP) at different time points in the same mouse (postnatal day 3, p3 and p40) we are able to selectively label adult-born or perinatal born GCs. Morphological analysis confirmed that adult-born GCs are located mainly in the inner GC layer, whereas p3 GCs are located mainly in the external part of the GC layer. Next, to investigate the functional connectivity between M/TCs and adult-born and perinatal born GCs we injected Pcdh21: :Cre transgenic mice with AAV vectors encoding for genetic encoding calcium indicators (GECIs) at p3 and p40, and with AAV encoding for ChETA. In these mice we photo-stimulate MCs-TCs and record calcium activity in GCs born at different time points. Lastly, we are in the process of collecting large 3D electron microscopy volumes from the same tissue to explore the connectivity amongst these and additional neuron types. These experiments will help us to investigate the specificity of connectivity between MCs, TCs and adult-born and perinatal born GCs, and therefore they help us to better understand the role of adult neurogenesis. Acknowledgements: Supported by intramural NINDS research program. FCOI Disclosure: None.

#243 **POSTER SESSION VI**

The Amphibian Olfactory System as a Model to Study Axonal Growth and Synaptogenesis In Vivo

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The olfactory system has the extraordinary capacity to generate new neurons throughout the lifetime of an organism. Neuronal stem cells in the epithelia of the olfactory organ continuously give rise to new sensory neurons that extend their axons into the olfactory bulb, where they face the challenge to integrate into existing circuitry. Because of this particular feature, the olfactory system represents a unique opportunity to monitor axonal wiring and guidance, and to investigate synapse formation. Here we developed a procedure for *in vivo* labeling of sensory neurons and subsequent visualization of axons in the olfactory system of larvae of the amphibian *Xenopus laevis*. We adopted the *in vivo* electroporation technique to stain sensory neurons in the olfactory organ. This technique is optimal for delivering fluorophore-coupled dextrans or other macromolecules into living cells. Stained sensory neurons and their axonal processes were then monitored in the living animal either using confocal laser-scanning or multiphoton microscopy. By reducing the number of labeled cells to few or single cells per animal, we were able to track single axons into the olfactory bulb and to monitor their morphological changes over weeks by conducting series of *in vivo* time lapse imaging experiments. While the described technique exemplifies the labeling and monitoring of sensory neurons of the olfactory system, it can also be adopted to other cell types within the olfactory and other systems. Our next step will be to study odorant responses of identified axon terminals of sensory neurons using functional calcium imaging in the *in vivo* system. Acknowledgements: Supported by Cluster of Excellence and DFG Research Center Nanoscale Microscopy and Molecular Physiology of the Brain (CNMPB) and DFG Schwerpunktprogramm 1392. FCOI Disclosure: None.

#244 **POSTER SESSION VI**

The Developmental and Physiological Properties of a Novel Flight-to-Olfactory Corollary Discharge Neuron

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Corollary discharge (CD) circuits provide information from motor to sensory centers to filter/modify sensory responses caused by an animal's own behavior (reafference). Active odor sampling (sniffing or wing beating) are behaviors that cause sensory reafference in the absence of odor but little is known about how these CD circuits modify olfactory responses. The goal of this study was to characterize the development and physiological activity of a novel CD circuit, composed of

two mesothoracic to deutocerebral histamine (MDH) neurons, which project from the mesothoracic ganglion (MTG), where flight motor patterns are generated, to the antennal lobe (AL) in *Manduca sexta*. Using immunolabeling, we determined that MDH cells appeared nearly fully developed in preflight larval stages. However, custom affinity purified HA_B receptor antibodies revealed that cells present in the adult AL that receive the histamine (HA) signal were either not present or not expressing HA_B receptors suggesting no functionality during larval development. Next, we ablated MDH axons 6 days prior to HA immunolabeling. Results of this experiment completely eliminated AL HA immunoreactivity indicating that MDH cells provide the only source of AL HA. Finally using intracellular techniques, we determined that MDH neurons are spontaneously active, tonically spiking (~20-30Hz) and change their behavior during fictive flight. Cell identity was confirmed by comparing dye fill and HA immunolabeling. These results indicate that MDH circuit development is finalized as moths emerge into flying adults and cells within the AL begin to express HA_B receptors at this time, suggesting MDH likely modulates olfactory processing during flight. Acknowledgements: RDC009417. FCOI Disclosure: None.

#245 **POSTER SESSION VI**

HCN Channels Mediate Rhythmic Activity in Arthropod Olfactory Receptor Neurons

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Some primary olfactory receptor neurons (ORNs) from animals as phylogenetically diverse as arthropods, amphibians, and mammals are spontaneously rhythmically active. Brief intermittent odor stimuli entrain the inherent rhythm of these 'bursting' ORNs (bORNs), in contrast to evoking the more traditional phaso-tonic responses of canonical ORNs. As a first step towards understanding the mechanism underlying bORN rhythmicity, we explored the potential role of hyperpolarization-activated (HCN) channels, PAIH channels, in burst generation in lobster bORNs. Using electrophysiological and calcium imaging, we characterized the biophysical and pharmacological properties of HCN channels expressed by bORNs and demonstrated that pharmacological treatment targeting HCN channels disrupted bursting. These results suggest that HCN channels contribute to burst generation in lobster bORNs. In support of that conclusion, immunohistochemistry and in situ hybridization localize PAIH expression to bORNs. In addition we identified two splice variants of PAIH that potentially contribute to the bursting activity of lobster bORNs. By functionally characterizing individual ORNs in conjunction with single cell RT-PCR we are testing whether PAIH alternative splicing could qualitatively and/or quantitatively determine the specific functional properties of lobster bORNs. Acknowledgements: Supported by the National Institute on Deafness and Other Communication Disorders through award (DC011859). FCOI Disclosure: None.

#246 **POSTER SESSION VI**

Identification of key residues involved in activation of G-protein Coupled Odorant Receptors

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The complexity of the odor chemical space and the large number of ORs associated to their combinatorial activation make understanding odor coding an enormous challenge. More specifically, being able to predict the behavior of an olfactory receptor in front of an agonist, an antagonist or a non-agonist remains to be done. Using a joint approach combining molecular modeling and experimental data on the mouse OR 256-3, we have built a model that can capture the active or inactive state of these proteins. By combining site-directed mutagenesis, heterologous expression, and atomic-level model that samples an active state in constitutively active mutants, we show that: i) some residues control the accessibility to the binding cavity ii) the toggle-switch for sensing odorants is an aromatic residue in the 'FYGT' motif in TM6 iii) the ionic-lock is made-up between the 'DRY' motif in TM3 and a positively charged 'R/K' residue in TM6. Such powerful approaches will help unravel odor-coding in the nervous system and facilitate the understanding of general rules of odor-induced activation of the olfactory neurons. Acknowledgements: This work was supported by grants from the National Institute on Deafness and Other Communication Disorders, National Institute of Health (DC011554 and DC006213 to M.M., and DC005782 and DC012095 to H.M.), APEX Region PACA to J. G. (OLFACTOME) and from the Foundation Roudnitska under the aegis of Fondation de France to C.A.D.M. C.A.D.M. also thanks GIRACT for a PhD bursary. FCOI Disclosure: None.

#247 **POSTER SESSION VI**

Utilization of a Confetti Cre Reporter System to Analyze Olfactory Neurogenesis

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Previously, we reported that c-Kit+ progenitor cells produce olfactory receptor neurons and Bowman's glands. Whether c-Kit+ cells are lineage committed or multipotential, and if they function as immediate precursors, amplifying progenitors, and/or reserve cells, has not been determined. Accordingly, we utilized a multicolor inducible reporter approach to address these questions. Using c-$Kit^{CreERT2/+}$;R26R-confetti mice, low-dose tamoxifen induction permitted the visualization of sparse,

non-overlapping, single color cell clusters by confocal microscopy. Several new findings emerged. Interestingly, c-Kit+ cells occasionally produced neuronal clones comprised of >10 cells, indicating that some c-Kit+ cells function as either amplifying progenitors or more upstream precursor cells, rather than undergoing only a terminal mitosis. Single dose tamoxifen treatment of adults at varying times after methimazole-induced lesion revealed that c-Kit+ activity is most robust at 7 days post-injury. Also, we found that microvillar cells, an important population known to regulate basal cell proliferation, arise from c-Kit+ progenitors. Microvillar labeling was confirmed by morphology and cell type-specific marker expression. The majority of clusters were comprised by one cell type, indicating that most c-Kit+ progenitors behave as lineage-committed cells. In addition, Bowman's glands appeared to arise as clonal units. Finally, quantification of label from mice receiving a single tamoxifen pulse followed by long survival times suggests that some c-Kit+ cells yield progeny cells that continue to produce neurons over a long time course. In summary, application of the multicolor Cre fate mapping technique provides novel information regarding olfactory progenitor cell activity.
Acknowledgements: Supported by NIH DC013556 (BJG).
FCOI Disclosure: None.

#248 **POSTER SESSION VI**

Receptive range analysis of a mouse odorant receptor subfamily

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The recognition of odorant molecules by odorant receptors (ORs) is a critical early step in olfaction. Mammals deploy a large array of ORs, a family of G-protein coupled receptors located on the cilia of olfactory sensory neurons, to detect and distinguish a vast number of odorant molecules. ORs vary widely in the type of odorant structures recognized and in the breadth of molecular receptive range (MRR), with some ORs recognizing a small group of closely related molecules and other ORs recognizing a wide range of structures. While closely related ORs have been shown to have similar MRRs, the functional relationships among less closely related ORs are unclear. We screened a small group of mouse ORs (MORs) with a diverse panel of 54 odorants to identify a new odorant-OR pairing. MORs were expressed in *Xenopus* oocytes, together with G olf and the human cystic fibrosis transmembrane regulator, to allow assay of odorant responses by two-electrode voltage clamp electrophysiology. We found that MOR263-3 responded to unsaturated aldehydes and ketones. We then extensively screened MOR263-3 and a series of additional MORs related to MOR263-3 in various ways. MORs related by phylogenetic analysis (MOR263-2, MOR263-9 and MOR263-10) had MRRs that overlapped with the MRR of MOR263-3, even with amino acid identity as low as 48%. MOR171-17, predicted to be functionally related to MOR263-3 by an alternative organizational scheme, but with only 39% amino acid identity, responded to an odorant ((-)-fenchone) that was distinct from the odorants that activated MOR263 subfamily members. Our results support the use of phylogenetic analysis to predict functional relationships among ORs with relatively low amino acid identity.
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#249 **POSTER SESSION VI**

The Anatomical Basis for Modulatory Convergence in the Antennal Lobe of Manduca sexta

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The importance of a sensory stimulus changes based on the physiological state of an animal. Sensory systems must therefore change how they respond to input based on context. This is often achieved in each network through the release of neuromodulators by widely projecting neurons. Neuromodulators alter network function by changing the biophysical properties of individual neurons and the synaptic efficacy with which individual neurons in the network communicate. However, the mechanisms by which sensory networks integrate multiple modulatory inputs is not well understood. Here, we characterized the relative glomerular distribution of two extrinsic neuromodulators, serotonin (5-HT) and dopamine (DA) in the antennal lobe (AL) of the moth *Manduca sexta*. Using immunocytochemistry and mass dye fills, we characterized the innervation patterns of both neuromodulators relative to each other, olfactory receptor neurons (ORNs), local interneurons (LNs) and projection neurons (PNs). We found that 5-HT and DA neurons innervate overlapping, yet distinct functional regions within glomeruli. We observed nearly complete overlap of 5-HT-ir with PNs and little with ORNs, suggesting that 5-HT modulates PNs and LNs directly but not ORNs. DA-ir overlapped with PNs, LNs and ORNs suggesting that dopamine has the potential to modulate all three cell types. Furthermore, the branching density of each neuromodulator in each ordinary glomerulus differed. 5-HT exhibited more proximal and dense arborizations, while DA-ir processes extended distally and were sparser. Our results suggest that 5-HT and DA target functionally distinct glomerular regions, yet still likely target partially overlapping set of neurons.
Acknowledgements: Start-up funds from WVU ECAS to AMD.
FCOI Disclosure: None.

#250 POSTER SESSION VI

Metabolites as preferred ligands for the human olfactory receptors outside "the olfactory box"

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Introduction: The existence of ORs outside the olfactory epithelium has been first documented in the mammalian germ cells. The authors suggested their possible role in chemotaxis during fertilization. Further studies led to the identification of many different ORs expressed in non-olfactory tissues. What if any is the functional role of these ORs? Our hypothesis is that these ORs can be activated by various metabolites and thus have an active role in various physiological and patho-physiological conditions. In order to unravel their function, we are going to identify their biologically relevant ligands. Methodology: We selected following groups of human ORs: 1. Evolutionary the most conserved (OR51E1, OR51E2 and OR6B1). 2. The most abundant and broadly expressed (OR51E1, OR51E2, OR2A4 and OR2W3). 3. The most highly expressed (OR2W3-thyroid, OR51E2-prostate and OR4N4-testis). We are using in silico approach to make homology models of ORs. These models are screened against a human metabolome library. Top candidate ligands will be validated in in vitro expression system using a two-electrode voltage clamp electrophysiology. Each receptor will be expressed in X. oocytes and exposed to the selected metabolites. Results: Homology model of OR51E2 was made based on active structure of beta-adrenergic receptor (4LDO) and screened against a library of 2552 metabolites. Top candidates included androgens, estrogens and steroid derivatives. In addition, using in vitro system, we confirmed that this receptor is activated by short chain fatty acids. Significance: Identification of biologically relevant ligands of ORs expressed in non-olfactory tissues will provide functional data for further studies aiming at identifying new biological functions of these conserved, highly and broadly expressed GPCRs. Acknowledgements: University of Miami funds.
FCOI Disclosure: None.

#251 POSTER SESSION VI

Structure-activity relationship and evolution of musk odor receptors in mammals

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Musks have been widely used for fragrance and medicine since antiquity because of their fascinating scent. Many synthetic compounds with musk odors have been produced to replace a rare natural musk compound, muscone, that was originally discovered in the stink glands of male adult musk deer. Some synthetic musks, however, are prohibited to be used due to their toxicity or sensitization, and thus, fragrance manufactures have sought to develop safe, easily-synthesized, and good-quality musk compounds. We recently reported muscone-responsive olfactory receptors (ORs) in mice and humans, MOR215-1 and OR5AN1, respectively (Shirasu et al. Neuron 2014). To elucidate the characteristics of these ORs in more detail, in this study we investigated structure-activity relationships of musk ORs in six mammals with 25 musk odors using the luciferase reporter gene assay in HEK293 cells. These musk ORs exhibited unique and distinct ligand spectra, and some of them including the human OR5AN1 also responded to nitro musks that have distinct chemical property from muscone. Our results support the Amoore's stereochemistry theory of olfaction in which musk compounds were predicted to be fitted into a disk shape. Moreover, the structure-activity relationships of OR5AN1 were in a good agreement with our sensory perception, suggesting that OR5AN1 is the crucial musk receptor in humans. The unique pharmacology of OR5AN1 obtained in this study provides important information that helps the development of commercially useful novel musk odors. Acknowledgements: JST, ERATO Touhara Chemosensory Signal Project, Japan.
FCOI Disclosure: None.

#252 POSTER SESSION VI

Electrophysiological analysis of odor response in mice lacking novel protein ROOK and OMP

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We recently identified a novel protein we named Regulator of Olfactory Kinetics (ROOK), which functions to "slow down" olfactory transduction kinetics. Mice mutant for ROOK have a faster olfactory response when measured by electroolfactogram, with a rising phase that is twice as fast as controls and terminate their responses more quickly. Interestingly, another component of the odor detection/signal transduction cascade, Olfactory Marker Protein (OMP), is known to "speed up" olfactory transduction kinetics and its deletion results in decreased amplitude, slower onset and slower termination. We generated mice that are mutants for both ROOK and OMP in order to explore any possible physiological interaction between these proteins with opposing roles in signal transduction. We find that double mutants display largely normal olfactory signal transduction kinetics, with a rising phase and amplitude that is most similar to control animals. Interestingly, termination of the olfactory response in ROOK/OMP mutants is a combination of phenotypes. The initial part of response termination (200ms or ~20% of response termination) in double mutants is fast, like in control mice, while the second part of termination is slow, like in OMP mutant mice. These results suggest that OMP and ROOK may act in the same portion of the signal transduction pathway and have a physiological interaction. Acknowledgements: NIH R01 DC007395. FCOI Disclosure: None.

#253 POSTER SESSION VI

Lamin B1 is required for olfactory sensory neuron development

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The nuclear lamina is a ubiquitous structure in metazoan cells yet has been implicated in cell type-specific processes including chromatin organization, gene expression, and differentiation. Proper neuronal development in the cortex is dependent on expression of a major structural component of the nuclear lamina, Lamin B1, but the mechanism of action and requirement of Lamin B1 in other neuronal systems is currently unknown. We investigated the role of Lamin B1 in olfactory sensory neuron development in the adult mouse olfactory epithelium, where local populations of progenitors are responsible for replacement of neurons in response to normal turnover and damage. We used mouse genetics to knock out Lamin B1 in an olfactory sensory neuron progenitor population, the horizontal basal cells, and monitored development of lineage-labeled mutant neurons and supporting cells after chemically-induced regeneration in adult animals. Lamin B1 mutant lineages display a decrease in mature neuron markers, with no decrease in immature neuron markers or change in progenitor proliferation or gross tissue morphology. These results suggest that Lamin B1 is not required for early stages of olfactory sensory neuron development, but may be necessary for neuronal maturation and/or maintenance. This work aims to uncover the role of a major component of the nuclear lamina in olfactory sensory neuron maturation and odorant receptor expression. Acknowledgements: NIH DC007395B. FCOI Disclosure: None.

#254 POSTER SESSION VI

Cyclophosphamide-induced loss in the murine olfactory epithelium and vomeronasal organ

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Chemotherapy patients often experience profound changes in their ability to taste and smell during/after drug therapy. Cyclophosphamide (CYP), one of the first chemotherapy agents, is known to disrupt taste by cyctotoxic effects and disrupt the taste cell replacement cycle. However, little is known about the effects of this drug on the olfactory system. Since the sense of smell depends on the presence of olfactory neurons that undergo replacement similar to the taste system, we asked if a single injection of cyclophosphamide would affect olfactory neurons? We examined the effects of CYP on olfactory neurons located in the main olfactory epithelium (MOE) and the vomeronasal organ (VNO). We used an antibody to Ki67, a protein only expressed in cells undergoing division (G1, S, G2, mitosis). Male mice were given a single, IP injection of CYP (75 mg/kg) and sacrificed from 1 day to 45 days post-injection. Mice were perfused with 4% paraformaldehyde, decalcified with EDTA, cryto-protected, sectioned and incubated with a Ki67 antibody (Thermo scientific). There were clear differences between the MOE and the VNO across all the time points. At 1 day post injection the MOE looked damaged, especially in the dendritic region while the VNO was structurally unaffected. However both tissues showed a decrease in KI67 protein label compared to controls. By day 2, neither tissue showed any Ki67 labeling. By day 4, the MOE directly above the VNO appeared not to contain olfactory neurons and no Ki67 label was observed. While the VNO was intact as every time point the size seemed to decrease and all Ki67 labeling was absent from days 4-6 post injection. Recovery was complete by 30 days. So far our data suggest that the olfactory tissue in the main olfactory epithelium was more affected by CYP than the VNO. Acknowledgements: NIH NIDCD 1R01DC012829. FCOI Disclosure: None.

#255 POSTER SESSION VI

Calcium-Activated Chloride Channels in Isolated Mouse Vomeronasal Sensory Neurons

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The main function of the vomeronasal organ is the detection of pheromones, which deeply affect animal physiology and behavior. Although some steps of the transduction cascade are known, the complete signaling pathway remains elusive. The binding of pheromones to receptors in microvilli of vomeronasal sensory neurons activates a phospholipase C signaling cascade that produces the opening of TRPC2 channels and the entry of Na+ and Ca^{2+}. In this study, we have used whole-cell and inside-out patch-clamp recordings to provide the first functional characterization of currents activated by Ca^{2+} in isolated mouse vomeronasal sensory neurons. In whole-cell with different free Ca^{2+} concentrations in pipette in the absence of intracellular K^+, we recorded outwardly rectifying currents in 70% of the neurons. The average current in 1.5 μM Ca^{2+} was -382 pA at -100 mV. Ionic substitutions indicated that currents were carried by Cl^- ions and that the permeability ratio sequence for anions was SCN^- (2.6) > Cl^- (1.0) > gluconate (0.1). The Cl^- blockers niflumic acid and $CaCC_{inh}$-A01 caused a reversible current inhibition at all voltages, while anthracene-9-carboxylic acid (A9C) had an anomalous effect, as previously observed in Ca^{2+}-activated Cl^- channels in other cells. Inside-out patches from dendritic knobs confirmed the presence of Ca^{2+}-activated Cl^- channels in the knobs and/or microvilli of mouse vomeronasal sensory neurons and showed that the Ca^{2+} concentration for half-maximal current activation was slightly voltage-dependent decreasing from 1.5 μM -100 mV to 1.1 μM at +100 mV. Our results show that the measured properties are similar to those of TMEM16A and TMEM16B, further supporting the hypothesis that these proteins are responsible for Ca^{2+}-activated Cl^- channels in mouse vomeronasal sensory

neurons. Acknowledgements: This study was supported by grants from the Italian Ministry of Education, University and Research(to AM) and from the Fondazione Compagnia di San Paolo, Torino (to AB). SP is a recipient of an EU Marie Curie Reintegration Grant (OLF-STOM n.334404). FCOI Disclosure: None.

#256 **POSTER SESSION VI**

Phosphodiesterase 5A Regulates the Vomeronasal Pump

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The vomeronasal organ (VNO) is a specialized olfactory structure that detects pheromones to mediate innate social and sexual behaviors in many animals. In mice, the VNO is a bone-encapsulated tubular structure located in the anterior portion of the nasal cavity. Pheromones are thought to be delivered to the VNO lumen via a blood vessel-based pumping mechanism and are then detected by vomeronasal sensory neurons (VSNs) located in the neuroepithelium of the organ. However, it is not well understood how the vomeronasal pump is regulated at the molecular level. We have identified expression of phosphodiesterase 5A (PDE5A) in the blood vessel of the VNO in mice. PDE5A transcripts localize most abundantly to endothelial cells (CD31 positive cells) of the blood vessel, but are also found in smooth muscle cells (smooth muscle actin positive cells) at a lower level. PDE5A is known to regulate blood vessel constriction and dilation in several organs via the breakdown of cyclic GMP. To assess the role of PDE5A in regulating the VNO pump, we pharmacologically inhibited PDE5A with sildenafil citrate. Inhibiting PDE5A led to reduced entry of rhodamine dye into the VNO and a reduced number of activated VSNs upon pheromone stimulation, labeled by the phosphorylation of ribosomal protein S6. Sildenafil citrate treatment also altered innate pheromone-based behaviors in male mice. These results are consistent with the notion that inhibiting PDE5A leads to reduced VNO pump activity. Overall, this study shows that PDE5A plays a role in pheromone sensing by regulating the vomeronasal pump. Acknowledgements: NIH DC007395B. FCOI Disclosure: None.

#257 **POSTER SESSION VI**

Comparative biophysical characterization of formyl peptide receptor expressing neurons in the mouse vomeronasal organ

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In many vertebrates, the mouse vomeronasal organ (VNO) serves as the chemosensory structure that detects both hetero- and conspecific social cues. The vomeronasal sensory epithelium harbors at least three different neuronal subpopulations that are distinguished by expression of either type 1 or 2 vomeronasal receptors (V1R/V2R) or members of the formyl peptide receptor (FPR) family. While various neurophysiological properties of both V1R- and V2R-expressing neurons have been described using genetically engineered mouse models, the basic biophysical characteristics of the more recently identified FPR-expressing vomeronasal neurons have not been studied. Here, we employ a transgenic mouse strain that expresses an enhanced variant of yellow fluorescent protein driven by the *Fpr-rs3* promoter (Fpr-rs3-i-Venus) to identify and analyze FPR-rs3 expressing neurons in acute VNO tissue slices. Single neuron electrophysiological recordings thus allow comparative characterization of the biophysical properties inherent to a prototypical member of the FPR-expressing subpopulation of VNO neurons. In this study, we provide an in-depth analysis of (a) passive membrane properties, (b) several types of voltage-activated ionic currents, and (c) action potential discharge patterns in fluorescently labeled versus unmarked vomeronasal neurons. Our results reveal both similarities and some differences in the basic (electro)physiological architecture of transgene-expressing and -nonexpressing neurons. Taken together, our findings confirm the suitability of this transgenic mouse model for future studies addressing more specialized issues in vomeronasal FPR neurobiology. Acknowledgements: This work is supported by the Deutsche Forschungsgemeinschaft SPP 1392: "Integrative Analysis of Olfaction" and the Volkswagen Foundation (83533). FCOI Disclosure: None.

#258 **POSTER SESSION VI**

Lingual ephrin-A's repel embryonic geniculate neurites in vitro

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Taste axons of the geniculate ganglion innervate pre-gustatory epithelium in fungiform papillae with high fidelity, and somatosensory axons from the trigeminal ganglion innervate the neighboring epithelium. Diffusible factors such as neurotrophins and semaphorins have key roles in guiding these afferents, but non-diffusible cues are also likely to be involved. Ephs and ephrins are cell surface molecules that act as ligands and receptors for one another, initiating signaling cascades that can cause repulsion, stabilization or growth promotion of axons. There are two classes of Ephs and ephrins: Ephrin-A's are lipid-linked proteins that interact predominantly with EphA's, whereas ephrin-B's are transmembrane proteins that interact predominantly with EphB's. During intralingual axon targeting in rats and mice, anti-ephrin-A3 labels the lingual epithelium, and anti-ephrin-A1 labels the vertical musculature. Antibodies against EphA5 and EphA7 labeled afferents within nascent fungiform papillae. To determine if ephrin-A's are capable of guiding axons we grew explants of geniculate and trigeminal ganglia on coverglasses coated with stripes of ephrin-A-Fc fusion proteins. Ephrin-A's -1, -2, -3, and -4 repel geniculate neurites in vitro. Preliminary data indicate that trigeminal neurites are also repelled by these ephrins, though the repulsion is less robust.

Together, these data are consistent with a guidance role for ephrin-A's during pathfinding and targeting of gustatory and somatosensory axons in the tongue. We previously demonstrated that ephrin-B2, which is also expressed along the dorsal epithelium of the tongue during innervation, is repellent in vitro. We are determining if combining ephrin-As and –B's in the same stripe results in additive or synergistic repellent effects. Acknowledgements: Portions of this work were supported by 1R15DC010910-01. FCOI Disclosure: None.

#259 **POSTER SESSION VI**

Effects of Cyclophosphamide on Quinine Preference in C57BK/6J Mice

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Patients undergoing chemotherapy often report disturbances in basic tastes, including bitter. Previous studies with mice (Mukherjee & Delay, 2011; Mukherjee et al., 2013) found that an acute injection of cyclophosphamide (CYP), a chemotherapy drug, has direct effects on taste epithelium and taste specific behaviors. In these studies CYP disrupted the cell replacement cycle of taste sensory cells (TSCs) and caused a loss of fungiform papilla immediately after injection and a loss of TSCs in circumvallate taste buds 8-12 days after injection. These effects were also responsible for deficits in umami and sweet taste detection when assessed by time intense behavioral methods. This study examined the effects of CYP on bitter taste. It was hypothesized that a single dose of CYP would cause disturbances in bitter taste function of mice. The effects of CYP on quinine preference of mice were assessed daily using a brief access test paradigm. C57BK/6J mice were water deprived and trained to lick in a Davis Rig. Quinine hydrochloride concentrations during training and testing were 0.03, 0.1, 0.3, 1, and 3 mM. Once licking was stable, half of the mice received a single 75/kg IP injection of CYP and the other half received equal volumes of saline. Our results show that CYP-treated mice increased their lick rates for 0.3, 1.0, and 3.0 mM quinine 5-14 days post-injection. These increases in lick rates for quinine are indicative of a decreased sensitivity to bitter stimuli. Currently, pilot studies are underway to determine the effects of CYP on detection threshold of quinine. Future studies will focus on bitter taste discrimination. Acknowledgements: This study was supported by NIH grant R01DC012829 awarded to ERD. FCOI Disclosure: None.

#260 **POSTER SESSION VI**

Genetic diversity and evolution of bitter taste receptor genes (TAS2Rs) in wild chimpanzees

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Mammals recognize poisonous compounds in the diet using bitter taste receptors (TAS2Rs) expressed in their taste organs and avoid ingesting poisonous foods. We previously revealed that primates (especially humans, apes and Old World monkeys) have expanded *TAS2R* gene repertoires and that nucleotide sequences of 28 *TAS2R* genes in chimpanzees (*Pan troglodytes*) are eco-evolutionarily diversified among subspecies, suggesting that frequent adaptive evolution occurs in primate *TAS2R*s during the speciation and population fragmentation (Hayakawa et al. 2012 *PLOS ONE*; 2014 *Mol. Biol. Evol.*). In this study, to investigate ecological backgrounds of this *TAS2R* diversification, we determined genetic diversity of some *TAS2R* genes in two wild West African chimpanzee (*P. t. verus*) populations in Guinea (Bossou and Mount Nimba) and two wild East African chimpanzee (*P. t. schweinfurthii*) populations in Tanzania and Uganda (Mahale Mountains National Park and Kalinzu Forest Reserve, respecitvely), using DNA from noninvasively collected samples such as feces and urine. As a result, subspecies differentiation (West vs. East) was observed in *TAS2R*s of the wild chimpanzees as previously reported in captive chimpanzees. For example, pseudogenized alleles of *TAS2R38*, which cause less sensitivity to bitterness of phenylthiocarbamide, were found only in West African chimpanzees. Moreover, haplotype frequency distributions are different between populations in the same subspecies (Bossou vs. Nimba and Mahale vs. Kalinzu), indicating that population-specific genetic drift and/or natural selection occur in chimpanzee *TAS2R*s. In future, we will associate these population specificities of *TAS2R*s with each feeding environment by chemical analyses of their diets and functional assays of population-specific haplotypes. Acknowledgements: Grant-in-Aid for JSPS Fellows (12J04270). FCOI Disclosure: None.

#261 POSTER SESSION VI

Development of a High Throughput Sensory Assay for Bitter Taste

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Following an approach similar to that of our earlier procedure (Palmer et al, 2013) we have developed a high throughput sensory assay for characterization of bitter taste. A cohort of 4 male Sprague Dawley rats was trained to lick solutions dispensed in 96-well plates then perform an operant taste discrimination task of lever-pressing reinforced by delivery of 45 mg grain-based pellets. Rats readily discriminated the taste of quinine from that of 100 mM sucrose, achieving 90% quinine-appropriate responding by day 5 of training. Addition of 100 mM NaCl then was added, followed by 10 mM citric acid, to the training regimen, which continued until quinine-appropriate responding returned to 90% consistently. Then trials of water finally were added. Although rats continued to accurately discriminate quinine from NaCl, sucrose, and citric acid, initial responses to water predominated on the quinine lever, a result consistent with those of other published bitter taste tests and often interpreted as indicating that water imparts a bitter taste quality. Examination of licking on water trials revealed an association between low lick rates for water (< 5 licks/trial, equivalent to quinine) and inappropriate quinine-lever presses, whereas higher rates (approximately 10-15 licks/trial) occasioned appropriate non-quinine lever presses. Extending the inter-trial interval from 30 to 60 seconds increased water lick rates and shifted lever presses to the appropriate non-quinine lever, ultimately achieving >90% accuracy in the final discrimination of bitter vs. non-bitter taste and water. Our results suggest that the taste kinetics of quinine (and possibly other bitter taste standards) can impact responses on subsequent non-quinine trials, potentially complicating interpretation of bitter taste quality measurement. Acknowledgements: Supported by internal corporate funds. FCOI Disclosure: R.K.P. and D.J.L are employees of Opertech Bio and M.F and A.D are employees of Diana Petfood, both of which are commercial enterprises. R.K.P. is co-founder and stockholder of Opertech Bio and is the lead inventor listed on patents covering the technology described in the abstract.

#262 POSTER SESSION VI

Umami-bitter interactions: the suppression of bitterness by umami peptides via human bitter taste receptor

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Taste-taste interactions often showed in human psychophysical studies. Considering that each tastant in foodstuffs individually stimulates its responsible gustatory systems to elicit relevant taste modalities, taste-taste interaction should be performed in taste receptor cell-based assay. While umami substances have been proposed to suppress the bitterness of various chemicals in human sensory evaluation, the bitter-umami interaction has not been explored in bitter taste receptors, TAS2Rs. We investigated umami-bitter taste interactions by presenting umami peptides with bitter substance (salicin) on Ca^{2+}-flux signaling assay using hTAS2R16-expressing cells. Five representative umami peptides (Glu-Asp, Glu-Glu, Glu-Ser, Asp-Glu-Ser, and Glu-Gly-Ser) derived from soybean markedly attenuated the salicin-induced intracellular calcium influx in a time-dependent manner, respectively, while Gly-Gly, a tasteless peptide did not. The efficacies of Glu-Glu suppressing salicin-induced activation of hTAS2R16 were higher than that of probenecid, a specific antagonist of hTAS2R16. According to Ca^{2+}-flux signaling assay using the mixtures of salicin and umami peptides, all five umami peptides suppressed salicin-induced intracellular calcium influx in a noncompetitive manner. These results may provide evidence that umami peptides suppress bitter taste via bitter taste receptor(s). This is the first report which defines the interaction between bitter and umami taste in taste receptor level. Acknowledgements: This study was supported by the Korea Food Research Institute (E0131201). FCOI Disclosure: None.

#263 POSTER SESSION VI

The taste quality of nonesterified fatty acids differs from prototypical bitter compounds

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Central to the concept of whether "fat" constitutes a primary taste is whether or not the oral sensation of non-esterified fatty acids (NEFA) is qualitatively different from other taste sensations. An earlier study indicated NEFA taste differed from sweet, sour, salty and umami, but perceptually overlapped with bitter. As this overlap could reflect a similar hedonic impression, a lack of discriminating lexicon, or actual similarity, we further tested whether humans perceived supra-threshold concentrations of NEFA differently from a variety of bitter tastants. Participants were screened on their ability to correctly identify the linoleic acid emulsion (0.018M) compared to a blank and were given 12 samples: 2 blanks, 7 bitter compounds (caffeine, sucrose octaacetate, 6-*n*-propylthiouracil (PROP), two concentrations each of quinine and urea) and 3 NEFA (C10:1, C18:1, C18:2). Nose clips, opaque lids/cups, and carbohydrate thickeners were used to minimize odor, visual, and textural cues respectively. Participants sorted the samples into groups based on similarity of the sensations. Next, they selected the two groups most similar to each other and combined them. This combining continued until only two groups remained. Data were used to generate dissimilarity matrices for each participant and bootstrapping was used to generate multidimensional scaling maps with kernel density for each sample. Data indicate some strong overlap between C18:1 and C18:2, and low density

perceptual overlap among C18:1, C18:2, and urea; high density peaks display clear differences between the NEFA and other bitters compounds. PROP non-tasters may perceive C10:1 as neutral (overlap with blanks and PROP) and may experience greater perceptual overlap among all the bitter samples compared to PROP tasters. Acknowledgements: USDA Hatch Grant 208684. FCOI Disclosure: None.

#264 **POSTER SESSION VI**

Feline bitter receptors TAS2R38 and TAS2R43 have response profiles distinct from their human homologues

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Bitter taste perception is mediated by members of the highly divergent TAS2R receptor family. While the molecular specificity of human TAS2Rs have been delineated in detail, less is known about the specificities of TAS2Rs from other species. We have initiated work to understand bitter taste perception and TAS2R function in domestic cats. Phylogenetic relationships among specific cat taste receptor sequences have been compared with other carnivores; however, no studies have reported taste perception through functional expression of cat taste receptors. Domestic cats are obligate carnivores and their receptor Tas1r2, associated with the human perception of sweet, is present only as a pseudogene. The perception of bitter by cats may also differ from that of other mammals. To understand the bitter taste perception of cats we functionally expressed two uncharacterized cat bitter taste receptors and determined their responsiveness to known human bitter compounds using a cellular assay. We demonstrate that the response profiles of the cat bitter receptors Tas2r38 and Tas2r43 are distinct from those of their homologous human receptors. Human and cat TAS2R38 both respond to phenylthiocarbamide (PTC), the prototypical TAS2R38 ligand, but show drastically different sensitivity to other known TAS2R38 ligands. Similarly, both human and cat TAS2R43 are responsive to aloin but differ in sensitivity to other known ligands. Based on this knowledge we can begin to understand what compounds cats can detect through bitter receptors and improve palatability of therapeutic and dietary products with bitter components. Future work will explore the behavioral response of cats to identified ligands. Acknowledgements: Supported by AFB International. FCOI Disclosure: None.

#265 **POSTER SESSION VI**

Bitter taste receptor agonists mitigate asthma phenotype in murine models

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Previous study from our laboratory demonstrated the expression bitter taste receptors (BTR) on airway smooth muscle (ASM) and established bronchodilatory effect of BTR agonists. Pre-clinical studies using integrated animal models are needed to establish the effectiveness of BTR agonists in the treatment of asthma. Here, we aimed at determining the effect of BTR agonists on allergen-induced airway inflammation, hyperresponsiveness and remodeling, salient features of asthma. Airway inflammation was induced in naïve mice using intranasal house dust mite (HDM) or aerosol ova-albumin (OVA) challenge for 3-4 weeks. Select group of mice were treated with chloroquine or quinine by intranasal (HDM animals) or aerosol (OVA animals) route 30 min prior to allergen challenge. At the end of the treatment protocol, airway responsiveness, inflammation (using bronchoalveolar lavage fluid (BAL)) and remodeling (histology and protein markers) were assessed. HDM or OVA challenge in mice resulted in airway inflammation as evidenced by increased infiltration of immune cells in the lungs. Airway inflammation was significantly reduced in mice pre-treated with BTR agonists, chloroquine and quinine. Measurement of cytokines/chemokines such as IL4, IL5, IL13, TNF-α, CCL3/4, IL8, RANTES, and Eotaxin in the BAL revealed effectiveness of BTR agonists in inhibiting allergen-induced key pro-inflammatory response. HDM and OVA challenge resulted in activation of pro-fibrotic signaling (TGFβ1) and remodeling (assessed by histology and marker proteins such as myosin heavy chain, a-actin, collagen and fibronectin) in the lungs, and chloroquine and quinine treatment effectively inhibited allergen-induced airway remodeling. These data establish the role of BTR agonists in mitigating the development of allergic asthma in murine models. Acknowledgements: American Asthma Foundation. FCOI Disclosure: None.

#266 **POSTER SESSION VI**

Diet induced obesity modulates turnover and receptor expression of murine taste buds

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An ever-growing epidemic, over two-thirds of adult Americans are overweight, with more than one-third classified as obese. Obesity is associated with an increased risk of diabetes, heart disease, and high blood pressure, among other serious health problems. Psychophysical research on obese humans, and behavioral recordings of obese animal models, have shown that obesity promotes an alteration in taste sensitivity and preference, when compared to lean individuals. These effects are reversible

with weight loss, suggesting that taste is modulated continuously by the host's state of obesity. The mechanism by which obesity modulates the sense of taste is still a subject of much debate. To investigate this phenomenon, we exposed a cohort of mice to a 35% fat diet for a period of five weeks, enough to induce a state of obesity. We enzymatically collected taste buds and non-taste tissue from lean and obese mice, and measured relative gene expression between the populations using qRT-PCR. Analysis revealed decreased expression of many cellular markers of gustatory tissue in the obese cohort, including several taste receptors and downstream effectors, signifying a decrease in sensitivity to stimuli. Immunohistochemical analysis of sectioned taste tissue revealed that obese mice display fewer overall numbers of taste buds, suggesting a dysfunction in taste bud turnover. Confirming our hypothesis, we found expression of a number of markers of cellular proliferation and taste bud differentiation to be down-regulated in obesity. These results suggest a possible mechanism for how chronic exposure to a high fat diet can alter the cellular makeup of taste tissue, and sheds light on how obesity leads to the modification of taste sensitivity, and feeding preference in human populations. Acknowledgements: Cornell University College of Agriculture and Life Sciences Startup Grant. FCOI Disclosure: None.

#267 **POSTER SESSION VI**

Nonivamide, a TRPV1 Agonist, promotes Body Weight Maintenance in healthy overweight Subjects

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Capsaicin, the major pungent compound of red pepper, has been shown to support a healthy body weight by regulating mechanisms of satiety and thermogenesis (Yoshioka, M. et al., Br J Nutr 1999, 82, 115-123; Westerterp-Plantenga, M. S. et al. Int J Obes (Lond) 2005, 29, 682-688). However, due to its pungency evoked by activation of the TRPV1 channel, the dietary intake of capsaicin is limited. Our study aimed at elucidating whether dietary intake of a less pungent structural capsaicin analog, nonivamide is able to support body weight maintenance by enhancing plasma concentrations of satiating hormones. The here presented results from a randomized, placebo-controlled, cross-over intervention study revealed that a bolus administration of 0.15 mg nonivamide reduced ad libitum energy intake from a standardized breakfast while increasing plasma serotonin concentrations in moderately overweight men. To validate the observed effects a 12-week, placebo-controlled, randomized human intervention trial with a daily amount of 0.15 mg nonivamide was conducted. A significant group difference in total change in body fat mass (- 0.61 ± 0.36 % for the nonivamide treated group and + 1.36 ± 0.38 % for control treatment, $p < 0.05$) was determined, although changes in body weight did not reach the level of statistical significance. Moreover, the daily administration of nonivamide resulted in elevated plasma serotonin levels following a 75g glucose challenge compared to control treatment. In summary, nonivamide has been demonstrated to affect peripheral serotonin concentrations and total body fat, indicating a promising therapeutic potential in strategies that help to maintain a healthy body weight. Acknowledgements: The financial support by the Austrian Federal Ministry of Economy, Family and Youth and the Austrian National Foundation for Research, Technology and Development and the Symrise AG is gratefully acknowledged. FCOI Disclosure: The authors Sabine Widder, Jakob P. Ley, and Gerhard E. Krammer are employees at the Symrise AG, Holzminden, Germany.

#268 **POSTER SESSION VI**

Pathways of Associations between Taste-related Risk Factors, Regional Taste Function and Adiposity in Adult Women

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Perceived intensity of taste and irritation on the anterior tongue is variable and, in small clinical studies, explain differences in food preference and adiposity. Anterior tongue and whole mouth intensity of concentrated quinine and salt were part of the new chemosensory protocol in U.S. National Health and Nutrition Examination Survey (NHANES) 2013-14. Here we mined an existing database of 407 ostensibly healthy women (35±17 y) who underwent similar protocol to identify paths of association between self-reported taste-related risks (otitis media, head trauma, tonsillectomy), bitter and salt intensity, fungiform papillae number, and adiposity with structural equation modeling. Thirty-nine percent of the women were overweight/obese; 21% had elevated waist circumference. The taste risk factors explained depressed bitterness on the anterior tongue, either alone or relative to whole mouth bitterness or saltiness, and these taste markers were significantly associated with greater waist circumference. Age, lower papillae number and taste risk factors also directly associated with greater waist circumference. In multivariate models, the bitter taste markers showed the best statistical fit, explaining up to 16% of variance in waist circumference, including mediating the association between head trauma and waist circumference ($b=-0.03$, $p < 0.05$). The salt indices by themselves did not explain significant variability in adiposity. To conclude, our findings support quinine as good marker to examine taste-health associations, the added value of regional and whole mouth taste assessment, and that altered taste or risk factors for taste alterations increase risk of elevated adiposity. Similar analytical approaches can test for taste-diet-health associations in the nationally representative NHANES 2013-2014 data. Acknowledgements: NIDCD/NIH.
FCOI Disclosure: None.

#269 POSTER SESSION VI

Early consequences of a high-fructose diet on olfaction

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The influence of nutritional status on olfactory processes has been thoroughly investigated over the last few years. It is now well-established that both nutritional status and hormones implicated in food metabolism can effectively modulate the olfactory system from the single neuron to the behavior. Thus, it seems likely that metabolic disorders such as type 2 diabetes (T2D) can induce olfactory dysfunctions. Indeed T2D patients display poor olfactory performances although the direct effects of diabetes on olfaction were not yet demonstrated. Here, we investigated the modulation of olfaction in young adult C57Bl/6 male mice caused by a high-fructose diet (HFruD), known to induce T2D in rodents. The development of T2D was monitored by metabolic approaches (weight gain, food and water intake, glycemia, insulinemia and glucose tolerance test). Animals displayed hyperglycemia, hyperinsulinemia and glucose intolerance after only 4 weeks of HFruD indicating a prediabetic state. In addition, after 4 weeks of HFruD, animals exhibited a decrease in olfactory capacities for both neutral and food odors (assessed by habituation/dishabituation test and buried food test respectively). General electrical responses of the olfactory mucosa (measured by electro-olfactogram) were reduced in HFruD animals. These behavioral and physiological effects persisted after 8 weeks of HFruD. Using immunohistochemistry, we observed a reduction of apoptosis in the olfactory mucosa indicating that HFruD modifies cell dynamics. Our results demonstrate that olfaction is modified by HFruD in rodents. Functional, anatomical and behavioral changes occurred in the olfactory system even before the complete development of the disease. Our data suggest that olfactory dysfunctions may be due to metabolic changes during the onset of T2D.
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#270 POSTER SESSION VI

Taste Phenotype Explains Adiposity and Cardiovascular Disease (CVD) Risk Factors Among Females Via Dietary Quality Constructed From a Liking Survey

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A more healthy diet (ie, high dietary quality) lowers risk of CVD, a leading cause of mortality. As sensory factors drive dietary choices, this observational study aimed to test if chemosensory phenotype predicted adiposity and CVD risk factors via dietary quality in 110 non-smoker females (mean age=45±6 yrs). Following our previous research, we constructed a reliable dietary quality index (Healthy Eating Preference Index, HEPI) from survey-reported liking of food groups (sweet, fruit/vegetable, fat, salty, protein) and variety of healthy foods. Latent variable analysis formed reliable taste (intensities of anterior tongue quinine and whole mouth quinine and propylthiouracil) and smell (intensities of eight orthonasally-perceived odors) variables. Half of the study sample had elevated CVD risk factors (serum lipids, blood pressure, central adiposity). Structural Equation models were used to assess the effect of the taste and smell variables on central adiposity and CVD risk factors considering age and dietary restraint. Although the taste and smell variables were highly correlated, the taste variable explained more variance in CVD risk factors. One main path was mediation of taste to adiposity and CVD risk factors via HEPI, significant for HDL-cholesterol. Another path was taste predicting HEPI, in turn, predicting central adiposity and then CVD risk factor, significant for fasting triglycerides and systolic blood pressure. A significant direct association was found between the taste variable and LDL-cholesterol. In summary, a simple liking survey is a reliable proxy of dietary quality to connect taste with meaningful health outcomes. Taste variation influenced CVD risk factors via differences in diet healthiness. Factors that mediate taste-serum lipid associations deserve further investigation. Acknowledgements: USDA/Hatch.
FCOI Disclosure: None.

#271 POSTER SESSION VI

Otitis media, food preferences and weight gain in college students

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Background: Otitis media (middle ear infection) can damage the chorda tympani taste nerve as it passes through the middle ear to the brain. This reduces taste perception on the anterior tongue but can intensify other oral sensations (release of inhibition). Otitis media is associated with increased weight. Data from

preschoolers (Peracchio et al, 2012) and adults (Bartoshuk et al, 2012) suggests that greater liking of sweet/fat foods explains the relation between otitis media and elevated adiposity. The purpose of the present study was to determine whether this association exists in college-age adults. Methods: Body mass indexes (BMIs) were calculated for subjects who had never experienced otitis media (N=249) and those with moderate-to-severe histories (N=36). All subjects rated liking/disliking for 65 food items using a global hedonic intensity scale (variant of the hedonic general Labeled Magnitude Scale) for which 0="strongest disliking ever experienced," 0=neutral and 100="strongest liking ever experienced." Results: Multiple regression revealed significant contributions of gender (p=.02) and otitis media group (p=.02) to BMI. Factor analysis produced a reliable sweet/fat cluster (alpha=.87) of 10 foods (cookies, cake or pastries; ice cream; sugar; sweets, candy; milk chocolate; oreo cookies; chips; fried food; cheddar cheese; spaghetti with marinara sauce). Mean liking for the sweet/fat foods was 30.6 for those with no otitis media and 36.0 for those with a history of otitis media. Distributions for these two otitis media groups were significantly different (Chi Square=5.9, p=.015). Conclusion: Our data suggest that greater liking for sweet/fat foods explains some of the association between otitis media and elevated adiposity in preschoolers, college students and adults (into their 80s). Acknowledgements: none. FCOI Disclosure: None.

#272 **POSTER SESSION VI**

Changes in Sensory Perception and Liking for Sweet and Savory-Fat Foods During a 6-Month Behavioral Weight Loss Intervention in Women: A Preliminary Report

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Rapid weight loss following bariatric surgery alters taste function; however, it is unclear whether dieting to lose weight has the same effect. As part of a study on the role of PROP status on weight loss, we assessed changes in sensory perception and liking for sweet and fat foods in women (BMI=34.9 kg/m^2; age=44.6) who were classified as non-tasters (n=21) or super-tasters (n=34) based on their ability to taste 6-n-propylthiouracil, and then randomized to a low-fat (LF) or low-carb (LC) diet. At 0, 3 and 6 mo., they used 15cm line scales to rate intensity of sweetness, creaminess, thickness, strawberry flavor and overall liking of strawberry milk with 0%, 15% and 30% sucrose as well as intensity of clinginess, spiciness, oiliness, overall flavor and overall liking of salad dressing with 10%, 30%, 50% oil. Mean weight loss at 6 mo. was 8.25%. Data were analyzed by repeated measures ANCOVA with weight loss as a covariate. Sweetness perception did not change for strawberry milk, but discrimination of strawberry flavor between 15% and 30% sucrose milk improved at 3 (p=0.02) and 6 mo. (p=0.002). 15% sucrose milk was preferred across the trial, but liking decreased in the LC group by month 6 (p=0.03). There were no changes in the fat-related attributes of salad dressing over time; however, the 50% oil sample was most liked at 0 mo. and least liked by 6 mo. (p=0.002). Super-tasters gave higher ratings for spiciness (p=0.001) and overall flavor (p=0.001), and the LF group gave higher ratings for overall flavor (p=0.001) and overall liking (p=0.03) than the comparison groups. These preliminary findings suggest that weight loss from dieting decreases liking of sweetened milks and salad dressings. A LC diet may have a particular influence on liking of sweetness. Supported by Am. Heart Assoc. 12GNT12060259. Acknowledgements: American Heart Association 12GNT12060259. FCOI Disclosure: None.

#273 **POSTER SESSION VI**

Glucose homeostasis and body composition in Tas1r3-knockout mice: effects of age and fasting

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In addition to being expressed in taste bud cells, the sweet taste receptor proteins T1R2 and T1R3 are also expressed extra-orally, where they are involved in control of intestinal glucose absorption, pancreatic insulin production, and adipogenesis. T1R2/T1R3-mediated effects of fructose or non-caloric sweeteners on insulin production by β-cells *in vitro* depend on concentration of glucose in the medium, which suggests that these effects in vivo may differ in fed or fasting states. Therefore, the goal of the present study was to examine how fasting affects glucose homeostasis in mice with deletion of the *Tas1r3*-gene encoding the T1R3 protein. Male mice from the *Tas1r3*-knockout strain C57BL/6J—*Tas1r3*^{tm1Rfm} (*Tas1r3-/-*) and from the control C57BL/6ByJ strain with the intact gene (*Tas1r3+/+*) that ranged in age from 12 to 120 weeks were used in the study. *Tas1r3-/-* mice in non-fasted state had reduced glucose tolerance in intragastric (IG) and intraperitoneal (IP) tests and lower IP insulin tolerance. However *Tas1r3-/-* and *Tas1r3+/+* mice did not differ in IG glucose tolerance after an 18-h fasting. Compared with *Tas1r3+/+* mice, in *Tas1r3-/-* mice food deprivation caused a greater decrease of the blood glucose and a smaller reduction in the body weight. Consistent with this, *Tas1r3-/-* mice had more body fat. *Tas1r3-/-* and *Tas1r3+/+* mice had similar non-fasted liver weight, and in fasted state they did not differ in plasma glucagon levels, suggesting that T1R3 is not involved in glycogenolysis. To evaluate gluconeogenesis, we examined pyruvate tolerance after 18 h fast, which showed that differences between *Tas1r3-/-* and *Tas1r3+/+* depend on age. These data suggest that T1R3-dependent mechanisms are involved in control of insulin sensitivity and gluconeogenesis. Acknowledgements: NIH Grants R03DC013526 (AAB and VAZ) and NIH R01DC00882 (AAB). FCOI Disclosure: None.

#274 POSTER SESSION VI

Odorant receptor expression in aged mice following genetically-mediated lesion

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Several repositories of neuronal stem cells are resident in the nervous system. One population is found in the peripheral olfactory system, and generates excitatory projection neurons that extend a long axon from the neuroepithelium lining the nasal cavity to the olfactory bulb. Basal stem cells have been known for more than 30 years to generate sensory neurons in the young adult epithelium, but the ability of their counterparts in aged tissue to recapitulate the epithelium is relatively unexplored. In addition, the gene expression profile of odorant receptors may vary with age. Here we probe the ability of the stem cell to generate a diverse array of neurons expressing the appropriate repertoire of odorant receptors in aged animals. To this end we generated a line of mice, iDTR x OMP-cre, whereby a Cre-mediated excision of a STOP cassette renders mature neurons sensitive to diphtheria toxin (DT) via activation of the DT receptor. This method permits a specific and reversible ablation of mature (OMP-expressing) neurons upon DT administration but without damage to potential synaptic targets in the OB or to other cell types found in the olfactory epithelium. We administered either DT or saline to male mice of several age groups (2-18 months) for six days. 30 days following ablation, to allow for complete degeneration and subsequent recovery of olfactory epithelia, RNAs were harvested and prepared for microarray analysis. Results reveal, that when examining the cohort of odorant receptor genes expressed following recovery from lesion, those regenerated at young ages do not significantly differ from those regenerated in aged animals. These results imply that the regenerative potential of the neuronal stem cell in aged animals is intact and is capable of generating a wide array of sensory neurons. Acknowledgements: NIH Grant DC012567. FCOI Disclosure: None.

#275 POSTER SESSION VI

Aging affect olfactory perceptual learning and olfactory bulb neurons structural plasticity

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Too often depreciated in humans, the sense of smell is important in everyday life. Indeed, olfaction plays a key role in nutrition, emotions, detection of dangers and social interactions. Like other sensory processes, olfaction undergoes aging, which is characterized by a decrease of olfactory performances (such as olfactory detection, discrimination, identification, hedonics and memory). These deficits lead to alterations of quality of life and can induce depression, anxiety and denutrition. Therefore understanding the underlying mechanisms is necessary to develop efficient therapeutic strategies to promote healthy aging. We used the mouse model to access the neuronal network and identify the cellular bases of olfactory aging. We studied olfactory perceptual learning in young (2 months), middle-aged (12 months) and aged (24 months) mice and the underlying changes in neuronal morphology. We showed an alteration of olfactory learning in old mice, which is correlated to alterations of neuronal structural plasticity. Surprisingly, middle-aged mice performed as well as young mice despite already present neuronal modifications. Our results suggest that the brain is structurally affected at early stages of aging but might develop compensating strategy in order to fight against aging and delay the decrease of olfactory performances. Acknowledgements: CNRS Lyon 1. FCOI Disclosure: None.

#276 POSTER SESSION VI

Men are more susceptible to age-related central olfactory functional decline: An Olfactory fMRI Study

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The effect of aging on olfactory system functioning has been extensively studied. However, whether this age-related olfactory change is due to central or peripheral functional decline is still unclear. The aims of this study were to evaluate the effect of age on central olfactory system function using fMRI and also to determine if gender differences exist within this effect. Young and older subjects underwent a novel olfactory fMRI paradigm that was designed to isolate and assess the function of the central olfactory system on a 3T system, along with a behavioral olfactory test (UPSIT). This paradigm included both *odor* and *no-odor* conditions paired with the same visual cue ("Smell?"). While the *odor* condition involves both peripheral and central olfactory functioning, the *no-odor* condition would presumably only involve central processing. In the older group, age was found to negatively correlate with the BOLD response to odor sensory processing in the odor condition ($r=0.475$, $p< 0.05$). However, we found a stronger negative correlation ($r=0.707$, $p< 0.0001$) with age in the *no-odor* conditions, indicating involvement of the central olfactory system in aging possibly due to odor memory processing. UPSIT scores were found to positively correlate with this BOLD response in the olfactory areas across all subjects ($r=0.331$, $p< 0.05$). Interestingly, the aging effect was found to be more pronounced in men compared to women in olfactory-related areas ($p< 0.05$), including the primary olfactory cortex and hippocampus. These results reveal a prominent effect of aging in the central olfactory system and that men appear be more susceptible to this effect compared to women. In addition, these findings provide valuable normative

aging data of the studies of olfactory deficits in many neurodegenerative diseases. Acknowledgements: University Funds. FCOI Disclosure: None.

#277 **POSTER SESSION VI**

Olfactory impairment in old age: prevalence and risk factors

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Loss of olfactory function is frequently observed in the elderly, but the underlying mechanisms are poorly understood. The present project used The Swedish National Study on Aging and Care in Kungsholmen (SNAC-K) to assess effects of known clinical, lifestyle and genetic risk factors for cognitive decline on olfactory performance. A total of 2439 participants without dementia, aged 60-90 years, completed a cued odor identification test and a questionnaire on their olfactory functioning. The prevalence of hyposmia (identification score< 11 out of 16) in the sample was 23.5% (574 participants); 4.5% of participants (n=111) were classified as functionally anosmic (score below 6). Awareness of sensory deficits was strongly correlated with test performance. Removing effects of age and gender, participants with Parkinson's disease and depression demonstrated significant losses in olfactory function. Current and lifetime smoking were related to increased risk of olfactory dysfunction, whereas moderate alcohol consumption was associated with a reduced risk. No effects of hypertension, high cholesterol levels, physical inactivity or diabetes were observed. *APOE* ε4 and *BDNF* val/val carriers had a higher risk of olfactory dysfunction independent from current clinical or lifestyle risk factors. Simultaneous presence of risk factors was linearly associated with an increased risk for olfactory loss. The 398 participants without any risk factors showed a significantly lower risk of hyposmia (17.3%, n=69), while maintaining comparable levels of functional anosmia (4.2%, n=11). Medical care providers should be alert to these potential cumulative effects of risk factors contributing to olfactory dysfunction during old age. Acknowledgements: Swedish Council for Working Life and Social Research Swedish Research Council, the Regional Agreement on Medical Training and Clinical Research (ALF) Between Stockholm County Council and Karolinska Institutet. FCOI Disclosure: None.

#278 **POSTER SESSION VI**

ApoE-e4 Mediates the Association Between Episodic Memory Decline and Olfactory Identification Deficit

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Episodic memory decline, olfactory identification deficits and the ApoE-e4 allele constitute risk factors for incident Alzheimers' Disease (AD). However, the relationships among these three risk factors are poorly understood, in part due to the paucity of large longitudinal datasets that involve such assessments. The present study used data from the Betula study (n=1225), which involves memory testing every five years. Participants completed an odor identification test, were genotyped for the ApoE gene, and had completed episodic memory testing for a 10-year period (3 testing occasions) leading up to the olfactory assessment. The episodic memory measure was a composite of five tasks, and decline was defined as an estimated change >1SD below the age norm. Participants were thus classified as "decliners" (n=125) or "non-decliners" (n=1100). Results showed that decliners had a poorer olfactory identification than non-decliners. However, when ApoE-e4 was taken into consideration, the association between memory decline and odor identification deficit was only present in ApoE-e4 carriers, whereas odor identification in memory decliners without e4 reached the same level as that of non-decliners. Future research on the role of olfaction in age-related memory impairment and dementia should consider the mediating role played by the ApoE-e4. Acknowledgements: The Swedish Research Council. FCOI Disclosure: None.

#279 **POSTER SESSION VI**

Olfactory training with older people

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Aim and Background: Reduction in olfactory function affects quality of life and enhances likelihood of depression. Further, there is evidence that olfactory abilities decrease with age. Several studies indicate that regular short-term exposure to odors (so-called "smell training") improves general olfactory function. The aim of this study was to investigate whether the positive effects of smell training extend to subjective well-being and cognitive function. Material and Methods: Eighty four healthy participants (aged 50 to 84 years, M= 61.1, SD 8.7) randomly assigned to an olfactory training group (N=58) and a control group (N=26) completed testing in our ongoing study. Olfactory and cognitive function, as well as subjective well-being, were

tested at baseline and after 5 months. In the meantime, the training group completed daily olfactory training while the control group completed daily Sudoku problems. Results and Conclusion: A significant improvement of olfactory threshold and discrimination ability was found in the training group compared to the control group ($p < 0.001$). Furthermore, participants of the training group improved in executive function ($p < 0.001$), felt younger and more active ($p=0.014$) after training. No such effects were observed in the control group. In a subgroup of participants characterized by subclinical depression at the first appointment, the olfactory training had a positive effect on the severity of depression ($p= 0.002$). Based on these results, olfactory training seems to constitute an inexpensive and simple way to improve quality of life in elderly people.
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#280 **POSTER SESSION VI**

Olfactory Threshold and Odor Discrimination in Children and Adolescents

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The evaluation of olfactory function in terms of olfactory threshold and odor discrimination in adults is widely performed using the "Sniffin' Sticks" test battery. Although well feasible for adults, the test seems rather long and complicated especially for young children. Therefore this study investigated whether the same test can be used to evaluate olfactory function in children and compared the results to a modified version of the "Sniffin' Sticks" test. We included 119 (m=59, f=60) healthy children aged 5-17 years (mean: 11.5±3.7). Children were grouped according to age (group 1: 5-8, group 2: 9-11, group 3: 12-14, group 4: 15-17 years). Odor threshold and odor discrimination were tested using "Sniffin' Sticks". Each child underwent the original test-version with "Sniffin' Sticks" triplets (three-alternative forced choice) as well as a modified version using just 2 "Sniffin' Sticks" (two-alternative forced choice). To evaluate the reliability of both methods, all participants were tested twice on separate days. Higher scores were obtained with the 2 stick-method for both threshold and discrimination compared to the 3 stick-method. Scores of both testing methods correlated positively with age. Regarding the test-retest-reliability, the 3 stick-method showed a positive correlation between the two test days for both odor threshold ($r=0.375$; $p < 0.001$) and discrimination ($r=0.531$; $p < 0.001$). With the 2 stick-method, a correlation between the two test days was only found for odor discrimination ($r=0.417$; $p < 0.001$) but not for odor threshold ($r=0.133$; $p < 0.148$). These data show that olfactory testing of children depends on age. Both odor detection threshold and odor discrimination scores showed a better test-retest correlation using the three-alternative compared to two-alternative forced choice method.
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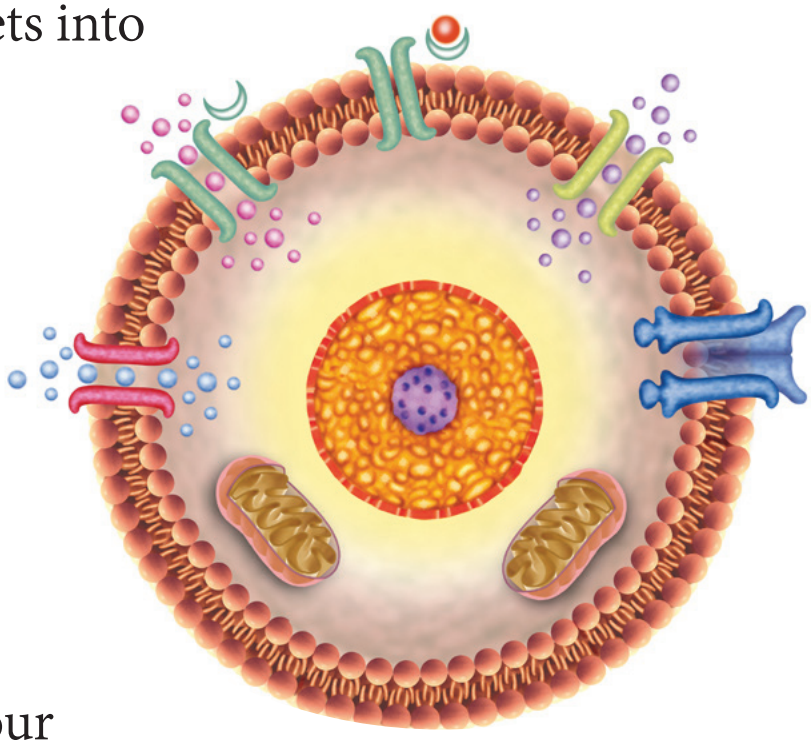

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