44th Annual Meeting of the Association for Chemoreception Sciences
April 20-23, 2022
Bonita Springs, FL

Printable Program & Abstracts

Wednesday, April 20, 2022

8:30 - 12:00 PM	Calusa FGH
INTERNATIONAL SOCIETY OF NEUROGASTRONOMY SYMPOSIUM AM SESSION	

8:30 **Welcome And Introduction.**
Tim McClintock
University of Kentucky

8:45 **Key Food Odorants And Their Receptors – Perfect Matches For Our Complex Sense Of Olfaction**
Dietmar Krautwurst
Liebnitz Institute for Food Systems Biology

9:15 **Mom, Am I Full Yet?**
Nisha Pradhan
University of Colorado

9:45 **Coffee Break**

10:15 **Bugs, Epialleles And Ancient Vines: A Multifocal View On Wine Quality**
Carlos Rodriguez Lopez
University of Kentucky

10:45 **A Chef'S Perspective**
Fred Morin, Chef

11:15 **Panel Discussion**

12:00 - 4:00 PM	Great Egret
Executive Committee Meeting (Invite Only)	

12:00 - 1:15 PM	Lunch On Own
Lunch On Own	

1:15 - 4:45 PM	Calusa FGH
INTERNATIONAL SOCIETY OF NEUROGASTRONOMY SYMPOSIUM PM SESSION	

1:30 **Delicious: How An Evolutionary Perspective Changes Our Perspective On Flavor"**
Rob Dunn
North Carolina State University

2:00 **The Power Of Hunger**
Amber Alhadeff
University of Pennsylvania and Monell Chemical Senses Center

2:30 **Coffee Break**

3:00 **The Flavor, Nutrition & Sound Of Wheat**
Merri Metcalf
Washington State University

3:30 **Progress In The War Against The Tasteless Tomato**
Harry J. Klee
University of Florida

4:00 **Panel Discussion**

4:45 **Closing Remarks**
Dan Han
University of Kentucky

4:00 - 4:30 PM	Driftwood
Pyrfume Code-fest Orientation	

Pyrfume is a project which uses computation methods to predict how a molecule smells based on its structure. If you are a programmer or coder-curious, come and get starter code, and orientation to the datasets, and meet our team of teachers. Baby coders are welcome!
Get an orientation, sample code, and access to eight new datasets that can be used to predict how a molecule smells from its structure, and code with us (R, Python, all languages welcome).

4:30 - 5:00 PM	Calusa Terrace
Diversity Fellowship Meet n' Greet (Invite Only)	

5:00 - 6:00 PM	Calusa ABCD
AChemS Welcome/Awards Ceremony	

6:00 - 7:00 PM	Calusa ABCD
Keynote Lecture	

6:00 **From Fixation To Exploration: An Integrative View Of Oculomotor Function**
Susana Martinez-Conde
SUNY Downstate Health Sciences University

During visual exploration, saccadic eye movements scan the scene for objects of interest. During attempted fixation, the eyes are relatively still but often produce microsaccades. Though exploration and fixation have traditionally been regarded as two distinct oculomotor behaviors, an alternative model is that fixation and exploration are not dichotomous, but the two extremes of a functional continuum. I will present data from healthy subjects as well as patients suffering from neurological disease, supporting the view that visual fixation is functionally equivalent to visual exploration on a spatially focused scale. I will also discuss my lab's research using microsaccade production to better understand the role of area V1 in the perceptual distinction between self-motion and world-motion. Finally, I will present our latest findings on what oculomotor behavior might reveal about the perception of art.

7:00 - 9:00 PM	Waterfall Pool Deck
Welcome Banquet	

Thursday, April 21, 2022

7:30 - 9:00 AM	Estero Foyer
Continental Breakfast	

8:00 - 10:00 AM	Estero Ballroom
Poster Session I	

100 **Intraoral Thermal Processing In The Gustatory Cortex Of Awake Mice.**

Cecilia/G Bouaichi, Roberto Vincis
Florida State University, Tallahassee, FL, United States

In the past decades, many electrophysiological studies in behaving rodents have described how neurons in the gustatory cortex (primary taste cortex, GC) process taste information. In addition, a growing body of experimental work in humans and primates - as well as pioneering works in anesthetized rats - indicates that GC neurons respond also to non-gustatory components of intraoral stimuli, including temperature, a salient sensory feature of food and beverages. While these data implicate the GC as a potential key brain region for the integration of taste and thermal orosensory inputs, they stop short of supplying a fine-grained analysis of its neural responses, and many questions remain. Here, using fiber-photometry and extracellular recording (tetrodes and silicon-based probes), we aim to provide a complete neurophysiological assessment of how thermal orosensory inputs shape GC activity in alert mice. Specifically, we tested 1) whether and how neurons in the GC of actively licking mice are modulated by changes in the temperature of chemically inert drinking solutions and 2) if thermal responses are organized across the dorso-ventral axis (granular, dysgranular, and agranular) of the GC. Licking and neural activity was recorded in mice trained to experience (on a fixed ratio schedule) a drop of cool (14°C), ambient (25°C), or warm (36°C) water. Overall our results show that GC processes thermal intraoral information at both a population and single-unit level without an apparent topographical organization. In conclusion, our data shows that temperature is a salient intraoral cue represented in the taste cortex and suggests the GC is cortical region important for the integration of thermal and chemosensory stimuli present in food and beverages.

101 **Understanding The Neuronal Substrates Of Sensorimotor Transformations Via A Novel Closed-Loop Olfactory Task (*Smellocator*) For Mice**

Marie Dussauze[1, 2], Priyanka Gupta[1], Uri Livneh[1], Dinu F Albeanu[1]
[1]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, United States, [2]CSHL School of Biological Sciences, Cold Spring Harbor, NY, United States

Through experience, the brain learns the reciprocal relationship between sensory inputs and movements to build internal models that predict the sensory consequences of its actions (sensorimotor predictions). While odor sampling is tightly coupled to motor actions, it remains unknown how olfactory representations are modulated by movement and whether such modulation supports sensorimotor predictions. To investigate sensorimotor predictions both at behavioral and circuit-level, we developed a novel closed-loop task (Smellocator) where head-fixed mice learn to steer a lever to control the lateral location of an odor source. Mice thus learn to link motor action to well-defined sensory expectations (odor location). Mice master the task quickly (~2 weeks, >90% accuracy), perform numerous trials per session (~600) and show precise movements. In expert mice, we violate learnt sensorimotor expectations by transiently decoupling the stimulus from the current action (open-loop replay, halting of odor source, changing the gain), thus creating sensorimotor errors. Additionally, across longer timescales, we invert the sensorimotor mapping (direction of odor movement) to engage sensorimotor adaptation. Strikingly, mice readily counter these sensorimotor errors and display precise corrective movements which provide a behavioral read-out of their internal model. To understand the circuit mechanisms which enable this behavior, we monitored activity in the olfactory cortex (Anterior Olfactory Nucleus, Piriform Cortex) using chronically implanted tetrode drives. Using a cross-area comparative approach and neuronal ensemble analysis, we are assessing whether olfactory cortical activity better represents the degree of mismatch between sensory inputs and sensorimotor expectation, or sensory inputs *per se*.

102 **Grey Matter Atrophy Of Olfactory-Related Structures In Mild Cognitive Impairment: Preliminary Results From The Cima-Q Cohort**

Benoit Jobin[1,2,3], Benjamin Boller[1,2], Johannes Frasnelli[1,3], And The CIMA-Q Group[2]
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Olfactory impairment is a clinical biomarker of Alzheimer's disease (AD) and is already present at the mild cognitive impairment (MCI) stage of the disease. Olfactory impairment may be due to early neuronal damage that occurs within limbic regions in patients with AD. We aimed to evaluate if olfactory-related regions are atrophied within the MCI stage of AD. Using voxel-based morphometry (VBM), we compared grey matter density of olfactory-related structures between a group of 93 participants with subjective cognitive decline (SCD) (mean age: 72.15, SD: 4.74) and a group of 40 patients with MCI (mean age: 72.08, SD: 5.51)from the Consortium for the early identification of Alzheimer's disease–Quebec (CIMA-Q). VBM analyses were done using the region of interest (ROI) approach with a specific mask resulting from a recent activation likelihood estimation meta-analysis that provided an activation probability map of the functional anatomy of the olfactory system (Torske et al., 2021). VBM analysis reveals smaller bilateral grey matter volume within bilateral piriform cortex and amygdala (left peak: MNI -28 −4 -14, k = 91, T = 3.75, pFWE corr. = 0.019) (right peak: MNI 28 -4 12, k = 226, T = 4.14, pFWE corr. = 0.009s) in MCI patients compared to participants with SCD. Grey matter neurodegeneration of limbic olfactory-related structures such as the piriform cortex and the amygdala occurs within the mild cognitive impairment stage of AD.

104 **Extrasynaptic $Gaba_A$ Receptors In The Gustatory Cortex And Taste-Dependent Impulsive Behavior**

Priscilla E. Yevoo, Alfredo Fontanini, Arianna Maffei
Dept. of Neurobiology and Behavior, SUNY Stony Brook, Stony Brook, NY, United States

Impulsivity is a trait that can be advantageous; though, when excessive, it becomes maladaptive. Heightened impulsivity is a predictor and consequence of many neuropsychiatric conditions, including eating disorders. Structural and chemical anomalies in the insular cortex (IC) are linked to abnormal impulsive behavior (IB). A portion of IC - the gustatory cortex (GC) - processes taste, reward expectation, and decisions about food intake, making it a relevant model for studying cortical mechanisms underlying IB related to feeding. Here, we test the hypothesis that altered levels of tonic, neurosteroid-modulated, GABAergic inhibition in GC contribute to taste-dependent impulsive choices. We use immunohistochemistry to assess the expression of neurosteroid-sensitive extrasynaptic $GABA_A$Rs and show that these receptors are present in GC. To explore the receptor's functionality, we use patch-clamp recordings from GC neurons in acute slices and observe an increase in tonic currents following neurosteroid application. We then examine the behavioral effects of local neurosteroid infusion in GC. Employing the Go/No-Go task, we evaluate mice's ability to inhibit licking for sucrose in response to a No-Go cue. Our data show that infusions of neurosteroids modulate licking behavior, implying that GC plays a role in mediating ingestive behaviors and that tonic inhibition is likely to control licking to predictive cues. This work provides novel insights into differences in neural network physiological properties underlying taste-dependent IB. A precise understanding of the mechanisms underlying inhibitory control deficits will provide novel biomarkers for detecting, preventing, and treating impulsivity-related neuropsychiatric disorders.

105 **A Novel Approach To Cue-Guided Taste Association Training**

Emma A. Barash, Daniel Å. Svedberg, Hannah F. Germaine, Donald B. Katz
Brandeis University, Waltham, MA, United States

Survival is inextricably tied to consumption decisions; toxic foods can lead to illness/death, while nutrient-rich foods promote good health. Thus, it is useful to associate cues (ex. the color of a fruit) with a post-consumption outcome (ex. eating a red fruit made me sick) to guide approach-avoidance decisions. While cue-driven-association research is common, little research focuses on the role of chemosensation in cue-driven foraging/consumption. Therefore, we developed a novel experimental framework dedicated to this question. Results from cue-“food reward” association tasks in literature show that rats learn associations in ~11 - 15 days, but the tasks used are often minimally complex. We aimed to not only improve upon the learning rate, but do so in a complex multi-step response task. We designed a paradigm with a cue-trigger-retrieval-reward sequencing, with visual-auditory cues that pair with unique tastants – citric acid, water, and sodium chloride – ranging from least to most palatable, respectively. Our findings indicate that the optimal procedure to increase speed of learning involves first training rats the “retrieval-reward” component with a neutral palatability water reward, then expanding to “cue-trigger-retrieval-reward” with varying palatability rewards. The preliminary results show a fast learning rate as well as expected behavior with rats displaying a decreased latency to response for cues indicating higher palatability rewards, and the inverse for cues indicating lower palatability rewards. In the future this paradigm will be expanded to include electrophysiological interrogation of neural representations of anticipation, decision, and response in the gustatory cortex to understand the neural underpinnings of the differential behavior for different palatability cues.

106 **Olfactory Training In Menopausal Women: Piloting An Alternative Design**

Cristina Jaén, Steven Rowe, Robert Pellegrino, Pamela Dalton
Monell Center, Philadelphia, PA, United States

Women show heterogeneous changes in olfactory acuity in the 5th decade of life that are associated with the peri- and post-menopausal phases. Women suffering smell loss due to hormonal changes have not been investigated for effective treatments. We evaluated 28 peri- post-menopausal women aged 50-65 for their sensitivity to 3 low (LMW) and 3 high (HMW) molecular weight odorants as well as 2 LMW and HMW odor mixtures, before and after a modified, remotely-monitored olfactory training method (sorting 3 different concentrations of training odorants into ascending intensity). There were two treatment groups (training on the LMW (n= 9) or HMW set of odorants (n= 9)) and a Control group (performing a visual exercise, n=10) that trained for 36-42 sessions (in a period of 5-8 weeks). In addition to thresholds, odor identification, odor memory, and salivary hormones

(estradiol, progesterone, and testosterone levels) were quantified. The LMW treatment group improved their odor thresholds, however, training with individual odorants did not improve sensitivity towards the odor mixtures, nor improve odor memory or odor identification. We also observed an overall negative correlation between testosterone levels and improvement in threshold acuity. Despite the limited improvement in olfactory sensitivity, the modified training method resulted in good compliance and could be deployed for use in future studies.

107 **Effects Of Diversity In Olfactory Environment On Children'S Sense Of Smell**

Lenka Martinec Nováková
Department of Psychology and Life Sciences, Faculty of Humanities, Charles University, Prague, *, Czech Republic

Diversity in children's everyday olfactory environment may affect the development of their olfactory abilities and odor awareness. To test this, we collected data on olfactory abilities using the University of Pennsylvania Smell Identification Test and on odor awareness, that was assessed with Children's Olfactory Behaviors in Everyday Life Questionnaire and Odor Awareness Scale (OAS), in 150 school-age children. Parents completed an inventory on children's exposure to a variety of odors and on their own odor awareness using the OAS. The effects of age and executive function on the children's performance were controlled for. It was found that the children's odor identification scores differed as a function of parental odor awareness. Although these effects were rather small, they were commensurate in size with those of gender and age. Future studies should consider the long-term impact of perceptual learning out of the laboratory and its consequences for olfactory development.

108 **The Impact Of Taste Experience On Long-Term Taste Learning And Memory Persistence**

Annika Patterson, Carl Hayes, Veronica Flores
Furman University, Greenville, SC, United States

Most living beings view the world through the lens of their acquired knowledge and experiences, adapting to their environment to survive and thrive. Previous work has shown that experience with innocuous tastes strengthens a future aversion towards a novel taste (Flores Et, al 2016, 2018, 2021). This study continues to explore the influence of innocuous taste experiences on the persistence of this strengthened conditioned taste aversion (CTA) over time. Naive rats were given salty and sour tastes followed by a CTA towards novel sucrose. In aiming to understand the strength of memory persistence we tested the rats at intervals of 24 hours, 48 hours, 1 week, or 2 weeks post-CTA. By examining behavioral consumption data and neural markers of immunohistochemistry we can determine the strength and persistence of the learned association. We specifically examined Neuronal PAS domain protein 4 (NPAS4) activity. NPAS4 is an immediate-early gene whose activity is specific to synaptic plasticity mechanisms, indicative of the learning process. By accounting for NPAS4 within the gustatory cortex (GC), a region of neural tissue linked to taste processing, we can determine GC's role in memory and innocuous experience-dependent learning. We hypothesize that the group receiving innocuous taste experiences will show a stronger learned association and that memory for the aversion will persist for longer amounts of time than the water exposure group. Our preliminary results begin to confirm that the innocuous taste exposure group showed a stronger persistence of the aversion towards sucrose at 2 weeks and that NPAS4 activity correlated with this behavior. These results support and build on past literature and give us a better understanding of how these innocuous taste experiences enhance aversion learning and memory as well as the role of GC in CTA plasticity mechanisms.

109 **Individual Variability In Olfactory Measurements**

Steven T. Rowe, Robert Pellegrino, Paul M. Wise, Pamela Dalton
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Studies have shown considerable variation in olfactory measures of acuity. The present study examines how a diverse group of participants perform over time on detection and recognition thresholds. Using gas-phase precision olfactometry, we are able to examine previous trends found in olfactory threshold literature. Twenty-eight participants were selected based on sensitivity to n-butanol (low, medium, or high sensitivity). They completed two phases of testing with an average of 73 days between phases. Phase 1 consisted of 10-18 sessions, in which detection and recognition thresholds were obtained across a possible 4 blocks of testing per odorant. Each block consisted of 8 stimulus presentations in a 3-alternative forced choice ascending method of limits design. Phase 2 had ten sessions of the same design. Individuals' performance improved rapidly, reaching a plateaued capacity by session 3, with recognition improving more drastically than detection acuity. Individuals maintained this heightened acuity after a break between testing phases. Contrary to previous studies, variance in performance was greater between subjects than within individual subjects. This disagreement in variance decreased over time. Our study highlights the importance of training on olfactory tasks to improve data quality.

110 **Identification And Investigation Of The Role Of An Amygdalar-To-Ventral Striatum Circuit In The Context Of Odor Memory Formation**

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Odors are potent triggers of emotional responses. Subserving this, the amygdala is well known for its critical role in influencing olfactory behavior, yet the precise cellular mechanisms whereby the amygdala influences odor-

evoked affective responses remain elusive. Using retrograde viral tracing in combination with transgenic mice, we have identified two distinct populations of amygdala projection neurons, specifically in the lateral portion of the basolateral amygdala (BLA), which innervate the tubular striatum (TuS; also known as the olfactory tubercle). These neurons express either the *drd1* or *drd2* gene, which encode for the dopamine D1- or D2-like receptor, respectively. Using anterograde viral tracing we found that both cell types innervate the entire span of the TuS. Interestingly, we found that three times more *drd1* neurons innervate the TuS than *drd2*. Furthermore, while the BLA is comprised of both GABAergic and glutamatergic neurons, and both have been shown to project outside of the BLA, through both RNAscope and immunohistochemistry, we found that TuS projecting D1 and D2 receptor expressing BLA neurons appear to be solely glutamatergic. Since work by several groups, including ours, has established that the TuS is a key region for linking odor information with learned responses, our ongoing work aims to resolve the potential contributions of these distinct neural circuits on olfactory memory formation and to define the synaptic basis for the interaction between the BLA and TuS.

111 **Computational Molecular Interaction Maps Of Signaling Events Within The Olfactory Epithelium**

Federica Genovese[1], Shailendra Gupta[2], Suchi Smita[2], Dominique Fastus[2], Krishna Pal Singh[2], Matti Hoch[2], Olaf Wolkenhauer[2,3], Antonella Di Pizio[3]

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In the olfactory epithelium (OE), multiple mechanisms, like odor detection, cell regeneration, and differentiation are vulnerable to a variety of external and/or internal factors. However, the understanding of the cell-to-cell communications and molecular events associated with these mechanisms are still not fully characterized. To provide a global vision of the OE and cross-talks between its different cell types, we prepared maps related to signaling and molecular events in sustentacular cells, microvillous cells, Bowman's glands, trigeminal nerve fibers, horizontal basal cells, globose basal cells, and olfactory sensory neurons accessible via an interactive, searchable, web-based platform through MINERVA, a well-established tool used for the presentation of disease maps (https://www.sbi.uni-rostock.de/minerva/). The molecular single-cell and interaction maps we developed will serve to conceptually visualize and analyze complex mechanisms within single cell types as well as among different cell types. The developed maps provide various entry points to the users to access the manually curated information at the cellular, process/pathway, and molecular level. The maps are designed with the aim to serve heterogeneous communities involved in olfaction including clinicians, research scientists, systems biologists, and industrial partners. In the web platform of the maps, users can identify and prioritize diagnostic/therapeutic markers associated with various olfactory diseases. For this, we developed various user-friendly plugins that help in mapping and analyzing experimental and clinical data directly onto the map. Here we provide a quick overview of manually annotated known signaling events within OE cells and highlight knowledge gaps that need further investigation.

112 **Investigation Of Nasal Solitary Chemosensory Cells By Light Sheet Microscopy**

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Solitary chemosensory cells (SCCs) are specialized epithelial chemosensory cells that can be found throughout the respiratory epithelium of the nasal cavity. Activation of these cells has been linked to irritant-induced inflammation which involves a signaling cascade comprised of known chemoreceptors (e.g. bitter taste receptors), the canonical taste-signaling molecules α-gustducin and phospholipase C β2 (PLCβ2), and transient receptor potential cation channel subfamily M member 5 (TRPM5). Our previous work has identified regional heterogeneity of SCCs based on molecular markers, density, and innervation patterns. However, these studies relied on IHC on tissue sections that represent <1% of the volume of the mouse nasal cavity creating potential subsampling issues. Here, we present a high throughput protocol to effectively map the majority of the nasal cavity using immunofluorescence. Whole mouse nasal regions were fixed with 4% paraformaldehyde and preserved using SHIELD. SHIELD-preserved samples were removed of light scattering lipids using an active protocol involving a sodium dodecyl sulfate based buffer combined with electrophoresis (LifeCanvas Smart Clear II). After detergent removal, samples were passively immunolabeled. Cleared samples were then incubated in refractive-index matching solution to achieve a uniform refractive index (~1.42). Index-matched samples were mounted in refractive index matching solution with 2% agar in a custom imaging chamber and imaged on a Nikon A1R laser scanning microscope, or custom-built light sheet microscope. For this study, we used TRPM5GFP mice to identify SCCs and demonstrate the capability of imaging large, heterogenous tissue types using optical clearing and lightsheet microscopy. This work was funded by NIH/NIDCD R21DC018864 to EDL.

113 **A 3D Transcriptomics Atlas Of The Mouse Nose Sheds Light Into The Anatomical Logic Of Smell**

Mayra L. Ruiz Tejada Segura[1,2,3], Eman H. Abou Moussa[4], Elisa Garabello[5,6], Thiago S. Nakahara[7], Melanie Makhlouf[4], Filippo Valle[5], Susie S. Y. Huang[4], Joel D. Mainland[8,9], Michele Caselle[5], Matteo Osella[5], Stephan Lorenz[4,10], Johannes Reisert[8], Darren W. Logan[10], Bettina Malnic[7], Antonio Scialdone[1,2,3], Luis R. Saraiva[4,8,11]

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Odor detection is initiated by the activation of olfactory receptors (ORs) expressed in olfactory sensory neurons (OSNs), which populate the main olfactory epithelium (MOE). Most mature OSNs express a single intact OR gene in a monoallelic fashion, and OSNs expressing the same OR are organized across multiple, partially overlapping spatial zones along the dorsal-ventral axis of the MOE. Many studies have focused on the molecular mechanisms and organizational strategies underlying mammalian olfaction, but due to the complexity of the system several key questions remain unanswered. For example, what is the total number of existing spatial zones in the MOE? What is the putative functional role of these zones in olfaction? To tackle these and other questions, we generated a browsable, genome-wide 3D transcriptomic atlas of the mouse MOE. First, using this 3D map of gene expression, we were able to identify new zonal markers, and to computationally define the expression zones for hundreds of ORs and other genes. Second, we successfully validated some key expression patterns through *in situ* hybridization. Third, we combined the 3D atlas with published single-cell RNA-seq datasets to get further insights into the spatial gene expression patterns of other cell types populating in the MOE. Last, we found out that the spatial pattern of ORs correlate with the mucus solubility of the odorants they detect; providing direct evidence for the chromatographic theory of olfaction. In conclusion, our results allowed for a better understanding of the organizational strategies of the mouse olfactory system, and it serves as a resource/platform for future studies focused on deconstructing mammalian olfaction.

114 **Investigation Of Ca^{2+}-Activated Ion Channels In Mouse Vomeronasal Sensory Neurons**

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The mouse accessory olfactory system regulates inter-and intraspecific communication. A multitude of semiochemicals serve as social cues and are detected by vomeronasal sensory neurons (VSNs) in the vomeronasal organ. Each VSN expresses a distinct member of one receptor gene superfamily (V1R, V2R, or FPR-rs) in its apical microvillar membrane. Here, receptor activation triggers a G-protein coupled signaling cascade resulting in Ca^{2+} influx and signal amplification via Ca^{2+}-activated Cl^- efflux. However, our knowledge of Ca^{2+} signaling and its compartmentalized function(s) in VSNs is limited. Therefore, we investigated Ca^{2+}-activated channels in different VSN compartments, focusing on somata. To isolate currents elicited at the soma, we combined targeted Ca^{2+} uncaging with whole-cell electrophysiology, Ca^{2+} imaging, and pharmacology. This approach revealed distinct Ca^{2+}-activated potassium and chloride currents. Notably, individual VSN current profiles appeared heterogeneous, potentially reflecting subpopulation-specific channel repertoires. Together, our data extend the established concept of VSN Ca^{2+} signaling by emphasizing additional functions of Ca^{2+}-dependent channels in VSN somata.

115 **Investigating Antiviral Response Of Hsv-1 Infection In The Olfactory Epithelium**

Sandy Vang[2,3], Laetitia Merle[2,3], Arianna Gentile Polese[2,3], Christy Niemeyer[1], Diego Restrepo[2,3]
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The COVID-19 pandemic has shed light on post-viral loss of smell, but the antiviral response of the olfactory epithelium (OE) during viral infection remains unclear. The OE is located in the nasal cavity and is constantly exposed to airborne pathogens. Additionally, it is not known how the OE and cells within this unit contain viral infection and limit viral spread to the brain. One pathogen of interest is herpes simplex virus-1 (HSV-1). HSV-1 is a common and lifelong virus that is contracted either orally or intranasally. HSV-1 can travel along the trigeminal cranial nerves and establishes latency within the trigeminal ganglion. Upon reactivation events, HSV-1 can induce pathological changes in the brain that are hypothesized to promote Alzheimer's disease (AD). Olfactory dysfunction is a common and early symptom of AD, but whether HSV-1 promotes AD-related olfactory deficits remains debatable. We investigated how the OE induces an antiviral response in the presence of HSV-1 (10^6 pfu per animal, McKrae strain) in 7, 24, and 72 hours post-infection in C57BL/6 male and female mice. We characterized the infection pattern across the nasal cavity. Surprisingly, we found few infection spots. The infection spots were located in the respiratory and olfactory epithelium, as well as in Steno’s gland. Infected areas were associated with tissue degeneration and macrophages infiltration. HSV-1 was also rarely found in the olfactory bulb (OB). We hypothesize that rapid OE degeneration prevents HSV-1 from entering the brain through the OB. However, the tissue degeneration in the respiratory and olfactory epithelium might give HSV-1 access to trigeminal fibers endings, allowing the viral spread to the trigeminal ganglion. Our data indicate OE viral response protects the central nervous system from infection.

117 **A Tas2R-Mediated Signaling Pathway In Nasal Solitary Chemosensory Cells Triggers Mice Avoidance Behavior To Inhaled Nebulized Irritants Without Involving The Taste And Olfactory Systems.**

Ranhui Xi[1,2], Sean McLaughlin[3], Ernesto Salcedo[4], Marco Tizzano[1]

[1]University of Pennsylvania, Philadelphia, PA, United States, [2]Sichuan University West China Hospital of Stomatology, Chengdu - Sichuan, *, China, [3]Brain Institute, Florida Atlantic University, Jupiter, FL, United States, [4]University of Colorado Anschutz Medical Campus, Aurora, CO, United States

The nasal epithelium houses a population of solitary chemosensory cells (SCCs) that express T2R (bitter) taste receptors and taste transduction signaling components. These cells are innervated by peptidergic (subP) trigeminal polymodal nociceptive nerve fibers and use acetylcholine as the neurotransmitter. Nasal SCCs respond to bitter compounds including bacterial metabolites evoking protective respiratory reflexes, as well as innate immune and inflammatory responses (Tizzano 2010, Saunders 2014). Here we tested whether SCCs were implicated in aversive behavior to specific inhaled nebulized irritants using a custom-built dual-chamber forced choice device. Mice behavior was recorded, and a MATLAB script was used to analyze the time spent in each chamber. When 10 mM denatonium (DEN) or cycloheximide was nebulized in one of the two chambers, wild type mice exhibited an aversion to the irritant mist chamber and spent most of the time in the control chamber (saline). Moreover, WT mice avoided the irritant more quickly with subsequent exposures (time points: naïve, 1hr and 6hrs). TrpM5-, Gnat3- and Skn1a-KO mice showed no aversion to the irritant at 2mM and developed an attraction at 10mM. This was attributed to the irritant's smell component as demonstrated by ablation of the olfactory epithelium post methimazole IP treatment. The P2X2/P2X3-KO mice that lack the taste but not the SCC pathway were used to show noninvolvement of bitterness in the aversive behavior. Like WT mice, P2X2/P2X3-KO showed aversion to DEN excluding a bitter taste-mediated aversion. The results demonstrate that activation of SCCs leads to a rapid aversive response to certain classes of irritants. This SCC-mediated avoidance behavior represents an important defense mechanism against inhalation of noxious chemicals.

118 **Characterization Of Chemosensory Dysfunction In Individuals Affected By Long-Term Covid-19 Via Direct And Self-Reported Methods**

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Chemosensory dysfunction (CD) is one common post-acute sequela of COVID-19 (PASC). Depending on the type of test used to measure it (self-report vs. direct test), the reported degree of CD in PASC can be inconsistent. We aimed to quantify within the same group of participants smell, taste and chemesthesis in PASC by both self-report and direct methods. 112 participants (81 women, mean age=42±14 y/o, duration of CD since COVID-19 diagnosis=245±120 days) completed the Smell-&-Taste-Check by the *Global Consortium for Chemosensory Research* which includes self-reports on smell, taste and chemesthetic abilities as well as intensity ratings of unstandardized smell, taste and chemesthetic stimuli (household items). Participants also completed SCENTinel, a validated, direct rapid smell test. PASC duration did not modulate olfactory performance at self-reported abilities (smell: p=0.35; taste: p=0.69; chemesthesis: p=0.93), intensity ratings (smell: p=0.93; taste: p=0.57; chemesthesis: p=0.78) and SCENTinel (p=0.18). We found a positive association between self-report, unstandardized direct test and validated direct test for smell (self-report vs. intensity rating: r=0.73, self-report vs. SCENTinel: r=0.59; intensity rating vs. SCENTinel: r=0.53; all ps<0.001), indicating moderate to large agreement across measures. Performance to SCENTinel was significantly associated with self-reported anosmia (70%) and hyposmia (20%) (p=0.04). A positive association between self-reports and intensity of household items was also retrieved for taste (r=0.55, p<0.001) and chemesthesis (r=0.49, p<0.001). We confirm in an independent sample the impairment of smell, taste and chemesthesis in PASC by using and comparing self-report and direct methods and we contribute to the discussion on best practices to monitor CD in PASC.

119 **Enhancement Of Taste By Retronasal Odors In Wolfram Syndrome**

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Wolfram syndrome (WFS) is a rare genetic disease with a wide spectrum of symptoms, including, impaired vision, audition, and olfaction. We recently found that in a young cohort of WFS patients (~15 yrs. old), olfactory impairment was related to smell identification (not insensitivity), and gustation was overall well conserved. Due to the documented smell dysfunction, we hypothesized that retronasal smells would not enhance taste intensity in participants with WFS. Last year we presented preliminary findings that provided support for this hypothesis, albeit when contrasting data in 27 WFS participants to data from a much older, historical, healthy control (HC) group (HC: 40±14 yrs. vs. WFS: 20±6 yrs.). Here we provide a new analysis of the data when compared to an age equivalent HC group (n=30, 19 ±3 years). We assessed taste and smell intensity using the general Labeled Magnitude Scale and used solutions of sucrose with strawberry extract, citric acid with lemon extract, sodium chloride in a vegetable broth, and caffeine in coffee. Participants taste these solutions and rated perceived intensities with and without noseclips. We found that compared with the age-equivalent HC group, ratings of smell intensity were reduced by ~half in the WFS group (all P<0.01); however, in contrast to our hypothesis (and previous preliminary findings), retronasal smell similarly increased taste intensity of sucrose, sodium chloride, and caffeine solutions in WFS and age-equivalent HC groups (main effect of noseclip for all P<0.03). In conclusion, because of blunted smell intensity, the perception of flavor is decreased in patients with

WFS, but taste-smell central interactions appear well preserved. Future studies exploring age-related differences in the enhancement of taste by retronasal odors are warranted.

120 **Effects Of Olfactory Training On Olfactory Dysfunction Induced By Covid-19**

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Purpose: Upper respiratory infections are among the main causes of olfactory dysfunction (OD). This symptom is also seen in 60% of people with COVID-19 and may last beyond resolution of the infection. The most promising intervention for post-viral OD is olfactory training (OT), which involves exposing the olfactory system to a range of odors daily. This approach would promote the regeneration of olfactory receptor cells, but its effectiveness must be verified in patients with post-COVID-19 OD. Methods: This randomized clinical trial compared the effectiveness of OT vs placebo in the treatment of patients with post-COVID-19 OD. 25 participants were recruited in each group ($\alpha = 0.05$; $1\text{-}\beta = 0.9$). OT protocol consisted of sniffing 4 scents (rose, orange, clove and eucalyptus) for 5 minutes twice daily for 12 weeks. Olfactory function was assessed before and after the training using (1) a validated odor identification test (UPSIT-40) and (2) a 10-point visual analog scale. Results: OT did not influence the UPSIT score. However, time had a significant effect on self-evaluation of smell function and a trend was observed for group*time. Also, participants from the intervention group exhibited a significant increase in their self-evaluation of smell (before: 3.8 (1.9) points; after: 5.4 (1.8) points; p=0.002) while no such effect was observed in the control group (before 3.5 (1.8) points; after: 4.2 (2.4) points). Conclusions: This study highlights an increase in subjective, but not objective olfactory function when performing OT for 12 weeks. As this is a phase one clinical study, more data is required to assess the efficacy of OT for treatment of post-COVID-19 OD.

121 **A Selective Deficit In Episodic Memory Of Odor Percepts Increases Risk Of Progression To Diagnosis Of Amnestic Mci Or Alzheimer&Rsquo;S Disease In Cognitively Normal Seniors**

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There is an unmet need for affordable, noninvasive biomarkers and prediction algorithms to identify healthy preclinical individuals at high risk for progression to amnestic-MCI (aMCI) or Alzheimer's disease (AD). While olfactory deficits have been described across the clinical spectrum of AD, the use of olfactory impairment as a biomarker for progression of preclinical and clinical stages of AD has been limited by the baseline variance of olfactory function in humans. Our team developed an algorithm that combines performance of odor identification and odor discrimination to set a personalized threshold for an odor percept memory test. Using the Percepts of Odor Episodic Memory (POEM) test battery, we tested 201 older individuals who were cognitively normal (CN) or expressed subjective cognitive concerns (SCC), to determine whether odor percept memory deficits predict progression to an aMCI or AD diagnosis. A Cox Proportional Hazards model was used to evaluate the relationship between olfactory deficits and clinical progression. Participants identified with an odor memory deficit using the POEM algorithm (28%) were more likely to progress to aMCI or AD over four years with 47% deemed "at risk" being diagnosed with aMCI or AD relative to 9% not identified as "at risk" (HR=3.45, (95% CI: 1.13-10.72; *P*-value=.0278)). Thus, episodic odor memory deficits predict faster progression to the diagnosis of aMCI or AD in cognitively normal elderly individuals. Validation of these findings in independent cohorts will enable the POEM algorithm to serve as a biomarker for cognitive decline in healthy seniors to reduce the screen failure and increase the effect size of research studies and clinical trials.

122 **Scentinel 1.1 Rapidly Screens For Parosmia**

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After smell loss, many individuals develop parosmia, which is the distorted perception of the quality of some odors. Directly testing for parosmia would help understand its prevalence in the population, however there is only one test, which cannot be self-administered, that screens for parosmia. To fill this gap, in the 1.1 version of SCENTinel, a rapid smell test to screen for smell loss, we included a hedonic subtest to screen for parosmia in addition to the prior validated subtests detection, intensity, and identification. The hedonic subtest calculates a hedonic score, which is the difference between the pleasantness of the odor smelled minus the pleasantness of the imagined smell of unpleasant odor. Subtests are measured for one of four different odors (popcorn, coffee, flower, and bubblegum). The overall SCENTinel 1.1 score discriminates parosmia (n=77) from hyposmia (n=84; AUC=0.89) and from anosmia (n=51; AUC=0.82). Hedonic score and parosmia frequency were negatively associated (r=-0.2; p<0.001). Those with parosmia report a significantly lower hedonic score as compared to those without parosmia for all odors (p<0.001), but do not differ in the hedonic rating of an imagined unpleasant odor (p=0.27). We conclude that SCENTinel 1.1 is a direct smell test that can rapidly and accurately screen for parosmia.

123 **A Quick Home Test To Objectify Olfactory And Taste Dysfunction : Validation Of The Chemosensory Perception Test**

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Chemosensory testing is important to monitor COVID-19 and other health conditions. We developed the Chemosensory Perception Test (CPT), a semi-objective self-administered test of orthonasal olfactory, retronasal olfactory, and gustatory function that can be used remotely in the context of COVID-19, using common North American household items as stimuli. Here we compare CPT scores with participants' subjective complaints and with standardized testing. In Experiment 1, we administered the CPT and the Sniffin'Sticks Test (SST) to 36 participants (20 M, 16 F, mean age: 68.6; olfactory dysfunction - OD: 17); in Experiment 2, we administered the CPT, the University of Pennsylvania Smell Identification Test (UPSIT), the Brief Waterless Empirical Taste Test (B-WETT), and a chemosensory questionnaire to 85 participants (22 M, 63 F, mean age: 45.5; OD: 42). In Experiment 1, all CPT scores significantly correlated with SST scores, with orthonasal (ϱ=0.837, p<.001) and retronasal scores (ϱ=0.732, p<.001) presenting the strongest association. The ability of the orthonasal CPT score to correctly identify olfactory dysfunction was excellent (AUROC: 0.923 [95% CI, 0.822-1.000], p<.001). In Experiment 2, the orthonasal CPT score was the only one correlated with UPSIT (ϱ=0.364, p<.001) and B-WETT scores (ϱ=0.277, p=0.011). The ability of the orthonasal CPT score to correctly identify participants' olfactory status was good according to their subjective olfactory complaint (AUROC: 0.829 [95% CI, 0.746-0.913], p<.001), but poor according to objective olfactory testing (AUROC: 0.648 [95% CI, 0.530-0.767], p=.018). Our results suggest that orthonasal and retronasal CPT scores are a promising tool in the context where remote testing is necessary. However, the gustatory CPT score's usefulness remains to be proven.

124 **Defects In Olfactory Bulb Presynaptic Vesicle Trafficking And Reduced Neurogenesis Cause Olfactory Deficits In A Mouse Model Of Parkinson&Rsquo;S Disease**

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Parkinson's Disease (PD) is a neurodegenerative disorder characterized by the early emergence of olfactory deficits. Despite the efforts made to understand the relationship between loss of smell and PD, the mechanisms that lead to this dysfunction remain unclear. In this work, we provide mechanistic insights for the cause of olfactory deficits by using a mouse model of PD that expresses the human A30P α-synuclein (α-syn) mutation. We studied animals at 6-7 and 12-14 months of age as representative of pre-symptomatic and symptomatic progression stages of the disease, respectively. Our results show strong accumulation of pathologic α-syn in projection neurons along the entire central olfactory pathway at both stages, with no apparent morphological disruptions. However, an olfactory test shows that only animals at symptomatic stages display olfactory deficits, suggesting that α-syn pathology is required much earlier that the appearance of symptoms. In contrast, our analysis of olfactory bulb (OB) neurogenesis revealed a reduction of granule cells only at pre-symptomatic stages, indicating different vulnerabilities of olfactory structures to α-syn pathology. In concordance with these results, a proteomic evaluation of OB and olfactory/piriform cortex (PC) tissues showed a downregulation of proteins involved in synaptic transmission and vesicular transport in the OB, whereas minimal changes are detected in the PC. Collectively, our data suggest that the A30P α-syn mutation induces olfactory deficits by: (1) accumulating α-syn pathology in OB and PC projection neurons; (2) reducing OB neurogenesis; and (3) causing defects in the OB synaptic endo- and exocytosis vesicle trafficking.

125 **Analyzing Human Olfactory Epithelium In Presbyosmia: Towards Identification Of Treatment Strategies**

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Presbyosmia, or aging related olfactory loss, impacts a large proportion of the elderly population. There are currently no specific treatment options, largely due to incomplete understanding of the pathobiology of presbyosmia. The olfactory epithelium (OE) in the nasal cavity houses the olfactory receptor neurons and is subject to acquired damage, suggesting a likely site of pathology in aging. Adult stem cells reconstitute the neuroepithelium in response to cell loss under normal conditions. In aged OE, patches of respiratory-like metaplasia have been observed histologically, consistent with a failure in normal neuroepithelial homeostasis. We have focused on identifying cellular and molecular changes in presbyosmic human OE, combining psychophysical testing with olfactory mucosa biopsy analysis, single cell RNA-sequencing (scRNA-seq), and culture studies. We identified evidence for inflammation-associated changes in the OE stem cells of presbyosmic patients. The presbyosmic basal stem cells exhibited increased expression of genes involved in response to

cytokines or stress, or epithelial repair. We further explored intercellular signaling pathways between immune cells and OE cells identified in presbyosmic samples. Our data suggest aging-related inflammatory changes in OE stem cells may contribute to presbyosmia, via the disruption of normal epithelial homeostasis. Ongoing efforts to evaluate signaling pathways identified in presbyosmic analyses may identify therapeutic targets for restoration of olfaction.

126 **The Best-Laid Plans Of Human Researchers Often Go Awry: Learning From Failure In The Third *Covid-19 Wave (Jul- Nov 2021)***

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During the first wave of COVID-19, the Global Consortium for Chemosensory Research (GCCR) successfully launched a large crowdsourced survey to investigate the self-reported loss of smell, taste, and chemesthesis. Using a similar approach, we investigated longitudinal changes in chemosensory function. Participants rated self-reported function and the intensity of common household and food items for smell, taste, and chemesthetic sensations over 12 weeks. Six months after launching the study, we discovered only 35 participants had completed the first day (38.4 ± 10.6yrs; 80% females). These data confirm that during COVID-19, participants report experiencing a significant loss in smell, taste, and chemesthesis function, but not nasal blockage (mean change on a 100 pt scale for smell: -75.1 ± 27.7; taste: -54.7 ± 36.0; chemesthesis: -38.3 ± 35.3). There was a positive association between self-reported chemosensory function and intensity rating for all sampled stimuli (smell ($r = 0.50$; $p = 0.02$), taste ($r = 0.48$; $p = 0.03$), and chemesthesis ($r = 0.58$; $p = 0.01$)). The severity of COVID-19 (estimated by the number of total symptoms reported) was significantly associated with the self-reported change in function for chemesthesis ($F1,25 = 7.17$; $p = 0.01$) but not for taste or smell. In addition, there was a significant negative correlation between severity and intensity ratings for salty ($r = -0.51$, $p = 0.02$) and sour ($r = -0.45$, $p = 0.04$) but not for sweet and bitter. Despite the study being carefully designed and programmed, we were not able to engage participants repeatedly over time and could not directly answer our pre-registered questions. In the spirit of open science, we hope sharing our failure will allow the community to learn from this setback and utilize this knowledge and shared materials.

127 **Olfactory Dysfunction (Od) Is Associated With Postoperative Neurocognitive Disorder (Pnd) In A Population Of Older Patients Scheduled For Elective Non-Cardiac Surgery**

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Objectives:OD has a well-known link with cognitive decline and may also represent a biomarker of frailty. Yet, the two latter are thought to be preoperative risk factors for developing PND. The aim of this study was to evaluate whether preoperative OD is associated with PND.
Methods:We conducted a prospective observational study including 79 patients aged from 65 years old and scheduled for elective non-cardiac surgery under general anesthesia. Olfactory function was examined using the Sniffin' sticks extended test (threshold, discrimination and identification, TDI-score on 48 points). OD was defined as TDI-score below the 25th percentile for age and gender. Baseline preoperative cognitive function was examined using the Montreal Cognitive Assessment 22-item (MoCA-22) test. At 3 months postoperatively, patients received a telephone interview in which they performed the MoCA-22 and were asked about any change in subjective cognitive concerns. PND was defined as either subjective cognitive change and/or a decline of at least 1 standard deviation in the postoperative MoCA-22. Statistical analysis was carried out using Kruskal-Wallis and chi-square tests.
Results:Incidence of PND at 3 months was 25.3%. Subjective cognitive complaints were found in 22.8% (18/79) of patients whereas an objective impairment in cognitive function was detected in 5.1% of patients (4/79). TDI-score was associated with the presence of PND since the PND group had a significantly lower median TDI-score (25.75[22.7-29.5] vs 30.5[26.5-32.5], p=0.011). Indeed, 45% of the patients with OD presented PND compared to only 18.6% of the patients with preserved olfaction (p=0.019).
Conclusions:This study demonstrates a significant association between preoperative OD and the incidence of PND in older patients undergoing elective surgery.

128 **Inhibition Of Mitochondrial Division Alters Response To Chemical Attractants In A Eukaryotic Microbe.**

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Dynamin-Related Protein 1 (Drp1) is critical for mitochondrial fission, a requirement for cell division. The Drp1 inhibitor mDivi1 results in a predicted reduction in fission and changes in mitochondrial phenotype. We have examined the effects of mDivi-1 treatment on *Paramecium tetraurelia*. *Paramecia* are large, multinucleate cells covered in cilia that allow them to swim to areas rich in food. When *Paramecium* are starved, a programmed

rearrangement of the macronucleus is triggered, likely by a caspase. The outcome is an easily observed, fragmented nucleus. Treatment with mDivi-1 delayed entry into macronuclear rearrangement and resulted in alterations in mitochondrial phenotype. Mitochondria became less punctate and showed denser fluorescence patterns, indicating increased networking. The nuclear phenotype was also observed when cells were treated with an AMP Kinase inhibitor, indicative of the well-documented involvement of this kinase as an indicator of nutritional status and mitochondrial health. We hypothesized that inhibitor-induced maintenance of the pre-starvation nuclear arrangement, coupled with larger mitochondrial networks may result in a reduced response to chemoattractants. Our initial data show a reduced swimming speed in cells treated with mDivi when exposed to the *Paramecium* attractant acetate mimicking swimming speeds seen in well-fed exponential cultures. Increased swimming speed and decreased turning are indicators of response to chemoattractants. We plan to pursue other attractants to determine if simulated availability of nutrients via mitochondrial fission inhibition impacts other response patterns.

130 **Behavioral Responses Of California Sea Lions (*Zalophus Californianus*) To Odors Detected In The Air And Under Water**

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Pinniped species (fur seal, sea lion, seal, walrus) have been shown to use olfaction in social (i.e., mother-young recognition) and foraging contexts. However, if some studies have revealed that pinnipeds can discriminate between natural and artificial odor cues in the air, such abilities remain weakly documented. Here, we designed a study to test behavioral responsiveness of California sea lions (n = 5) living under human care at La Flèche Zoo (a park renowned for its quality and ethics in France) when confronted with odors either in the air, or under water. Two categories of odors were presented to the animals: social odors (familiar and unfamiliar sea lions, human odors - zookeepers -, odors of a terrestrial carnivore - tiger) and non-social odors (food - fish - odors and putative repellents as camphor and menthol). Video recording allowed us to analyze and quantify behavioral variables such as contacting the odor source, displaying oral movements, emitting vocalization, or producing bubbles according to the environment. The results pinpoint that California sea lions positively respond to odor cues in the air, especially to food and social odors (except for the tiger odor, here), sometimes displaying certain responses only to a specific category of stimuli, i.e. vocalizations during exposure to food odors. Strikingly, California sea lions may also respond to odors detected under water, by releasing bubbles directed to the source of food odors, a behavior that looks like “bubble sniffing”. Finally, the animals appeared on some occasions to express avoidance responses to camphor. Confirming and continuing this work could open the door to applications both in captivity (animal welfare, animal-human relationships) and in the wild (deterrent device to limit depredation or interaction with fisheries).

131 **World Taste And Smell Day Association: Towards The Public Elevation Of The Chemical Senses**

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The COVID-19 pandemic demonstrated on a global level how all too often the chemical senses are not fully appreciated, until they are diminished. In 2020, a group of concerned individuals with science, industry, communication and direct experience with smell/taste loss came together to establish a day where the world could meet to celebrate the role of taste and smell. The mission of the World Taste and Smell Day (WTSD) Association is to create and sustain an international day devoted to elevating the senses of taste and smell. On September 14, 2021 WTSD hosted an online Exploratorium of the Joy and Science of Flavor (https://tasteandsmellday.tumblr.com/). This community-fueled online exhibition and related global social media efforts touched over 25,000 people. WTSD also featured a global panel discussion, several events in China and raised nearly $10,000 to support the three primary patient advocacy groups. Here is an opportunity for scientists to share their priorities and insights into what science has to offer to the public to raise awareness about these senses. The results of anonymous polls conducted at AChemS 2022 will be presented live at the poster session and made available to the community thereafter. The aim is to learn what messages the scientific community sees as key to communicate the importance of smell and taste to society.

132 **A Layered, Hybrid Machine Learning Analytic Workflow For Mouse Risk Assessment Behavior**

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Accurate and efficient quantification of animal behavior has multiple benefits in the quest to understanding the workings of the brain. An emerging approach within the rapidly developing machine learning (ML) field is to combine multiple ML-based algorithms to support automated quantification of animal behavior from video recordings. These so-called hybrid models have emerged because of limitations associated with supervised (e.g., random forest, RF) and unsupervised (e.g., hidden Markov model, HMM) ML classifiers. For example, RF models lack an explicit accounting for temporal relationships across video frames, and HMM latent states are often difficult to interpret. We set out to develop a hybrid model that integrates aspects of RF and HMM models, and did so in the context of a study of threat assessment to predatory odors (e.g., reptile fecal extracts, trimethylthiazoline). We utilized DeepLabCut to estimate the positions of mouse body parts. Positional features

were calculated using DeepLabCut outputs and were used to train RF and HMM models with equal number of states, separately. The per-frame predictions from RF and HMM models were then passed to a second HMM model layer ("reHMM"). The outputs of the reHMM layer showed improved interpretability over the initial HMM output, and improved the capacity to analyze temporal aspects of behavior. Finally, we combined the reHMM model outputs with selected positional features to train a third-layer HMM model ("reHMM+"). This reHMM+ three-layer hybrid model unveiled distinctive behavioral patterns that mice displayed in the presence of predator odors. We conclude that this layered, hybrid machine learning workflow represents a balanced approach for improving the depth and reliability of ML classifiers in chemosensory and other behavioral contexts.

133 **Genetics Of Bitter Taste Sensitivity In People Of Different Ancestries**

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To learn more about bitter perception in genetically diverse human groups, we investigated person-to-person differences in the bitterness of five medications and the relationship of perception to bitter receptor genotypes. Among participants selected were those living in the US or Canada who were recent immigrants from many countries worldwide (N=223). We studied drugs used to treat diseases in low-resource settings (i.e., praziquantel, tenofovir alafenamide, amodiaquine, and moxifloxacin) plus propylthiouracil and collected a saliva sample from each participant for genetic analyses using the Global Diversity Array. Person-to-person differences in the bitterness of these medicines were common and the individual ratings of all drugs except propylthiouracil were highly correlated ($r=0.30\text{-}0.55$, $p<1x10^{-5}$), indicating a shared common determinant of these person-to-person bitterness differences. Consistent with this observation, ongoing genetic analyses show a similar pattern, with variants of bitter receptors explaining the individual differences in bitterness ratings for these four medications (albeit at a nominal significance; $p<0.05$, $n=124$). In particular, genetic variants in *TAS2R2P*, a gene originally but perhaps prematurely annotated as a pseudogene, are associated with bitterness perception for the same four medications. Data collection is ongoing to target additional diverse groups and provide insight into genetic and sensory relationships.

134 **Psychophysical And Psychohedonic Sweetness Functions Have A Similar Shape Across Familiar And Unfamiliar Foods In Dutch Consumers**

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People typically like sweet foods, but dislike unfamiliar foods. However, it is unclear whether or not psychophysical (concentration-intensity) and psychohedonic (concentration-pleasantness) sweetness functions have a similar shape across familiar and unfamiliar foods. The main objective of this analysis was to investigate the effect of familiarity on the psychophysical and psychohedonic sweetness functions in equivalent liquid, semi-solid and solid foods. Twenty eight participants (11 M, 17 F; mean age 23.4 ± 4.2 y) evaluated the familiarity, perceived sweetness intensity (both 100-unit VAS) and preference (Ranking on a Scale) of 3 familiar and 3 unfamiliar sweet foods, each varying in 5 levels of sweetness. Unfamiliar foods, created by the addition of unfamiliar flavourings and colourings, were perceived as being less familiar than familiar ones ($M_{familiar}=77.5$; $M_{unfamiliar}=46.4$, $F(1,139)=66.5$, $p<0.001$). Perceived sweetness intensity increased linearly across sweetness concentration levels for all foods (*concentration*, $F(4,803)=387.6$, $p<0.001$), with unfamiliar foods on average being perceived as sweeter than familiar ones across all sweetness levels (*familiarity*, $F(1,803)=17.1$, $p<0.001$). Preferences were generally higher for familiar foods (*familiarity*, $F(1,803)=38.1$, $p<0.001$) and differed across sweetness levels (*concentration*, $F(4,803)=24.9$, $p<0.001$). However, there were no effects of familiarity on the shape of the psychophysical sweetness (*concentration x familiarity*, $F(4,803)=0.9$, $p=.85$) nor on the psychohedonic sweetness function (*concentration x familiarity*, $F(4,803)=0.7$, $p=.55$). These results indicate that familiarity, manipulated by flavour and colour, affects sweetness intensity and liking, but not the shape of psychophysical and psychohedonic sweetness functions.

135 **Bitter-Sweet: An Examination Of Taste On Person Perception**

Rachel S. Herz[1], Caitlin M. Cunningham[2], Theresa L. White[2]

[1]Brown University, Providence, RI, United States, [2]Le Moyne College, Syracuse, NY, United States

Few studies have examined whether the basic tastes of a food that we consume alters interpersonal behavior. Eating candy has been reported to increase agreeableness (Meier et al., 2012), presumably due to the sweet taste, though past experiences with post-ingestive consequences from calories is also possible. Bitter drinks, such as tea, coffee, and grapefruit juice have been reported to increase hostile behavior (Sagioglou & Greitemeyer, 2014) and alter financial decision making (Cai et al., 2017). However, many questions remain in this area of research,

including the robustness of behavioral changes and whether effects are mediated by calorie consumption. Our pre-registered study was aimed at addressing these issues by examining how sweet and bitter tasting drinks that were either caloric or non-caloric would affect person perception and alter the interpretation of ambiguous emotions expressed by male faces. Participants were female undergraduates. In Experiment 1, we compared the effects of drinking a small amount of regular Sprite with diet Sprite. In Experiment 2 study, we compared regular tonic water with diet tonic. In both studies, club soda was the control beverage. In general, we found that neither taste nor caloric content altered facial expression interpretation. However, interesting trends for how bitter taste altered mood were observed in Study 2. This work has implications for how the tastes of the food that we consume may influence our interactions with others.

136 **The Integration Of Gustation And Odor Signals In Flavor Perception Over Time**

Emma Ornduff, Joost Maier
Wake Forest University, Winston-Salem, NC, United States

Multisensory integration is a phenomenon that allows organisms to combine information from multiple senses in order to better perceive the world around them and make more informed, adaptive decisions (Stein 2012). An example of this phenomenon is the combination of taste and smell, known as flavor. Our lab has previously shown that taste and smell are integrated to inform flavor liking in rats. However, perception is a complex process, and taste-smell interactions may contribute differently to different aspects of flavor perception. Here, we focus on flavor detection and identification. We first trained rats to associate one odor with quinine and another one with sucrose. After confirming associations, rats performed a series of two-bottle tests to assess their ability to detect threshold concentrations of taste and smell components of associated pairs, as well as congruent and incongruent mixtures of these components. If taste-smell associations facilitate integration as previously observed in other multisensory systems, congruent combinations of very weak gustatory and olfactory signals will work together to enhance the detection of flavor. The results show that congruent mixtures were reliably detected whereas individual gustatory and olfactory components and incongruent mixtures were not. These findings support the idea that multisensory flavor mixtures are perceptually more salient than the sum of their components.

137 **Functional Analysis Of Pharyngeal Gustatory Receptor Neurons In *Drosophila* Larva**

Seungyun Yu, Jaekyun Choi, Min Sung Choi, Jae Young Kwon
Department of Biological Sciences, Sungkyunkwan University, Suwon, *, South Korea

Chemosensation plays important roles in the survival of organisms by sensing the external and internal environments and maintaining homeostasis. Among the chemosensory organs, gustatory organs play major roles in making a decision on whether to intake nutritive foods or avoid toxic materials which have harmful effects on organisms. We investigated the function of the dorsal pharyngeal sense organ (DPS), a major internal gustatory organ of *Drosophila* larvae, to elucidate complex food quality coding mechanisms. Using optogenetics and calcium imaging, we confirmed that several DPS neurons are important for sensing potential toxic chemicals and are hardwired to mostly aversive behaviors. For example, DP2 responded to non-neutral pH solutions and elicited aversive behaviors when artificially activated. However, DP3 responded to several amino acids and showed both characteristics of aversive neurons and attractive neurons when artificially activated. Further neuronal inactivation experiments revealed that DP2 neurons are responsible for the avoidance of non-neutral pH conditions, while DP3 neurons are necessary for the sensing of several amino acids and eliciting either attractive or repulsive behaviors in response.

138 **Theta-Beta Coupling In Orbitofrontal Cortex Underlying Olfactory Predictive Coding**

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The orbitofrontal cortex (OFC) plays a major role in multisensory integration and predictive coding. However, it is unclear how the OFC integrates information from other brain regions to achieve its function, especially in olfaction. Low and high frequency oscillations have been suggested to support communication between brain regions and local computations, respectively. We hypothesized that the OFC achieves olfactory predictive coding through cross-frequency coupling between low and high frequency oscillations. We used intracranial EEG recordings from a cued odor-sampling task and computed the strength of theta-beta coupling in the OFC. We first examined the overall theta-beta cross-frequency phase-amplitude coupling using the modulation index over a broad time window (0–2 s after the cue). To characterize the temporal dynamics of this coupling, we then calculated the modulation index using a sliding time window. These analyses revealed that cue-induced cross-frequency coupling is maximal in the lateral OFC. Furthermore, theta-beta coupling peaked at around 1 s following the cue, which is slower than oscillatory power increases previously observed in piriform cortex (PC). We found that low frequency power increases in OFC following cues differed significantly based on the specific odor being cued (Rose or Mint), whereas this was not the case in PC. Future analyses will include determining the frequency and direction of oscillatory coherence between OFC and PC following cues, and decoding of cue identity across oscillatory frequencies, prior to presentation of odor. These findings suggest that the orbitofrontal cortex might encode the identity of predictive codes in the olfactory system.

139 **Integrative Structural Modeling Reveals Functional Molecular Switches Of Human G Protein-Coupled Bitter-Taste Receptors**

Sébastien Fiorucci[1], Cédric Bouysset[1], Ysul Kim[2], Jody Pacalon[1], MeeRa Rhyu[2], Jérôme Golebiowski[3], Jérémie Topin[1]

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On the human tongue, the bitter taste depends on a large family of 25 taste receptors type 2 (TAS2R) belonging to the G protein-coupled receptor (GPCR) family and classified distantly related to class A GPCR. To date, the experimental structures have not been determined for any TAS2R and key residues controlling their function are still under debate. Here we streamline the modeling of these receptors using an integrative approach combining sequence analysis, molecular modeling and site-directed mutagenesis followed by functional assays. We provide a general approach for modeling all mammal TAS2R and identify functional motifs or residues which are central to understand how we perceive bitterness. Above the protocol which is transposable to all TAS2R, the identification of functional molecular switches lays the groundwork for the rational design of chemical modulators of bitter taste receptors. Such ligands will be of broad interest beyond food science since bitter-taste receptors are ectopically expressed in other parts of the human body besides the tongue. [1] Topin et al. Functional molecular switches of mammalian G protein-coupled bitter-taste receptors. Cell. Mol. Life Sci., 2021, 78, 7605-7615.

140 **Inhibition Of Sweet Taste By The Nonsteroidal Anti-Inflammatory Drug Ibuprofen**

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Introduction: Sweet taste is mediated, in part, by the T1R2-T1R3 GPCR. Whereas agonists of this receptor are extensive, few antagonists have been identified. Lactisole is an antagonist of human sweet taste perception and contains a 2-methylacetic acid moiety that binds to the T1R3 receptor. This moiety is also in phenylpropanoic nonsteroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen, which inhibits the T1R2-T1R3 receptor *in vitro*. We hypothesized that these NSAIDs are antagonists of human sweet taste as well. Methods: To test this hypothesis, we asked subjects to rate the sweetness intensity of caloric (sucrose) and non-caloric (sucralose) sweeteners at the same sweetness intensity with and without a pre-rinse of ibuprofen. We tested two concentrations of ibuprofen against six concentrations of each sweetener. Results: Ibuprofen inhibited the sweetness of both sucrose and sucralose. 13.5 mM ibuprofen significantly decreased the perceived sweetness of sucrose at 300 and 646 mM ($p<0.005$) and sucralose at 430 and 927 μM ($p<0.05$). 57 mM ibuprofen significantly decreased the perceived sweetness of sucrose at 139, 300, and 646 mM ($p<0.001$) and sucralose at 93, 200, 430, and 927 μM ($p<0.001$). Conclusion: Ibuprofen inhibited sweet taste perception of sucrose and sucralose in a dose-dependent manner. Chronic NSAIDs, particularly ibuprofen, are known to have benefits in metabolic diseases such as diabetes, Alzheimer's, and cancer. This could be due to anti-inflammatory impact, T1R antagonism, or both. The T1R2/T1R3 receptor is expressed widely in metabolic regulatory tissues and may be inhibited by circulating levels of ibuprofen, which may help explain the metabolic benefits of its chronic use.

141 **Evidence For Gustatory Sensory Ganglion Neurons That May Selectively Signal Glucose**

Sebastien Hayoz[1], Gennady Dvoryanchikov[1], William M Connelly[2], Nirupa Chaudhari[1,3], Stephen D Roper[1,3]

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Sugars and artificial sweeteners activate Tas1R2+Tas1R3 to elicit sweet taste. Glucose also activates taste cells via transporter uptake and this pathway is thought to trigger cephalic phase insulin release (CPIR). Taste cells appear to co-express Tas1Rs and glucose transporters. We asked whether there are separate neural pathways in the periphery for sweet taste *versus* CPIR, given that these two different outcomes seem to originate from the same taste bud cell. We used *in vivo* Ca^{2+} imaging of geniculate ganglion cells in *Pirt*::GCaMP6s mice (n=7) to test if separate ensembles of ganglion neurons subserve these two pathways. We tested 4 different stimuli: fructose and sucralose, which elicit sweet taste via Tas1Rs; glucose, which elicits sweet taste via Tas1Rs *and* evokes CPIR; and SolCarb, (a poly-glucose that weakly elicits CPIR but does not stimulate Tas1Rs). We applied these stimuli to the mouse tongue at concentrations that elicited Ca^{2+} responses ($\Delta F/F$) of similar amplitude in geniculate neurons (1-way ANOVA, $p>0.05$): 200 mM glucose, 100 mM fructose, 16% SolCarb, and 0.5 mM sucralose. Hierarchical clustering analysis showed 2 populations of geniculate ganglion neurons: one activated by all 4 stimuli and one activated by glucose alone. We suggest that the neural pathways for "sweet" versus "CPIR" already begin to separate at the level of the geniculate ganglion. We also tested a panel of 5 prototypic basic tastes: 10 mM citric acid (sour); 300 mM sucrose (sweet); 100 mM MSG/1 mM IMP (umami); 250 mM NaCl (salty); and 1 μM cycloheximide/0.3 mM quinine (bitter). Interestingly, out of a population of 113 neurons, sugar-responsive ones were more broadly responsive ($p<0.05$) to basic tastants (H entropy value: 0.58±0.06 vs. 0.37±0.03) than neurons that did not respond to sugars.

142 **Mixture Suppression Primarily Occurs In Broadly Tuned Neurons In The Geniculate Ganglion**

Tao Huang, Robin Krimm
Department of Anatomical Sciences and Neurobiology, University of Louisville School of Medicine, Louisville, KY, United States

Taste experience usually arises from a mixture of multiple taste stimuli. Frequently, mixtures produce a reduced response (mixture suppression). In this study, we examined the variation of mixture suppression between individual geniculate neurons to binary taste mixtures of citric acid with NaCl and sucrose by using calcium imaging. The degree of suppression to citric acid/NaCl and citric acid/sucrose mixtures varied considerably across neurons. Broadly tuned neurons had greater mixture suppression than narrowly tuned ones (r = -0.53, p <0.001). Specifically, neurons responding to more taste qualities had greater suppression than those responding to fewer taste qualities (p <0.05). Mixture responses were suppressed to a greater extent in neurons that responded to both stimuli in the mixture compared to neurons that responded to only one of the stimuli in the mixture (142 mM NaCl/10 mM citric acid, p<0.001; 137 mM sucrose and 10 mM citric acid p<0.001). In fact, neurons that only responded to NaCl showed no mixture suppression to NaCl/citric acid mixture (p=0.72). Similarly, neurons that responded only to citric acid or sucrose did not show suppression to a citric acid/sucrose mixture (p=0.392 and p=0.337, respectively). This indicates that taste bud cells transducing both stimuli in the mixture must drive a functional response in the neuron for most mixtures to show suppression. One exception is that neurons responding only to citric acid were suppressed by the citric acid/NaCl mixture (p=0.007). Since Type III cells respond to both citric acid and NaCl, one explanation is that NaCl in the mixture inhibits citric acid responses of Type III taste bud cells. None of these results specifically support lateral cell-cell inhibition as a mechanism mediating mixture suppression.

143 **Adaptation Effects Between Sucrose And Citric Acid**

Jonas Yde Junge, Glenn Hjorth Andersen, Ulla Kidmose
Food Quality Perception & Society, iSense Lab, Department of Food Science, Aarhus, *, Denmark

Drinking is a dynamic process that generally encompasses more than one sip. The perception of beverages changes sip to sip as a result of adaptation effects. Adaptation effects are often studied at high concentrations, hence results are not readily translatable to common beverages with lower concentrations. The aim of this study was to investigate adaptation effects (adaptation, cross-adaptation, and post-adaptation taste of water) between sucrose (25.0 g/L) and citric acid (1.14 g/L) solutions at intensities analogous to those in commercial beverages. The study was conducted in 50 healthy adults (35 F, age: 25.4±3.6). Adaptation and test solutions were isointense. In control trials, participants directly received the taste stimulation. In the adaptation trials, participants kept the adaptation solution in their mouth for one minute and spat it out, before receiving the taste stimulation via a custom-built gustometer. Immediately after stimulation, participants rated the sweet and sour intensity using generalized labeled magnitude scales. Adaptation percentages are mean differences between adaptation condition and control condition (no adaptation), then divided by the mean of the adaptation condition. Adaptation to citric acid reduced perceived sourness of citric acid by 17%, enhanced perceived sweetness of citric acid by 9%, and enhanced the perceived sweetness of pure water by 8%. Adaptation to sucrose reduced the perceived sweetness of sucrose by 24%, enhanced sourness by 20%, and enhanced the perceived sourness of pure water by 16%. Sucrose adaptation showed a higher impact on the perception of both sweetness and sourness than citric acid. Both citric acid and sucrose adaptation resulted in cross-adaptation enhancement. In particular, the enhanced perceived sourness from sucrose was remarkable.

144 **Trpm4 Regulates Taste-Evoked Calcium Signaling In Broadly Responsive Type Iii Taste Cells**

Kathryn F Medler[1], Debarghya Dutta Banik[1, 2]
[1]University at Buffalo, Buffalo, NY, United States, [2]Indiana University School of Medicine, Indianapolis, IN, United States

Peripheral taste receptor cells use multiple signaling pathways to transduce taste stimuli into output signals that are sent to the brain. We recently identified a subpopulation of Type III taste cells that are broadly responsive (BR) to multiple taste qualities including bitter, sweet, umami, and sour. BR cells use a PLCβ3/IP_3R1 signaling pathway to detect bitter, sweet, and umami stimuli while a separate signaling pathway is used to detect sour stimuli. To date, the other components of the PLCβ3 signaling pathway in BR cells have not been identified. Our recent experiments have found that TRPM4, a monovalent selective TRP channel, is an important downstream target of the PLCβ3 pathway. Live cell imaging on isolated mouse taste receptor cells found that inhibition of TRPM4 activity abolishes taste-evoked sodium responses in BR cells. Interestingly, the taste-evoked calcium signals in BR cells are also significantly reduced when TRPM4 activity is inhibited. Since BR cells are a subpopulation of Type III taste cells, they have conventional chemical synapses and express voltage-gated calcium channels (VGCCs). Our data suggests that the membrane depolarization due to TRPM4 activity regulates at least some VGCC activity in BR cells. Unlike Type II taste cells that also detect bitter, sweet and umami tastants, the taste-evoked calcium signals in BR cells are comprised of both calcium release from internal stores as well as calcium influx through VGCCs. Our data indicate that TRPM4 links the initial taste-evoked calcium release to the subsequent calcium influx through VGCCs. Both of these calcium signaling events are needed to generate an appropriate output signal.

145 **Generation And Characterization Of Polarity Reversed Taste Organoids**

Jayaram Sakthi Prasad, Sunil K Sukumaran
Department of Nutrition and Health Sciences, University of Nebraska, Lincoln, NE, United States

Taste organoids cultured from primary taste stem/-precursor cells is an excellent model system to study various aspects of taste biology. However, in organoid culture using existing methods, the cilia that harbour the receptors

are oriented towards their inner core, limiting their application for tastant stimulation studies. The goal of our study is to develop polarity reversed taste organoids in which the cilia are oriented outward. Taste stem cells were isolated from circumvallate papillae of knock-in mice expressing a green fluorescent protein under the control of Leucine-rich repeat-containing G protein-coupled receptor 5 (Lgr5) promoter using fluorescent activated cell sorting. The initial stages of the culture employed Matrigel, as in the existing methods. To induce polarity reversal, Matrigel was removed on day 4 and the organoids were allowed to grow and differentiate in suspension cultures in low attachment plates while maintaining similar culture conditions. qRT-PCR and immunostaining were used to determine the expression of taste marker genes and proteins in polarity reversed and control organoids. The expression levels of most genes were largely comparable between the two models. After polarity reversal, the apical taste junction marker ZO1 is found to be localized on the surface of the organoids, whereas in regular organoids it was localized to the core, thus confirming successful polarity reversal. In future experiments, we will determine the cellular diversity of organoids using droplet-based scRNASeq. The suitability of these organoids for taste signalling studies will be determined using functional calcium imaging experiments. We believe that polarity reversed taste organoids represent a unique model system that can prove especially valuable for taste signalling studies.

8:00 - 10:00 AM	Great Egret
Olfactory Transcriptome Workshop	

A comprehensive understanding of brain cell types in the olfactory system is essential to understand how neural circuits generate olfactory perception and complex behaviors such as odor plume navigation. Identifying and characterizing brain cell types, with the means to target each cell type, will elucidate the functional interactions that give rise to the emergent properties of the olfactory system. Here we hold a workshop that will highlight current advanced methods for cell census of the olfactory bulb and epithelium and we seek to obtain feedback from chemical senses investigators. The workshop will include emerging technologies funded by the NIH's Brain Research through Advancing Innovative Neurotechnologies (BRAIN) Initiative - Cell Census Network (BICCN). The studies will raise the question of the relevance of the differences and similarities across species.

Chair(s): Diego Restrepo

10:00 - 12:00 PM	Calusa ABCD
TOWARD AN UNDERSTANDING OF THE GENERATION AND REGENERATION OF CHEMOSENSORY CELLS	

Chair(s): Peihua Jiang

10:00 **Toward An Understanding Of The Generation And Regeneration Of Chemosensory Cells**
Peihua Jiang
Monell Chemical Senses Center, Philadelphia, PA, United States

Taste receptor cells and olfactory sensory neurons turn over throughout life. Adult taste and olfactory stem/progenitor cells produce new cells to replenish taste and olfactory tissues, respectively. Despite these continuous processes, our daily experiences of smell and taste remain stable, largely due to the fact that the regulation of taste and olfactory tissue homeostasis is tightly controlled. Disruption of these processes may cause taste and smell disturbances. In this symposium, the invited speakers will provide us with an update on the understanding of the molecular and cellular basis for the regulation of olfactory and taste tissue homeostasis and regeneration. They will also discuss potential regenerative treatment strategies for taste and smell disorders.

10:10 **How And When To Fix A Broken Nose**
James E Schwob
Tufts University School of Medicine, Boston, MA, United States

Many forms of olfactory dysfunction, particularly the all-too-frequent age-related decline in ability and the loss that can occur after URI, are associated with pathological alterations of the normal composition of the olfactory epithelium. Two forms of epithelial abnormality are neurogenic exhaustion, in which the active stem and progenitor cell population of globose basal cells (GBCs) as well as their progeny, the olfactory sensory neurons (OSNs), become depleted, and respiratory metaplasia, in which what was olfactory epithelium adopts the cellular composition and appearance of respiratory epithelium. In both cases, the population of reserve stem cells, the horizontal basal cells (HBCs), persist but remain unhelpfully dormant. A potential strategy for repairing the periphery activates the HBCs and directs their progeny toward transdifferentiating into GBCs which then repopulate the OSNs. Key to the activation process is the master transcription factor DNp63, and it is necessary and sufficient to eliminate or diminish its amounts for activation of the HBCs to proceed. Efforts toward that end entail direct targeting of p63 expression and manipulations of Notch signaling and directed proteolysis of p63, both of which are known to participate normally in the regulation of its expression and concentration.

10:40 **Taste Homeostasis And Regeneration: Hedgehog Signaling And Antagonism**
Archana Kumari[1], Benjamin L. Allen[2], Charlotte M. Mistretta[3]
[1]Rowan University School of Osteopathic Medicine, Stratford, NJ, United States, [2]University of Michigan Medical School, Ann Arbor, MI, United States, [3]School of Dentistry, University of Michigan, Ann Arbor, MI, United States

Taste bud (TB) cells in gustatory papillae, fungiform (FP) and circumvallate (CV), renew every several days and reside in an epithelium of keratinocytes that also turn over. FP and CV require Hedgehog (HH) signaling for homeostasis, with loss of TB on pathway inhibition. Notably, after stopping exposure to the inhibitor drug sonidegib, we demonstrated restoration of CV TB, whereas only half of all FP TB regenerated. To entertain potential regenerative treatments, we are asking why the FP TB do not fully recover. We extended studies to HH-interacting protein (HHIP), a pathway target that is an endogenous, HH signaling antagonist in many organs. There was a novel, restricted expression pattern of HHIP in the non-taste filiform (FILIF) papillae only, even though HH signaling is inactive there. Further, we found HH co-receptor *Ptch1* expression similar to HHIP in FILIF. In pharmacologic and genetic models of HH pathway inhibition, there were ectopic HHIP and *Ptch1* expressions in the FP apex and FP assumed a conical, FILIF-like shape. Using chorda tympani/lingual nerve cuts for partial-to-complete inhibition of HH signaling in FP we observed opposing expressions between HHIP and HH-responding *Gli1lacZ* cells. Strikingly, in recovery experiments the antagonist was retained in the Atypical FP with no TB that were unable to recover. Overall, through HH antagonism, the pathway functions in basic taste organ homeostasis and regeneration. Further, with pathway disruption the innervation to gustatory papillae is retained and although taste sensation is lost with TB cell elimination, nerve responses to somatosensory stimuli

remain. Thus HH signaling has diverse roles in the multimodal taste organ sensations of taste, touch and temperature, all salient in patients during and after treatments that alter the pathway.

11:10 **Probing Restoration Of Taste Receptor Cell With Engineered Nanobodies**

Wan-Jin Lu, Yunxiao Zhang, Anping Li, Philip A. Beachy
Stanford University School of Medicine, Stanford, CA, United States

Few therapeutic options are currently available for patients with taste disturbance. Although some of the molecular and cellular basis for taste tissue regeneration have been characterized, applying key molecular pathways to stimulate regeneration will require precise methods of delivery that could stimulate residual stem and progenitor cells in the taste buds with limited off-target effects. Recent work has elucidated a critical role for Hedgehog signaling in taste organ maintenance and regeneration (reviewed in Mistretta and Kumari, IJMS, 2019). We further demonstrated that activation of Hedgehog pathway using a small molecule, SAG21k, could enhance regeneration of taste receptor cells in mice (Lu et al. PNAS, 2018). However, systemic delivery of Hh agonist produce numerous undesirable effects, including potentiating cancer, therefore therapeutic application has been difficult for lack of pathway-activating agents that are amenable to tissue targeting. We tested an alternate strategy by designing a synthetic nanobody to mimic the native Hedgehog protein without the lipid modification required for activity (Zhang et al. PNAS, 2020). As a single-domain protein, the nanobody is amenable to engineering therefore we added a collagen type I binding peptide to limit its distribution to the mesenchymal compartment for precise control of Hedgehog pathway activity. We found our engineered nanobody could concentrate and efficiently activate the Hedgehog pathway indicated by mesenchymal *Gli1* expression. Further work is required to determine the efficacy of nanobody in taste cell regeneration in other available injury models. Our work presents exciting promises in the usage of nanobody, especially when combined with tissue targeting strategies to precisely deliver factors with regenerative activities for the taste organ.

11:30 **Adult Olfactory Neurogenesis: Considerations In Mouse And Human**

Bradley J. Goldstein
Duke University, Durham, NC, United States

The olfactory epithelium in the nose houses the olfactory sensory neurons, serving as the peripheral organ for smell. Acquired injury or loss of olfactory neurons can occur due to trauma, infection or inflammatory insults. Fortunately, a remarkable repair capacity exists, in the form of basal stem cells capable of reconstituting the epithelium following damage. This system has been well-studied in rodent models, providing insights into the mechanisms maintaining normal neuronal turnover or wholesale neuroepithelial repair following experimentally-induced injury. However, acquired olfactory disorders occur in humans, suggesting that reparative mechanisms are imperfect. In an effort to better understand acquired olfactory disorders and consider possible treatment strategies, we have utilized new approaches to analyze human olfactory epithelium. Here, we will summarize findings investigating neurogenesis in rodent and adult human olfactory epithelium, with a focus on efforts to begin to translate advances to address human sensorineural olfactory disorders.

10:00 - 12:00 PM	Calusa FGH
TOP-DOWN REGULATION IN OLFACTION	

Chair(s): Elizabeth Hanson Moss & Joseph Zak

10:00 **Top-Down Regulation In Olfaction**

Elizabeth L Hanson Moss[1], Joseph D Zak[2]

[1]Baylor College of Medicine, Houston, TX, United States, [2]University of Illinois Chicago, Chicago, IL, United States

To appropriately detect, interpret, and respond to external stimuli, sensory processing needs to balance reliability with context and state-dependent flexibility. Flexibility of fixed circuits is enabled by top-down regulation – where higher-order brain regions project back to primary sensory areas to modulate circuit function. Olfactory circuits are the target of extensive top-down input from cortical and subcortical brain regions. Advances in genetic, imaging, electrophysiological, and computational approaches provide a window into the function of these top-down inputs, particularly in awake behaving animals. This symposium will highlight diverse modes of top-down regulation in olfaction and showcase current work advancing our understanding of how top-down regulation impacts olfactory processing.

10:10 **Long-Range Gabaergic Projections Contribute To Cortical Feedback Control Of Olfactory Processing**

Gabriel Lepousez, Camille Mazo, Enzo Peroni, Antoine Nissant, Pierre-Marie Lledo
Institut Pasteur, Laboratory for Perception & Memory, CNRS UMR3571, PARIS, *, France

In sensory systems, cortical areas send excitatory projections back to subcortical areas to dynamically adjust sensory processing and provide top-down modulation. In parallel to this classical cortical excitatory feedbacks, we will show in this presentation that the olfactory system also contains a previously unknown cortical inhibitory feedback circuit. Using viral-genetic tracing techniques, we identified a subpopulation of GABAergic neurons in the anterior olfactory nucleus and piriform cortex which directly innervate the olfactory bulb (OB). In the OB, these long-range cortical inhibitory inputs synapsed with both local and output neurons of the olfactory bulb. Optogenetic activation of cortical GABAergic projections caused a net subtractive inhibition of both spontaneous and odor-evoked activity in local interneurons as well as output projection neurons. Stimulation of GABAergic cortico-bulbar projections also entrained network oscillations in the communication band between the cortex and the OB. Targeted pharmacogenetic silencing of the cortical GABAergic outputs in the olfactory bulb impaired discrimination of similar odor mixtures. Lastly, analogous inhibitory cortico-thalamic projections were also present in the somatosensory system. Thus, cortical GABAergic feedback represents a new circuit motif in sensory systems involved in refining sensory processing and perception.

10:40 **Basal Forebrain Gabaergic Projections To The Olfactory Bulb Are Rapidly Recruited By Odor Encounters In A Stimulus Specific Manner**

Pablo S Villar[1], Ricardo C Araneda[1], Dinu F Albeanu[2]

[1]University of Maryland, College Park, MD, United States, [2]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, United States

Olfactory perception emerges from the interplay between stimulus-driven feedforward cortical activation and feedback signals that sculpt neuronal activity of early processing in the olfactory bulb (OB). The output of the OB is tightly regulated by descending signals arising from many brain regions, including a heterogeneous collection of GABAergic neurons in the basal forebrain (BF). To date, the dynamics of activation and functional contribution of these GABAergic projections to odor representations remain unknown. Here, we used multiphoton calcium imaging in awake mice to investigate the recruitment of activity in BF-OB GABAergic projections by odors. Odor presentation resulted in fast and sparse responses, in an odor and concentration specific manner. Across multiple odors, boutons responded by either enhancement or suppression of baseline activity, while mixed-type responses were rarely observed. This response mode specificity was maintained across orders of magnitude change in concentration. Our results indicate that upon olfactory-cortical activation, the basal forebrain GABAergic projections to the bulb are rapidly recruited and develop diverse and stimulus specific responses. Interestingly, the activation dynamics of GABAergic boutons in response to odor stimuli are similar to responses exhibited by glutamatergic feedback boutons originating in the piriform cortex. These results indicate that the bulb integrates fast excitatory and inhibitory descending signals that can rapidly modulate the output polarity. To determine how these signals support behavioral flexibility, in future experiments we will monitor both excitatory and inhibitory top-down feedbacks in mice performing rapid odor discriminations.

11:05 **Selective Attention To Odors Engages An Olfactory-Prefrontal Network**

Hillary L. Cansler[1,2], Estelle E. in 't Zandt[1,2], Waseh T. Khan[1,2], Minghong Ma[3], Daniel W. Wesson[1,2]

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Cognitive state profoundly impacts sensory processing and perception. In the olfactory system, the tubular striatum (TuS, also known as the olfactory tubercle) in particular exhibits attention-dependent encoding of odors in both humans and rodents. Here, we investigated interregional dynamics associated with odor-directed

attention in rats. First, we used multiple cell-type specific tracing methods, which showed that the TuS is a privileged recipient of dense glutamatergic input from the medial prefrontal cortex (mPFC). Retrograde tracing revealed that TuS-projecting neurons in the mPFC largely arise from the prelimbic and infralimbic regions. Injections of anterograde AAVs expressing synaptophysin-GFP or -mRuby in the prelimbic and infralimbic, respectively, revealed that these projections provide significantly denser input to the TuS than other olfactory regions. Next, we acquired multi-site local field potential recordings from the TuS, mPFC, and olfactory bulb (OB) of rats as they completed an olfactory selective attention task. We found that gamma band power was elevated during odor-directed attention in the OB and mPFC. We also observed greater coherence in the beta band between the OB-mPFC and the mPFC-OT as the rats attempted an intermodal attentional switch. Elevated coherence between OB-mPFC in the respiratory range was also present during an intermodal attentional switch. Lastly, we recorded sniffing to assess the influence of sniffing on respiratory theta coherence. We found that rats precisely structured their sniffing across task conditions, despite changing attentional demands and behavioral shifts. Together, these results suggest that top-down (e.g. mPFC) versus bottom-up (sniffing) mechanisms underlie the effects of odor-directed attention on olfactory processing in the brain.

11:30 **A Non-Canonical Pathway For Computing Odor Identity**

Dinu F Albeanu, Honggoo Chae, Arkarup Banerjee, Marie Dussauze
Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, United States

Elucidating neural circuits that enable robust odor identification remains a fundamental challenge in olfaction. Here, we assessed the interplay between feedforward and feedback signals across different olfactory areas in representing odor identity by leveraging cell type specific analysis. We monitored the responses of the two classes of bulb outputs, the mitral and tufted cells via multiphoton microscopy, as well as of neuronal ensembles from their major cortical targets, the piriform cortex and the anterior olfactory nucleus (AON) respectively using tetrode micro-drives in response to the same stimuli in awake mice. We find that tufted cells, which strongly innervate the AON, substantially outperform mitral cells in decoding both odor identity and intensity, as well as in intensity invariant decoding, acting in a largely feedforward fashion. Cortical-bulbar feedback signals from the piriform cortex and the AON preferentially regulate the activity of mitral versus tufted cells respectively, matching biases in feedforward connectivity and, furthermore, appear to perform different functional roles. Piriform cortex feedback specifically restructures the mitral cell responses leaving tufted cell responses largely unaltered, while feedback from the AON preferentially controls the gain of tufted cell odor representations. Finally, preliminary recordings indicate that the AON neural ensembles outperform piriform cortex ensembles in decoding both odor identity and concentration information, thus reflecting the differences observed in their tufted versus mitral cell inputs. These results indicate that, unexpectedly, the non-canonical tufted cell-to-anterior olfactory nucleus pathway plays a major role in mediating odor recognition and discrimination.

12:00 - 1:00 PM	Calusa Foyer
Chemical Senses Editorial Board Meeting (Invite Only)	

12:00 - 1:00 PM	Lunch On Own
Lunch On Own	

1:00 - 3:00 PM	Calusa ABCD
CHEMOSENSORY TESTING AND COVID-19: RESULTS OF THE NATIONAL INSTITUTES OF HEALTH RADX-RAD INITIATIVE	

Chair(s): Susan Sullivan & Steven Munger

1:00 **Chemosensory Testing And Covid-19: Results Of The National Institutes Of Health Radx-Rad Initiative**

Susan L. Sullivan[1], Steven D. Munger[2,3]

[1]Smell and Taste Program, National Institute on Deafness and Other Communication Disorders, Bethesda, MD, United States, [2]Center for Smell and Taste, University of Florida, Gainesville, FL, United States, [3]Dept. Pharmacology and Therapeutics, University of Florida, Gainesville, FL, United States

The goal of the National Institutes of Health's Rapid Acceleration of Diagnostics (RADx) initiative is to speed the development, validation and commercialization of tests for COVID-19 and/or SARS-COV-2. Within it, the RADx-Radical (RADx-rad) program targets support to new, non-traditional approaches including the use of screening and home-based sensory testing to address gaps in COVID-19 testing. In December, 2020, RADx-rad funded four teams that are exploring the use of chemosensory tests for COVID-19 screening and surveillance. In this symposium, members of each team will present results of those ongoing studies. (1) Kym Man will describe studies to develop, validate, and deploy a novel test that uses hard candy to independently assess smell and taste function in individuals who are at high risk for contracting COVID-19. (2) Pamela Dalton will discuss her team's project to test and deploy a rapid and objective measure of smell ability, the SCENTinel test, for widespread COVID-19 surveillance. (3) Mark Albers will present studies from his team to develop a native app and algorithms to conduct longitudinal COVID-19 smell tests for at-risk populations and to differentiate smell loss from COVID-19 relative to influenza. (4) Richard Gerkin will report on a multi-institution study to develop and implement novel objective, self-administered smell tests for the purpose of identifying individuals with COVID-19 as well as for use in population-level surveillance of COVID-19 spread. Together, these speakers will communicate the state-of-the-field for using chemosensory testing in COVID-19 screening and surveillance.

1:20 **Don Tucker Finalist: A Confectionary-Based Screening Tool For Assessing Chemosensory Loss In Covid-19 Patients**

Kym Man[1], Zhenxing Wu[2], Aayah Mohamed-Osman[2], Kai Zhao[2], Susan P. Travers[3], Christopher T. Simons[1]

[1]Department of Food Science and Technology, The Ohio State University, Columbus, OH, United States, [2]Department of Otolaryngology, The Ohio State University, Columbus, OH, United States, [3]Division of Biosciences, The Ohio State University, Columbus, OH, United States

To protect public health, simple and effective screening protocols are needed to identify suspected COVID-19 cases. Sudden chemosensory loss is a cardinal symptom of COVID-19. Using eight different fruit-flavored but visually identical hard candies, we developed an easy-to-use, prospective screening tool to monitor smell and taste function in at-risk subjects. Orthonasal aroma identification and perceived intensity were collected after unwrapping the candy; sweet, sour, and retronasal flavor intensity and identification were collected after placing the candy in the mouth. In Phase I, psychophysical testing was conducted over Zoom to assess chemosensory function of COVID+ subjects compared to controls without COVID using the candies and, as validation, the NIH toolbox. In addition to the toolbox's scratch and sniff smell identification and (1 mM) quinine bitter intensity assessment, we included taste identification and intensity assessment of a low concentration (1 mM) sucralose solution. In Phase II, a 2.5 months' supply of candy was given to subjects to assess and record smell and taste function on a daily basis via a customized smartphone app, which also records daily COVID-19 related health information, including exposure, symptoms, and COVID testing results. The utility and functionality of the test system was assessed on days 1, 28, and 56 using the System Usability Scale. To date, 160 subjects have been enrolled in Phase I (28 COVID+ and 132 control) and 600 subjects in Phase II, 305 of whom completed at least 10 daily testing sessions (mean 28.8 days). Updated results reflecting the frequency and magnitude of smell and taste loss in COVID+ individuals will be presented along with the potential utility of using candy as the basis for a home screening public health tool.

1:45 **Scentinel: A Rapid Smell Screening Test For Covid-19 And Beyond**

Pamela Dalton, Danielle Reed, Stephanie Hunter, Mackenzie Hannum, Maureen O'Leary, Nancy Rawson, Robert Pellegrino, Valentina Parma
Monell Center, Philadelphia, PA, United States

In response to the sudden loss of smell associated with the SARS-COV-2 virus and the need for early diagnostic

tests to limit the spread of COVID-19, we developed a rapid (<2 minutes), and inexpensive ($1/test) smell test to screen for sudden smell loss, a specific symptom of COVID-19. Building on our knowledge of the advantages and limitations of earlier smell tests, SCENTinel utilizes the Lift'n'Sniff technology to reduce the intensity variation associated with scratch and sniff tests. SCENTinel uses one of nine odorants, assesses three features of olfactory function: detection, rated intensity and identification. The test can be self-administered on a smart device by scanning a QR code or by entering the unique code into a web browser. The first version of the test successfully discriminated the performance of people with anosmia vs. normosmia and more recently it has been shown to discriminate hyposmia and parosmia. It has been utilized by over 5000 individuals and deployed in multiple venues, including COVID-19-PCR testing sites, among nursing home staff for pre-shift screening and in school children, to test for feasibility of use.

2:10 **Longitudinal At Home Smell Testing To Detect Infection By Sars-Cov-2**

Mark Albers
MGH / Harvard Medical School, Boston, MA, United States

Sudden loss of smell increases the odds of being infected with SARS-CoV-2. However, self-report of smell function is unreliable. In our mouse studies, sterile induction of anti-viral innate immune signaling in <1% of cells in the olfactory epithelium diminished odor discrimination and reduced odor-evoked local field potentials. RNA levels of all class I, class II, and TAAR odorant receptors were markedly reduced in olfactory sensory neurons in a non-cell autonomous manner. We hypothesized that smell loss evoked by SARS-CoV-2 infection was non-cell autonomous with reduced odorant receptor signaling. We revised our Alzheimer's disease smell test to a self-administered "at home" 5-minute objective smell test. In pilot studies, lower odor intensity ratings and odor discrimination deficits were present in COVID patients - consistent with a downregulation of odorant receptor signaling in these patients. Each participant's COVID status was validated by extracting results of clinical SARS-CoV-2 RT PCR assays from electronic health records. The COVID smell test predicted infection by SARS-CoV-2 better than symptom tracking alone (AUC = (0.85 vs. 0.66). We expanded this smell test to 6 smell cards, each with 3 different odors. These distinct smell cards afford longitudinal screening as often as several times per week and will provide data to construct personalized thresholds for changes in smell function - rather than population norms. This longitudinal test will also be deployed to track recovery of smell function in patients who have been infected with COVID and enrolled in the NIH Recover Cohort. In this presentation, we will share our progress on testing with this longitudinal test in patients at risk for COVID infection, patients recovering from COVID infections with or without persistent symptoms.

2:35 **Rapid Testing Of Odor Detection Threshold For Covid-19 Screening**

Richard C Gerkin[1], Elisabeth M Weir[2,3], Carolyn O Dirain[4], Jeb M Justice[4], Cara Exten[5], Steven D Munger[6,7], John E Hayes[1,2]

[1]School of Life Sciences, Arizona State University, Tempe, AZ, United States, [2]Sensory Evaluation Center, College of Agricultural Sciences, The Pennsylvania State University, University Park, PA, United States, [3]Department of Food Science, College of Agricultural Sciences, The Pennsylvania State University, University Park, PA, United States, [4]Department of Otolaryngology, Gainesville, FL, United States, [5]College of Nursing, University Park, PA, United States, [6]Center for Smell and Taste, University of Florida, Gainesville, FL, United States, [7]Department of Pharmacology and Therapeutics, University of Florida, Gainesville, FL, United States

The COVID-19 pandemic is the most devastating infectious disease outbreak in a century, and symptom-based screening may help quickly identify individuals with the disease. Sudden partial or complete olfactory loss is, at least for some variants of the virus, the single best predictor of COVID-19. However, existing options for identifying olfactory loss typically rely on odor identification tasks, which may risk conflating prior familiarity and/or odor naming with the underlying ability to smell, especially in children, the elderly, or others with language barriers. To overcome these problems, three of the authors created the Adaptive Olfactory Measure of Threshold (ArOMa-T). The ArOMa-T includes multiple concentrations of PEA, encapsulated in peel- and-burst labels on a bi-fold card that can be mailed. The card is paired with an adaptive algorithm, implemented electronically, that guides the user (or a proctor) through a sequence of trials where specific concentrations are selected based on prior responses. By skipping non-informative concentrations, this test rapidly determines the user's odor detection threshold, which is used to determine the presence (or absence) of smell dysfunction. We discuss the key ideas behind adaptive threshold testing and present the results of preliminary studies using the ArOMa-T and odor identification tests. In brief, the ArOMa-T takes <3 minutes to complete, exhibits internal reliability, and recapitulates well-established population-level differences in detection thresholds between sexes and between age groups, as well as evidence for heritability of detection threshold. These results support the use of the ArOMa-T in the field and may be valuable in settings where rapid and portable assessment of olfactory function is needed, including as a screening and community surveillance tool for COVID-19.

3:00 - 4:00 PM	Calusa ABCD
Oral Data Blitz	

Chair(s): John McGann

3:00 **Loss Of The Primary Ciliary Protein, Arl13B, In Immature Osns Impairs Neuronal Maturation**

Julien C. Habif[1,2], Kirill Ukhanov[1,2], Carlos de Celis[1,2], Chao Xie[1,2], Lian Zhang[1,2], Warren W. Green[1,2], Jeffrey R. Martens[1,2]

[1]Department of Pharmacology and Therapeutics, University of Florida, College of Medicine, Gainesville, FL, United States, [2]University of Florida Center for Smell and Taste, Gainesville, FL, United States

Ciliopathies are a class of inherited disorders induced by mutations of ciliary genes and manifest in dysfunction in various organs, including the olfactory system. It is believed that ciliopathy induced olfactory dysfunction is caused by defects in the multi-cilia of mature olfactory sensory neurons (mOSNs) which possess the machinery necessary for odorant detection. We show for the first time that immature OSNs (iOSNs) possess primary cilia that express ARL13B, a canonical ciliary marker. *Arl13b* encodes a small GTPase and is a causative gene for the ciliopathy, Joubert syndrome (JS). There is a profound gap in knowledge whereby the role of ARL13B in OSNs is unknown. It is also unclear if JS patients suffer from smell impairment. To explore the role of ARL13B in iOSNs, we derived a mouse model where *Arl13b* is excised from iOSNs (*123-Cre;Arl13b$^{fl/fl}$;123-Arl13b*). The OE of *123-Arl13b* mice showed a shift in neuronal population, with more iOSNs and less mOSNs compared to WT mice at 1 month of age. Also, at that age there was an increase in the basal stem cells and more overall proliferation. BrdU lineage trace experiments revealed that OSNs in *123-Arl13b* mice had a delay in maturation compared to the WT OSNs. Also, *123-Arl13b* mice had a decrease in the number and length of cilia of mOSNs and a reduced ability to detect odorants, as tested by electro-olfactogram recordings. Finally, *123-Arl13b* mice displayed severely deformed glomeruli, speaking to a role of ARL13B in glomerular innervation. Together, our findings demonstrate that ARL13B plays an important role in the maturation of OSNs and suggests that JS has penetrance in the olfactory system.

3:04 **A Tas2R-Mediated Signaling Pathway In Nasal Solitary Chemosensory Cells Triggers Mice Avoidance Behavior To Inhaled Nebulized Irritants Without Involving The Taste And Olfactory Systems.**

Ranhui Xi[1,2], Sean McLaughlin[3], Ernesto Salcedo[4], Marco Tizzano[1]

[1]University of Pennsylvania, Philadelphia, PA, United States, [2]Sichuan University West China Hospital of Stomatology, Chengdu - Sichuan, *, China, [3]Brain Institute, Florida Atlantic University, Jupiter, FL, United States, [4]University of Colorado Anschutz Medical Campus, Aurora, CO, United States

The nasal epithelium houses a population of solitary chemosensory cells (SCCs) that express T2R (bitter) taste receptors and taste transduction signaling components. These cells are innervated by peptidergic (subP) trigeminal polymodal nociceptive nerve fibers and use acetylcholine as the neurotransmitter. Nasal SCCs respond to bitter compounds including bacterial metabolites evoking protective respiratory reflexes, as well as innate immune and inflammatory responses (Tizzano 2010, Saunders 2014). Here we tested whether SCCs were implicated in aversive behavior to specific inhaled nebulized irritants using a custom-built dual-chamber forced choice device. Mice behavior was recorded, and a MATLAB script was used to analyze the time spent in each chamber. When 10 mM denatonium (DEN) or cycloheximide was nebulized in one of the two chambers, wild type mice exhibited an aversion to the irritant mist chamber and spent most of the time in the control chamber (saline). Moreover, WT mice avoided the irritant more quickly with subsequent exposures (time points: naïve, 1hr and 6hrs). TrpM5-, Gnat3- and Skn1a-KO mice showed no aversion to the irritant at 2mM and developed an attraction at 10mM. This was attributed to the irritant's smell component as demonstrated by ablation of the olfactory epithelium post methimazole IP treatment. The P2X2/P2X3-KO mice that lack the taste but not the SCC pathway were used to show noninvolvement of bitterness in the aversive behavior. Like WT mice, P2X2/P2X3-KO showed aversion to DEN excluding a bitter taste-mediated aversion. The results demonstrate that activation of SCCs leads to a rapid aversive response to certain classes of irritants. This SCC-mediated avoidance behavior represents an important defense mechanism against inhalation of noxious chemicals.

3:08 **Chemogenetic Inhibition Of Somatostatin Neurons In The Nucleus Of The Solitary Tract Differentially Modulates Bitter And Sweet Taste Signals**

Kalyanasundar Balasubramanian, Charlotte Klimovich, Sidney Li, Emma Gutarts, Susan Travers
Division of Biosciences, College of Dentistry, Ohio State University, Columbus, OH, United States

The rostral nucleus of the solitary tract (rNST) is the first central taste circuit and houses a network of heterogeneous neurons. A recent study (Jin et al., 2021) proposed that one genetically distinct neuron type, comprised of rNST neurons expressing somatostatin (Sst), exclusively processes bitter taste. However, in the caudal NST, Sst neurons are a mixed population of excitatory glutamatergic and inhibitory GABAergic cells, suggesting that rNST Sst neurons might be multifunctional. To explore this, we made injections of a cre-dependent AAV virus expressing an inhibitory DREADD (hM4Di) into the rNST of Sst-cre mice and then tested licking in response to concentration series of bitter (quinine) and sweet (sucrose) stimuli after I.P. injections of CNO or saline. Consistent with the previous report, inhibition of Sst neurons increased quinine licking (taste: water ratio, P=0.0004, N=6), suggesting that bitter signaling was suppressed. More surprisingly, sucrose licking also increased (taste-water licks, P=0.04, N=5), similar to our previous observations using DREADDs to suppress rNST GABA signaling. To explore the possibility that effects on bitter and sweet-driven behaviors

might arise from glutamatergic Sst versus GABAergic Sst neurons, we performed fluorescent in situ hybridization for Sst, VGLUT2, and VGAT in the rNST. Preliminary data based on counting 249 Sst-positive cells (2 mice, 4 rNST sections) show that ~2X as many Sst neurons are GABAergic (N=162) than glutamatergic (N=81). Thus, rNST Sst neurons are heterogeneous and have the potential for multiple functions. Ongoing optogenetic neurophysiological studies in mice expressing ChR2 in Sst neurons are directly assessing the gustatory response profiles of these cells and their ability to modulate taste activity in other neurons.

3:12 **Anatomical And Functional Dissection Of The Anterior Olfactory Nucleus To Nucleus Of Lateral Olfactory Tract Pathway In Mice**

Janardhan P Bhattarai, Yingqi Wang, Yun-Feng Zhang, Emma Janke, Wenqin Luo, Minghong Ma
Department of Neuroscience, University of Pennsylvania Perelman School of Medicine, , Philadelphia, PA, United States

The olfactory bulb projects to multiple olfactory cortical areas including the piriform cortex and anterior olfactory nucleus (AON), which play distinct roles in odor-guided behaviors. The function(s) of the AON and its connected brain regions are not well established. In order to gain genetic access to the AON neurons, we wished to identify a molecular marker for these neurons through a differential gene expression search in the Allen Brain Atlas. This search led to the identification of the neuromedin B receptor (NMBR) gene as the top candidate that is highly expressed in the AON compared to the rest of the brain. Using the CRISPR-Cas9 gene-editing approach, we generated an NMBR-Cre knock in mouse line. Anatomical tracing from the AON neurons revealed specific projection to the nucleus of lateral olfactory tract (NLOT), part of the cortical pallial amygdala. In addition, whole-cell patch clamp recordings combined with optogenetic activation showed that the AON/TT neurons make monosynaptic and polysynaptic connections onto NLOT neurons. Furthermore, in vivo fiber photometry revealed odor and/or sniff induced calcium signal elevation in the AON neuron axonal terminals in the NLOT of freely behaving mice. Finally, ablation of excitatory neurons in the NLOT not only impaired olfactory guided food search and social discrimination but also disrupted aversive behavior to a synthetic predator odor. Taken together, these results indicate that the AON/TTàNLOT pathway plays a critical role in olfactory-guided behaviors.

3:16 **Chemical Structure-Based Model Outperforms A Human Panelist On Odor Description Task**

Emily J Mayhew[1], Kelsie A Little[2], Matthew Andres[2], Britney B Nguyen[2], Richard C Gerkin[3], Joel D Mainland[2,4]
[1]Michigan State University, East Lansing, MI, United States, [2]Monell Chemical Senses Center, Philadelphia, PA, United States, [3]Arizona State University, Tempe, AZ, United States, [4]University of Pennsylvania, Philadelphia, PA, United States

If you wanted to know what a novel odorant smelled like, would you ask a person to smell and describe it or ask a model to predict it? The relationship between stimulus and odor percept is complex, and so predicting odor perception from molecular structure has been an enduring challenge in the field. Here, we combine state-of-the-art machine learning with a large set of high-quality psychophysical data to raise the ceiling of structure-odor predictive modeling performance. We trained a neural network (NN) on over 5000 molecules characterized in perfumery databases. The model reads in molecular structure and outputs odor label (e.g. grassy, fruity, sulfurous) probabilities. To prospectively validate this model, we purchased 400 structurally diverse molecules that do not appear in or resemble molecules in fragrance databases. Next, we recruited a cohort of human subjects, trained them using odor references to describe odors with a 55-label odor lexicon, and screened to retain only those subjects with high inter- and intra-rater agreement. The 15-subject panel evaluated each of the 400 molecules in duplicate using the rate-all-that-apply method, generating stable mean ratings (panel mean test-retest R=0.80). We find that predictions from molecular structure alone are sufficient to achieve super-human performance. Across all molecule by label combinations, the median panelist predicts the panel mean with an R of 0.47; the NN just surpasses this mark (R=0.49). On a per-molecule basis, NN-predicted labels more closely match the panel mean than the ratings of a single subject for 60% of molecules. Given the choice between the median subject and the NN, you should ask the NN.

3:20 **The Essence Of Male Scent Promotes Female Puberty And Estrus**

Xiaoyan Fu[1], Donghoon Lee[1,2], Bradley S Evans[3], Timothy E Holy[1]
[1]Department of Neuroscience, Washington University in St. Louis, St. Louis, MO, United States, [2]Starr Center for Human Genetics, Rockefeller University, New York, NY, United States, [3]Donald Danforth Plant Science Center, St. Louis, MO, United States

Pheromones are chemical signals that trigger a response in another member of the same species. In mice, it has been shown that exposure of females to male pheromones leads to puberty advance and estrus induction, phenomena known as the Vandenbergh and Whitten Effects, respectively. These effects can be triggered by conspecific male urine, suggesting that they are related to the chemical composition of this stimulus. Although these phenomena were among the earliest known examples of pheromonal actions, the identities of these chemical signals remain mysterious. In agreement with previous work, we found that these behavioral effects could be triggered by low molecular weight nonvolatile constituents of male mouse urine. By combining high-performance liquid chromatography, calcium imaging from the mouse vomeronasal organ, and mass spectrometry (MS), we identified physiologically active fractions of male urine. We then isolated two small molecules in male urine, termed Calin319 and Calin381, that accounted for much of the vomeronasal neuronal response to male urine. By MS-MS and MS^n and direct synthesis, we identified the structure of Calin319 as 2,6-dimethyl-2-heptyl glucuronide. We found that Calin319 and Calin381 were sufficient and necessary to advance

juvenile female puberty and induce female estrus. Besides acting as a primer pheromone, a blend of these two male compounds also acts as a releaser pheromone that resulted in increased investigatory behavior by female mice. These findings demonstrate that Calin319 and Calin381 are crucial male pheromones that regulate female reproductive behavior in mice. This study resolves the long-standing mystery of the molecular code of male urinary chemicals that control female gonadal function.

3:24 **Inhibition Of Sweet Taste By The Nonsteroidal Anti-Inflammatory Drug Ibuprofen**

Payton C Harmon[1], Daiyong Deng[1], Sarah M Sywanycz[1], Emily C Hanselman[1], Paul A S Breslin[1, 2]
[1]Rutgers University, New Brunswick, NJ, United States, [2]Monell Chemical Senses Center, Philadelphia, PA, United States

Introduction: Sweet taste is mediated, in part, by the T1R2-T1R3 GPCR. Whereas agonists of this receptor are extensive, few antagonists have been identified. Lactisole is an antagonist of human sweet taste perception and contains a 2-methylacetic acid moiety that binds to the T1R3 receptor. This moiety is also in phenylpropanoic nonsteroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen, which inhibits the T1R2-T1R3 receptor *in vitro*. We hypothesized that these NSAIDs are antagonists of human sweet taste as well. Methods: To test this hypothesis, we asked subjects to rate the sweetness intensity of caloric (sucrose) and non-caloric (sucralose) sweeteners at the same sweetness intensity with and without a pre-rinse of ibuprofen. We tested two concentrations of ibuprofen against six concentrations of each sweetener. Results: Ibuprofen inhibited the sweetness of both sucrose and sucralose. 13.5 mM ibuprofen significantly decreased the perceived sweetness of sucrose at 300 and 646 mM ($p<0.005$) and sucralose at 430 and 927 μM ($p<0.05$). 57 mM ibuprofen significantly decreased the perceived sweetness of sucrose at 139, 300, and 646 mM ($p<0.001$) and sucralose at 93, 200, 430, and 927 μM ($p<0.001$). Conclusion: Ibuprofen inhibited sweet taste perception of sucrose and sucralose in a dose-dependent manner. Chronic NSAIDs, particularly ibuprofen, are known to have benefits in metabolic diseases such as diabetes, Alzheimer's, and cancer. This could be due to anti-inflammatory impact, T1R antagonism, or both. The T1R2/T1R3 receptor is expressed widely in metabolic regulatory tissues and may be inhibited by circulating levels of ibuprofen, which may help explain the metabolic benefits of its chronic use.

3:28 **Response Profiles Of Vagal Gustatory Neurons**

Bryan Fowler, Saima Humayun, Shannon Landon, LIndsey Macpherson
University of Texas, San Antonio, TX, United States

There are two major pathways conveying gustatory information from the oral cavity to the brainstem: the anterior pathway in which geniculate ganglion neurons relay taste information from the fungiform and palate taste buds, and the posterior pathway in which petrosal neurons of the vagal complex relay taste signals from foliate and circumvallate taste buds. In addition to this anatomical segregation, there are also differences in the composition and proportions of types of taste receptor cells in these taste papillae, and they produce different downstream behavioral and reflex responses. While a wealth of data is now available to examine the tuning profiles of individual geniculate ganglion neurons, we currently lack equivalent information for the vagal ganglion neurons of the posterior taste pathway, making it difficult to evaluate how the activity of these neurons may contribute to downstream taste signaling pathways. We use in vivo calcium imaging to show that vagal neurons react to taste stimuli with similar response qualities to geniculate neurons, i.e. repeatable responses with narrow tuning profiles at moderate stimulus concentrations. However, the proportions of neurons responding to taste qualities are significantly different between the two ganglia, with relatively more vagal neurons responding to bitter or umami stimuli and fewer to salty or sweet stimuli than geniculate neurons. The surprising over-representation of umami-responding vagal neurons led us to explore the potential physiological relevance of activating this population. We find that MPG+IMP elicits more salivation at the posterior tongue than at the anterior tongue. Together, these data provide a better understanding of the response profiles and physiological roles of gustatory signaling through the posterior taste pathway.

3:32 **Trpm4 Regulates Taste-Evoked Calcium Signaling In Broadly Responsive Type Iii Taste Cells**

Kathryn F Medler[1], Debarghya Dutta Banik[1, 2]
[1]University at Buffalo, Buffalo, NY, United States, [2]Indiana University School of Medicine, Indianapolis, IN, United States

Peripheral taste receptor cells use multiple signaling pathways to transduce taste stimuli into output signals that are sent to the brain. We recently identified a subpopulation of Type III taste cells that are broadly responsive (BR) to multiple taste qualities including bitter, sweet, umami, and sour. BR cells use a PLCβ3/IP_3R1 signaling pathway to detect bitter, sweet, and umami stimuli while a separate signaling pathway is used to detect sour stimuli. To date, the other components of the PLCβ3 signaling pathway in BR cells have not been identified. Our recent experiments have found that TRPM4, a monovalent selective TRP channel, is an important downstream target of the PLCβ3 pathway. Live cell imaging on isolated mouse taste receptor cells found that inhibition of TRPM4 activity abolishes taste-evoked sodium responses in BR cells. Interestingly, the taste-evoked calcium signals in BR cells are also significantly reduced when TRPM4 activity is inhibited. Since BR cells are a subpopulation of Type III taste cells, they have conventional chemical synapses and express voltage-gated calcium channels (VGCCs). Our data suggests that the membrane depolarization due to TRPM4 activity regulates at least some VGCC activity in BR cells. Unlike Type II taste cells that also detect bitter, sweet and umami tastants, the taste-evoked calcium signals in BR cells are comprised of both calcium release from internal stores as well as calcium influx through VGCCs. Our data indicate that TRPM4 links the initial taste-evoked calcium release to the subsequent calcium influx through VGCCs. Both of these calcium signaling events are needed to generate an appropriate output signal.

3:36 **Olfaction, Cognitive States, Mortality, And Life Expectancies: A Multistate Survival Analysis**

Jamie E. Knight[1], Tomiko Yoneda[1], Nathan Lewis[1], Graciela Muniz-Terrera[1,2], David A. Bennett[3], Andrea M. Piccinin[1]

[1]University of Victoria, Victoria, BC, Canada, [2]University of Edinburgh, Edinburgh, *, Scotland, [3]Rush University, Chicago, IL, United States

This project aimed to investigate the extent to which olfactory ability, measured by a 12-item smell identification test, predicts transitions between clinically diagnosed cognitive states and death, as well as the degree to which olfaction is associated with cognitively unimpaired and total life expectancies in midlife to older adulthood (*N*=1501; 74% female). METHODS: Multi-state survival models (MSM) estimated the association of baseline olfaction on transition patterns through cognitive states (no cognitive impairment [NCI], mild cognitive impairment [MCI], dementia) and death. To estimate cognitively unimpaired and total life expectancies, multinomial regression models were fit using the hazard ratios (HRs) from the MSM's. RESULTS: Higher olfactory test scores were associated with a lower risk of transitioning from NCI to MCI (HR=0.86, 95% confidence interval 0.82-0.88) and from MCI to dementia (HR=0.89, 0.86-0.93). Additionally, better olfactory ability was associated with a greater likelihood of transitioning backwards from MCI to NCI (HR=1.07, 1.02-1.12). The MSMs suggest that the direct association between olfaction and mortality was not statistically significant after accounting for transitions through cognitive states. Higher olfactory test scores were associated with up to 6 additional years free of cognitive impairment, as well as 5 additional years of lifespan, compared to individuals with low olfaction. CONCLUSION: These findings suggest that higher olfactory ability is associated with a decreased risk of progressing forward through cognitive impairment, and that the association between olfaction and mortality likely occurs primarily through the pathway of neurodegeneration. These analyses highlight the differential role of olfaction as a risk factor for changes across cognitive states

3:40 **Computational Molecular Interaction Maps Of Signaling Events Within The Olfactory Epithelium**

Federica Genovese[1], Shailendra Gupta[2], Suchi Smita[2], Dominique Fastus[2], Krishna Pal Singh[2], Matti Hoch[2], Olaf Wolkenhauer[2,3], Antonella Di Pizio[3]

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In the olfactory epithelium (OE), multiple mechanisms, like odor detection, cell regeneration, and differentiation are vulnerable to a variety of external and/or internal factors. However, the understanding of the cell-to-cell communications and molecular events associated with these mechanisms are still not fully characterized. To provide a global vision of the OE and cross-talks between its different cell types, we prepared maps related to signaling and molecular events in sustentacular cells, microvillous cells, Bowman's glands, trigeminal nerve fibers, horizontal basal cells, globose basal cells, and olfactory sensory neurons accessible via an interactive, searchable, web-based platform through MINERVA, a well-established tool used for the presentation of disease maps (https://www.sbi.uni-rostock.de/minerva/). The molecular single-cell and interaction maps we developed will serve to conceptually visualize and analyze complex mechanisms within single cell types as well as among different cell types. The developed maps provide various entry points to the users to access the manually curated information at the cellular, process/pathway, and molecular level. The maps are designed with the aim to serve heterogeneous communities involved in olfaction including clinicians, research scientists, systems biologists, and industrial partners. In the web platform of the maps, users can identify and prioritize diagnostic/therapeutic markers associated with various olfactory diseases. For this, we developed various user-friendly plugins that help in mapping and analyzing experimental and clinical data directly onto the map. Here we provide a quick overview of manually annotated known signaling events within OE cells and highlight knowledge gaps that need further investigation.

3:44 **Plasticity Of Taste Progenitor Cells In Taste Epithelial Homeostasis**

Sushan Zhang[1, 2], Jennnifer.K Scott[1, 2], Linda.A Barlow[1, 2]

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In mice, the circumvallate taste papilla (CVP) contains hundreds of taste buds. Each taste bud comprises type I, II and III taste receptor cells (TRCs) that renew continuously from progenitor cells, subpopulations of which are LGR5+ and GLI1+ (Ren et al., 2014; Liu et al., 2013). To test the necessity of LGR5+ progenitors in TRC renewal, we ablated these cells by treating *Lgr5*$^{DTR\text{-}mGFP}$ mice with Diphtheria toxin (DT). At 24 hrs post-DT, LGR5-GFP+ cells and taste buds were almost completely gone; however, recovery began by 48 hrs when sparse LGR5-GFP+ cells and scattered KRT8+ TRCs were evident. By 72 hrs, numerous LGR5-GFP+ cells and multiple small KRT8+ cell clusters were detected that also expressed type I, II and III TRC markers. By 7 days post-DT, LGR5-GFP+ cells and taste buds had increased but were still fewer than found in controls. As LGR5-GFP+ and KRT8+ taste cells reappeared coincidentally and unexpectedly rapidly, we posited LGR5^{neg} progenitors were activated by DT injury. Ki67+ cells were increased 24 hrs post-DT, indicating a rapid proliferative response to LGR5+ cell killing. All TRCs differentiate from post-mitotic SHH+ taste precursor cells and in controls 3-4 *Shh*+ cells are found basally in each bud. At 24 hrs post-DT, however, *Shh*+ cell clusters spanned the height of the epithelium, presaging appearance of KRT8+ cell clusters at 72 hrs. Finally, *Gli1*+ cells were abundant at 24 hrs post-DT, leading us to hypothesize activated GLI1+ progenitors support accelerated taste epithelium recovery after LGR5+ cell killing. We will next test if persistent GLI1+ progenitors underlie

taste epithelium recovery by treating *Lgr5*$^{DTR\text{-}mGFP}$*; Gli1*^{CreER}*; Rosa*^{tdTomato} mice with DT and tamoxifen to track the contribution of GLI1+ cells to regenerating taste buds following LGR5+ cell ablation.

3:48 **_Arc_-Expressing Accessory Olfactory Bulb Interneurons Play A Role In Chemosensory Social Behavior**

Kelsey E. Zuk[1,2], Julian P. Meeks[3]

[1]UT Southwestern Medical Center, Graduate School of Biomedical Sciences, Dallas, TX, United States, [2]UT Southwestern Medical Center, Department of Neuroscience, Dallas, TX, United States, [3]University of Rochester, Departments of Neuroscience and Pediatrics, Rochester, NY, United States

The accessory olfactory system (AOS) is critical for the development and expression of sex-typical social behavior in terrestrial mammals. The first dedicated circuit in the AOS, the accessory olfactory bulb (AOB), exhibits cellular and network plasticity in both male and female mice after social experience. An AOB interneuron subtype, internal granule cells (IGCs), has been shown to selectively express the plasticity-associated immediate-early gene *Arc* following social experience. In this work, I sought to better understand how *Arc*-expressing IGCs shape AOB information processing and the display of social behavior. I used Arc-CreERT2 mice to selectively and permanently label *Arc*-expressing IGCs following male-male resident-intruder interactions. Using whole-cell patch clamp electrophysiology, I found that *Arc*-expressing IGCs displayed increased intrinsic excitability for several days after a single resident-intruder interaction. Further, Arc-expressing IGCs displayed a similar increase in excitability across a week of repeated resident-intruder interactions. During these repeated interactions, I found that resident mice increase their aggression. I then tested the hypothesis that *Arc*-expressing IGCs participate in increasing resident aggressive behavior. Using a combination of Arc-CreERT2 mice and chemogenetics, I found that disruption of *Arc*-expressing IGC activity during repeated resident-intruder interactions completely abolishes the increase in resident aggression. Taken together, this work demonstrates that *Arc*-expressing AOB IGCs participate in the establishment and expression of sex-typical social behavior. These findings increase our understanding of central chemosensory processing, experience-dependent plasticity, and the role of specific AOB cell types in mammalian social behavior.

3:00 - 3:30 PM	Calusa Foyer
Coffee Break	

4:00 - 4:45 PM	Great Egret
Pyrfume Code-fest Presentations	

4:45 - 6:00 PM	Calusa Foyer
Mentoring & Career Networking Social	

7:00 - 9:00 PM	Calusa ABCD
Polak Award Lectures	

Chair(s): Yanina Pepino

7:00 **Compensatory Mechanisms Underlying Functional Olfaction Despite Olfactory Bulb Deficiency**
Tamar Licht, Dan Rokni
Dept. of Medical Neurobiology, Hadassah Medical School, the Hebrew University, Jerusalem, *, Israel

The olfactory bulb (OB) is considered to be indispensable for the perception of odors. However, studies demonstrate normal olfaction in humans when the OB is absent. In trying to understand such an inexplicable observation, we used a transgenic mouse line that presents a major developmental deficit of the OB. This mouse line was designed for a brain specific, time-regulated blockade of the angiogenic factor VEGF and leads to a specific OB collapse when induced at embryonic day 13.5. Adult mice containing 5% - 30% of the original OB size were able to distinguish different enantiomers and to detect a specific odor in a mixture of up to 7 odors. Furthermore, males with as little as 5% of OB were able to mate and fertilize females. Electrophysiological recordings from the anterior Piriform cortex of awake mice revealed many sniff-locked neurons which responded to odors by excitation or inhibition. The changes in the anatomy of the olfactory system were further observed with transgenic reporter mice, immunohistochemistry, and viral injections. We found that olfactory sensory neurons in the olfactory epithelium send axons to the residual OB and to more posterior brain areas (such as the anterior olfactory nucleus) and seem to lose normal glomerular structure. Mitral and tufted cell numbers were dramatically reduced but their efferent connectivity to the PC was maintained. The granule cell layer was largely absent, as well as the rostral migratory stream. One remarkable observation was the presence of cells expressing mitral/tufted cell markers in the piriform cortex of some mutant mice, which are normally found only in the OB. In addition to demonstrating the great power of brain plasticity, this mouse model may also provide some insight into why humans with no OB still smell.

7:20 **Atp Release From Type Ii Cells Activates Type I Glial-Like Taste Cells**
Yuryanni Rodriguez[1], Vivien Makhoul[1], Andoni Asencor[1], Stephen Roper[1,2], Nirupa Chaudhari[1,2]
[1]Department of Physiology & Biophysics, University of Miami Miller School of Medicine, Miami, FL, United States, [2]Department of Otolaryngology, University of Miami Miller School of Medicine, Miami, FL, United States

Type II taste bud cells express TasR receptors and signal to nerves by secreting ATP in response to gustatory stimuli. In contrast, type I taste cells are thought to be passive and glial-like. We used Gad2;;GCaMP3 mice in which type I cells express GCaMP, to assess their participation in gustatory signaling. Our data indicate that type I cells respond to ATP secreted by type II cells during bitter taste stimulation. Specifically, the bitter compound cycloheximide (Chx,5 μM) elicited responses in type I cells in lingual slices of circumvallate papillae. Carbenoxolone (Cbx, 10μM), which blocks CALHM1/3 channels and decreases ATP release from type II cells, reduced type I cell responses by 83% ($p<0.001$, 2-tailed, ratio-paired t-test). As positive controls, ATP and ATP-ϒ-S continued to elicit type I cell responses in the presence of Cbx. These data suggest that there is type II-to-type I cell cross-talk during taste bud stimulation, similar to how astrocytes participate at CNS synapses. In a separate series, we examined if the taste-elicited secondary responses that we attributed to type I cells instead originated in type III cells (which may also express Gad2). We sequentially stimulated lingual slices with taste mix (3μM Chx, 1mM Den, 100 μM SC45647, 1mM saccharin) and KCl (50mM). Of the 58 cells that responded, 76% responded exclusively to taste mix, 17% only to KCl-mediated depolarization, and 7% to both. Thus, consistent with our earlier immunostaining validation, only a minority of type III taste cells weakly expressed GCaMP, not contributing appreciably to the secondary responses recorded here. Taste-evoked secondary responses in glial-like type I cells support the notion that these cells in taste buds may serve a more complex role in transmission to afferent nerves than previously recognized.

7:40 **A Persistent Behavioural State Enables Sustained Predation Of Humans By Mosquitoes**
Trevor Sorrells, Anjali Pandey, Adriana Rosas, Leslie Vosshall
Rockefeller University, New York, NY, United States

Predatory animals pursue prey in a noisy sensory landscape, deciding when to continue or abandon their chase. The mosquito *Aedes aegypti* is a micropredator that first detects humans at a distance through sensory cues such as carbon dioxide. As a mosquito nears its target it senses more proximal cues such as body heat that guides it to a meal of blood. How long the search for blood continues after initial detection of a human is not known. Here we show that a 5-second optogenetic pulse of fictive carbon dioxide induced a persistent behavioural state in female mosquitoes that lasted for more than 10 minutes. This state is highly specific to females searching for a blood meal and was not induced in recently blood-fed females or in males, who do not feed on blood. In males that lack the gene *fruitless*, which controls persistent social behaviours in other insects, fictive carbon dioxide induced a long-lasting behaviour response resembling the predatory state of females. Finally, we show that the persistent state triggered by detection of fictive carbon dioxide enabled females to engorge on a blood meal mimic offered up to 14 minutes after the initial 5-second stimulus. Our results demonstrate that a persistent internal state allows female mosquitoes to integrate multiple human sensory cues over long timescales, an ability that is key to their success as an apex micropredator of humans.

8:00 **Cyclic Oligosaccharides Are Sweet Stimuli**

Laura E Martin, Juyun Lim
Department of Food Science and Technology, Oregon State University, Corvallis, OR, United States

Oligosaccharides, a subclass carbohydrates, occur both naturally in foods and as a result of oral starch digestion. We have previously shown that humans can taste maltooligosaccharides (MOS), and that their taste detection is independent of the canonical sweet receptor. While MOSs most commonly occur in linear or branched forms, they can also exist in cyclic structures, referred to as cyclodextrins. Cyclodextrins are widely used in pharmaceutical and consumer applications due to their ability to form inclusion complexes with hydrophobic compounds (e.g., active ingredients). The aim of this study was to investigate how the structure of the MOS backbone (i.e., cyclic form) and the size (i.e., degree of polymerization; DP) affect their taste perception. Subjects were asked to discriminate cyclodextrins with DP of 6, 7, and 8 (i.e., α-, β-, and γ-cyclodextrin, respectively) as well as glucose at 75 mM from blanks. Samples were prepared with 5mM acarbose to prevent hydrolysis of glycosidic bonds by salivary α-amylase and with or without 1.4mM lactisole, a sweet receptor antagonist. Results showed that in the absence of lactisole, all four samples were detected at a significant level ($p < 0.05$) with similar discriminability. In the presence of lactisole, none were detectable to a significant degree ($p > 0.05$). These findings suggest that the cyclodextrins, unlike their linear analogs, are ligands of the human sweet taste receptor, hT1R2/hT1R3. Study findings will be discussed in terms of how chemical structures may contribute to tastes of saccharides.

8:20 **Child Food Neophobia And Olfactory Perception In Young Children: A Psychophysical Study**

Agnieszka Sorokowska, Dominika Chabin, Sabina Barszcz, Edyta Sperling, Aleksandra Kamieńska
Institute of Psychology, University of Wroclaw, Wroclaw, *, Poland

Child food neophobia, i.e., rejection or avoidance of novel foods in young age, is a prevalent problem that affects quality of children's diet and often impedes the development of healthy food preferences. Sensory sensitivity is hypothesized to be among the determinants/correlates of this problem, but existing research has rarely examined the association between olfaction and food neophobia in young children. In the current study, we decided to thoroughly investigate the relationship between different aspects of smell sensitivity and food neophobia in this age group. We tested food neophobia (Child Food Neophobia Scale; Pliner, 1994), olfactory identification skills (U-Sniff test; Schriever et al., 2018), thresholds for odor detection (Sniffin' Sticks Test; Hummel et al., 1997), and pleasantness assessments of 12 odors in a sample of 170 children (89 girls) aged between 3 and 10 years (M=6.02, SD=2.13). Food neophobia was associated with reduced perceived odor pleasantness (r=-.24, p=.002), and children with highest food neophobia levels tended to exhibit poorer thresholds for odor detection than children with lowest food neophobia levels (90^{th} percentile: 6.38±1.12 vs. 10^{th} percentile: 9.49±1.08; p=.058). We hope that our large-scale exploratory project involving standardized, psychophysical methods of olfactory testing will help gain better understanding of food neophobia mechanisms and aid in designing strategies leading to reduction of this problem.

9:00 - 11:00 PM	Estero Ballroom
Poster Session II	

200 **Plasticity In The Peripheral Taste System Of Rats With Consumption Of Chronic High Sucrose**

Iva Abrayam-Vesela#[1], Hayeon Sung#[2], Hannah Dirks[2], Carrie Ferrario[3], Charlotte Mistretta[1], Monica Dus*[2], Robert Bradley*[1]

[1]University of MIchigan, School of Dentistry, Ann Arbor, MI, United States, [2]University of Michigan, Department of Pharmacology, Ann Arbor, MI, United States, [3]University of Michigan, Department of Pharmacology and Biopsychology, Ann Arbor, MI, United States

Consumption of high sucrose diets is associated with increased risk for metabolic, heart and neurological diseases. Whereas accumulating evidence from humans, rodents and insects suggests that high dietary sucrose modifies sweet taste sensation, there is sparse understanding of any associated peripheral nerve or taste bud alterations. We fed male Sprague-Dawley rats with regular chow and 30% liquid sucrose (HS Group) or drinking water (Control Group) for 4 weeks. The effects of high sucrose consumption were measured in neurophysiological responses of the chorda tympani (CT) nerve to lingual stimulation with sucrose, NaCl, citric acid and HCl, MSG and quinine HCl. Compared to Control, HS rats had decreased CT responses to a range of sucrose stimuli from 0.15 to 2.0M. No effects were seen in responses to salt, bitter, MSG, or to somatosensory tactile and cold stimuli. Notably, effects were not observed to glucose, fructose, maltose or Na saccharin stimuli. Preliminary assessment of fungiform and circumvallate papillae revealed no alteration in the number or size of taste buds, or volume of innervation with diet treatment. However, using immunohistochemistry we observed a decrease in the number of PCLβ2+ taste bud cells in HS rats. Importantly, initial analyses indicate restoration in the altered CT responses and cell type numbers at 4 weeks after replacement of HS with drinking water. Our preliminary results indicate particular, selective effects in peripheral taste nerve responses and taste bud cells with high sucrose consumption. Future studies will be directed to understand mechanisms. Our work suggests that diet effects on the dynamic, peripheral sense of taste can promote metabolic and chronic disease by altering gustatory neurobiology.
#-equal contribution * correspondence

201 **Response Profiles Of Vagal Gustatory Neurons**

Bryan Fowler, Saima Humayun, Shannon Landon, LIndsey Macpherson
University of Texas, San Antonio, TX, United States

There are two major pathways conveying gustatory information from the oral cavity to the brainstem: the anterior pathway in which geniculate ganglion neurons relay taste information from the fungiform and palate taste buds, and the posterior pathway in which petrosal neurons of the vagal complex relay taste signals from foliate and circumvallate taste buds. In addition to this anatomical segregation, there are also differences in the composition and proportions of types of taste receptor cells in these taste papillae, and they produce different downstream behavioral and reflex responses. While a wealth of data is now available to examine the tuning profiles of individual geniculate ganglion neurons, we currently lack equivalent information for the vagal ganglion neurons of the posterior taste pathway, making it difficult to evaluate how the activity of these neurons may contribute to downstream taste signaling pathways. We use in vivo calcium imaging to show that vagal neurons react to taste stimuli with similar response qualities to geniculate neurons, i.e. repeatable responses with narrow tuning profiles at moderate stimulus concentrations. However, the proportions of neurons responding to taste qualities are significantly different between the two ganglia, with relatively more vagal neurons responding to bitter or umami stimuli and fewer to salty or sweet stimuli than geniculate neurons. The surprising over-representation of umami-responding vagal neurons led us to explore the potential physiological relevance of activating this population. We find that MPG+IMP elicits more salivation at the posterior tongue than at the anterior tongue. Together, these data provide a better understanding of the response profiles and physiological roles of gustatory signaling through the posterior taste pathway.

202 **Species Differences In The Expression Of P2X Receptors On Gustatory Nerves**

Brigit High, Thomas E. Finger
University of Colorado School of Medicine, Aurora, CO, United States

The trimeric ionotropic purinergic receptors P2X2 and P2X3 are essential for the transmission of taste information from taste buds to gustatory nerves. In mice, gustatory nerve fibers express both P2X2 and P2X3, so many receptors are likely P2X2/P2X3 heteromers. P2X3 is also expressed homomerically in nerve fibers innervating the large airways which mediate cough triggered by ATP release from local tissues. Broad P2X3 antagonists have been used successfully to treat chronic cough in humans but produce dysgeusia likely due to off-target effects on P2X receptors in taste nerves. One avenue of thought is that antagonists specific for P2X3 homomers might still reduce objective cough frequency in chronic cough patients while avoiding off-target dysgeusic effects. Since P2X subunit composition may differ across species, we used immunohistochemistry to investigate taste bud innervation in humans (31 total samples – 12 laryngeal, 19 fungiform) and in Rhesus monkey (5 monkeys, both laryngeal and fungiform tissues) to test whether the taste nerves in these species express both P2X2 and P2X3 as in mice. Antibodies to P2X2 and P2X3 were validated against hP2X2-expressing HEK cells and human gastrointestinal tissue containing P2X2-expressing neurons in the submucosal plexus. In fungiform taste bud samples from humans and monkeys, P2X3+ fibers extensively innervate taste buds as in mice. However, all Rhesus samples and most human samples lacked P2X2+ innervation. Of the 31 human subjects, only four (1 laryngeal, 3 fungiform) showed expression of P2X2 in nerve fibers innervating taste

buds. These findings suggest that for most humans, taste buds are innervated by nerve fibers expressing only P2X3 homomeric receptors and not P2X2/P2X3 heteromers.

203 **Gdnf Family Ligands In The Gustatory System**

Louis J. Martin, Brian A. Pierchala
Indiana University School of Medicine, Indianapolis, IN, United States

Neurotrophic factors are critical for the development and maintenance of the gustatory system. The glial cell line-derived neurotrophic factor (GDNF) family of ligands (GFLs) include four ligands: GDNF, neurturin (NTRN), artemin (ARTN), and persephin (PSPN). These ligands signal through a common receptor, Ret, which is expressed in geniculate ganglion (GG) neurons that respond to mechanosensory stimuli. To clarify the role of GFLs in taste, we utilized conditional reporter lines for each GFL. Mice were generated by inserting an internal ribosomal entry site (IRES2) fused with CreERT2 into the 3-prime region of NRTN, ARTN, or PSPN genes, allowing for Cre expression without affecting GFL expression. GDNF-IRES2-CreERT2 were provided by Frank Costantini. Animals were crossed with TdTomato mice to allow Cre-dependent RFP expression upon tamoxifen (TMX) administration. Mice were injected with TMX for 4-5 days and tissue was collected either one day or one week after the final injection. Tissue from the oral cavity was immunolabeled with K8, Tuj1, and RFP, and GG were immunolabeled with Tuj1, Phox2b, and RFP prior to super-resolution confocal imaging. We found that the ventral side of fungiform taste buds in GDNF- and ARTN-reporter mice contain large RFP+ cells with processes that ensheathe Tuj1+ axons. Although the tongue contains some RFP+ cells in the NTRN reporter, they are not consistently located near taste buds. No RFP+ cells were evident in the tongues of PSPN-reporter mice. We found similar patterns of staining in taste buds of the foliate, nasoincisor duct, soft palate, and circumvallate. There were no neuronal cell bodies labeled with RFP in the GG of any GFL reporter line. These findings suggest that GDNF and ARTN are important ligands that interact with Ret+ GG neurons near taste buds.

204 **Single-Cell Rnaseq Analysis Of Primary Or Immortalized Human Taste Bud Tissue-Derived (Htbec) Cell Cultures And Fresh Tissue From Human Fungiform Taste Papillae**

Danielle Reed[1], Cailu Lin[1], Robert Margolskee[1], Nancy Rawson[1], Erik Schwiebert[2]
[1]Monell Chemical Senses Center, Philadelphia, PA, United States, [2]Discovery Biomed, Birmingham, AL, United States

A current barrier in taste research is the lack of human taste bud tissue-derived cell lines responsive to appropriate stimuli, like molecules that are perceived as bitter. We collected taste tissue from human fungiform papillae and examined the fresh tissue directly, and we also created primary cultures of disassociated cells from human fungiform papillae tissue. These primary cultures were selected for their response to bitter stimuli and were later immortalized. We evaluated the response of the primary cultures and immortalized cells to bitter ligands and transcriptionally profiled cells from all the samples including the fresh tissue using single-cell RNASeq (scRNASeq) to determine how many cells had taste signaling molecules. After filtering the sequencing data for data quality, each sample yielded data on between 6,733 to 11,762 cells. In freshly isolated human fungiform papillae tissue, 3.6% of cells contained one or more bitter receptor cell marker genes, either a bitter taste receptor (TAS2Rs) or signaling molecules or both (e.g., *PLCB2*, *TRPM5*), whereas the percent of cells with these markers in primary and immortalized cell cultures ranged between 4.1% to 4.3%. No cells in any sample expressed *GNAT3* (gustducin). Follow-up studies using targeted, real-time, quantitative PCR (qRT-PCR) on the primary hTBEC cell cultures confirmed expression of some TAS2Rs, multiple PLCB subtypes, TRP channels (including TRPM5), and CALHM1 (see companion E. Schwiebert et al. abstract), suggesting that other critical taste transduction mRNAs are in the very low mRNA copy category and may be below the limits of detection using scRNASeq. Continuing studies focus on realizing hTBEC cell cultures that are robust *in vitro* human cell models for taste modulator research, such as medium-throughput screening for bitter blocker molecules.

205 **Single Cell And Spatial Rnaseq Of Murine Taste Papillae**

Sunil K. Sukumaran[1], Brian Lewandowski[2]
[1]University of Nebraska-Lincoln, Lincoln, NE, United States, [2]Monell Chemical Senses Center, Philadelphia, PA, United States

A detailed understanding the cellular diversity and spatial organization of taste papillae is an important and necessary step to construct testable models of taste information encoding and taste cell regeneration. Towards this end, we employed 10X Genomics single cell and spatial RNASeq to murine circumvallate (CVP) and fungiform papillae (FOP). About seven thousand cells each from with good quality data were obtained from both taste papillae using scRNASeq. Using cluster analysis, we identified all known and a few novel cell types. Prominent novel cell types identified in the CVP include an immature type II taste cell type and an antimicrobial peptide secreting cell type. In addition, type III cells in the CVP formed two distinct clusters which might represent functionally diverse cell types. As expected, type III and bitter taste cells were much rarer in the FFP. Trajectory analysis was used to generate a map of cell differentiation in both papillae. Both papillae had a diverse population of immune cells, that included monocytes/macrophages, dendritic (Langerhans) cells and T cells. The spatial RNA Seq lacked single cell resolution. However, this data set proved valuable for generating an overview of the organization of taste and non-taste cells in the papillae, including those in the non-taste lingual epithelium and lamina propria. Crucially, when integrated with the single cell RNASeq data, it generated a dramatically improved picture of distribution of taste cells within the papillae. We believe our dataset has the potential to generate crucial insights into taste biology in follow up hypothesis driven studies.

206 **Oral Sensory Neurons Of The Geniculate Ganglion That Express Tyrosine Hydroxylase Comprise A Subpopulation That Contacts Type Ii And Type Iii Taste Bud Cells**

TAO TANG, Brian Pierchala
Indiana University, Indianapolis, IN, United States

Oral sensory neurons of the geniculate ganglion innervate taste papillae and buds on the tongue and soft palate. Electrophysiological recordings of these neurons and fibers revealed complexity in the number of unique response profiles observed, suggesting there are several distinct neuronal subtypes. Molecular descriptions of these subpopulations have been slower to emerge. We report here the identification of a subpopulation of geniculate ganglion oral sensory neurons by expression of tyrosine hydroxylase (TH). TH-expressing geniculate neurons represent 10-20% of oral sensory neurons and these neurons innervate taste buds in fungiform and anterior foliate taste papillae on the surface of the tongue, as well as taste buds in the soft palate. While 35-50% of taste buds on the tongue are innervated by these TH+ neurons, 100% of soft palate taste buds are innervated. These neurons did not have extragemmal processes outside of taste buds and did not express the mechanosensory neuron-associated gene Ret, suggesting they are chemosensory and not somatosensory neurons. Within taste buds, TH-expressing fibers contacted both Type II and Type III receptor cells, raising the possibility that they are responsive to more than one taste quality. During this analysis we also identified a rare TH+ taste receptor cell that was found in only 12-25% of taste buds and co-expressed TRPM5, suggesting it was a Type II cell. Taken together, TH-expressing geniculate ganglion oral sensory neurons innervate taste buds preferentially in the soft palate and contact Type II and Type III taste bud receptor cells.

207 **Cortical-Bulbar Feedback Supports Behavioral Flexibility During Rule-Reversal**

Diego / E Hernandez Trejo[1], Andrei / Ciuparu[2], Pedro / Garcia da Silva[1,3], Cristina / M Velasquez[1,4], Martin / B Davis[1], Raul / C Mureșan[2], Dinu / F Albeanu[1]
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To survive, animals must flexibly adjust their actions to changes in their environment. Mice excel at the recognition of odorants in complex sensory environments; however, little is known on: (1)How odorant representations are modified upon sudden changes in stimulus contingency and (2)How changes in odorant representations causally relate to behavioral changes. The piriform cortex(PC) receives input from the olfactory bulb(OB) and association areas(e.g. orbitofrontal cortex) and sends rich top-down feedback that controls the activity of OB outputs in a cell-type specific manner. Thus, the PC is ideally positioned for integrating sensory input and experience-based sensorimotor predictions. To determine whether cortical feedback supports behavioral flexibility, we trained mice in an Odor/Sound guided Go/No-Go task with rule-reversal and monitored the activity of PC feedback boutons(GCaMP5) across OB layers using multiphoton microscopy(n=12). Within the same session, the reward contingency was switched (~4) across blocks of contiguous trials(~45), rewarding either the odor or sound cue. Feedback boutons displayed dense responses(55% boutons). Interestingly, the activity of individual boutons mirrored the session's block-structure and changed their response properties to the same sensory cue after each rule-reversal. Boutons slightly lagged in updating their response similarly to the animal's behavior. Classifiers(multi-layer perceptrons) trained to decode stimulus identity, trial contingency and behavioral outcome rapidly increased their performance for each variable during cue-delivery. Thus, cortical-bulbar feedback carries information related to stimulus identity, contingency, and behavioral outcome, which is readily re-formatted across different rules of engagement in evolving environments.

208 **Neural Mechanisms Involved In Contextual And Cognitive Load Influences On Odor Discrimination**

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Understanding the neural mechanisms involved in odor discrimination helps us understand both chemosensation and learning. Gamma oscillations (65-85 Hz) in the rat olfactory bulb (OB) Local Field Potential (LFP) are elevated during fine odor discrimination. These oscillations are necessary and sufficient for fine odor discrimination across rats, mice and honeybees. However, we do not know what cognitive aspects of the odor discrimination task drive increased gamma. Preliminary data suggest that cognitive load may be a factor, with easier loads making discrimination of similar odorants simpler than in high load conditions. We address interactions between cognitive load and odorant similarity using a paradigm in which context cues make an odor discrimination task easier or harder in a trial-by-trial or block fashion. Rats discriminate a pair of very different odorants (coarse) and a pair of very similar odorants (fine) in block and interleaved sessions in a 2-alternative choice task for sucrose reward. We record LFPs from the OB, piriform cortex and hippocampus while rats perform this task. We test the hypotheses that a) warning that a difficult discrimination is required will elevate gamma in anticipation of the odor, and b) informative vs. uninformative context cues decrease the difficulty of the fine discrimination requiring a smaller increase in gamma to do well in the task.

209 **Predicting Intensity Interactions In Odor Mixtures.**

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Most odors encountered in daily life are complex mixtures where molecules interact to overshadow, suppress,

inhibit and synergize with each other. Multiple models exist to predict the odor intensity of a mixture from the intensity of its components; however, these interaction models have not been compared systematically and are not based on biopsychical interactions. In this study, 15 panelists rated the intensity of binary mixtures where each component was presented at varying concentrations. An additive model consistently overestimated mixture intensity, as most mixtures were less intense than the strongest component. The intensity of the mixture was predicted by the intensity of the components as well as the odor threshold of the components, but was only weakly predicted by the maximum intensity of the components. These interactions suggest that most previous models, which only predict mixture intensity using the intensity of the components, would be improved by adding information about each components' concentration-intensity function.

210 **Designing A &Ldquo;Smell-Aid&Rdquo; Through Enhancing Intranasal Air And Odorant Delivery Patterns**

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Innovations to enhance sensory functions have importantly advanced human civilization, e.g. for vision: the microscope, telescope, and eye glasses; for hearing: stethoscope, hearing aids, etc.- they all serve to enhance the external stimuli to enable us to see or hear things that we wouldn't otherwise be able to. But we have no equivalent technology for sense of smell. We attempt to design prototypes of"Smell-Aid" that may enhance the odorant delivery to the olfactory epithelium, using: (a) a nasal foam plug with a diagonal channel embedded, confirmed by computer modeling that would direct air/odor flow upwards to the olfactory region; (b) a clip (similar to synchronized swimmers use) pinching a critical nasal valve region that may intensify the nasal airflow vortex to the olfactory region. Detection threshold to phenylethyl alcohol (PEA) were measured in 58 healthy controls, in counterbalanced order, without interventions (baseline) vs with a "pinch" and with the nasal plug inserted up or down. A significant correlation was found between degree of olfactory improvement and baseline olfactory sensitivity ($r=-0.41$, $p<0.05$), with most improvement in subjects with less sensitive smell function to begin with. This makes sense - as an analogy, corrective lenses may have limited effect on a perfect 20/20 vision but can significantly improve suboptimal vision. Thus, we divided the sample based on the median of PEA thresholds (16.5) into "average" (8-16.5, n=30) and "super smeller" (>16.5, n=28), and found that PEA thresholds were significantly improved in the average group (baseline 12.5+-2.8, pinch 14.75+-5.4, plug up 14.41+-4.9, $p<0.05$), but not among the "super smeller" nor in plug down condition. Novel approaches to enhance nasal airflow and olfactory odor delivery may one day lead to an OTC smell aid.

211 **Smr1 Expression Upregulates Across Development In Males But Not Females**

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There are sex differences in both salivary protein (SP) expression and the rate of acceptance of a bitter diet. Males, but not females, increase acceptance of a quinine containing diet within 4 days of exposure, and the acceptance is correlated with an increase in SP expression. We have identified SPs correlated with this increased diet acceptance, one of which is submandibular gland androgen regulated protein (SMR1) at the 23 kDa band. SMR1 is differentially expressed in males and females. Here, we aimed to determine the developmental profile of SMR1 expression in rats under control conditions. We asked if expression of SMR1 was under adult hormonal control or if differences between males and females were present at weaning. We collected saliva from male and female Long Evans rats kept on a rodent laboratory chow (Envigo 2018) between postnatal day (PD) 20 and 57. Across development males demonstrate a significant upregulation of the 23 kDa band (identified by western blot as SMR1) across time ($p <0.001$) while females do not ($p = 0.137$). Males and females did not differ during the early saliva collections, but we found a trend in the expression of the 23 kDa band between males and females on PD 36, 48, and 50 (p's = 0.08) and a significant difference at PD 57 ($p = 0.05$). Our data suggest that young animals show low expression of this protein, but it is upregulated around puberty in males. This is consistent with the proteins being activated by androgens at puberty.

212 **Assessing Retronasal Odor Perception And Its Relation To Eating Behavior Among Young Children**

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Flavor perception is a critical determinant of food choices, which directly relate to risk for disease. An understanding of flavor perception in young children would inform interventions for eating behavior in early life and reduce health risks later in life. Often referred to simply as "taste", flavor is in fact a multisensory experience that combines gustation and retronasal olfaction. Although taste preferences are stable from birth, the development of retronasal smell perception remains unknown, partly due to the difficulty in assessing sensory function in young children. The primary goal of this study is to implement a novel protocol for assessing flavor preference in toddlers to investigate development of retronasal odor perception. Subjects were recruited and tested in the local community. Young children ages 3 to 6 years old (n=50) and one of their parents (n=50) were asked to drink solutions containing either a taste or odor compound. Participants rated the solutions on a pictorial liking scale. Video recordings of facial and vocal responses to the solutions were also obtained. Ratings for sweet and bitter taste were stable with age, demonstrating validity of the rating scale. In order to examine changes in flavor perception with age, all solutions were analyzed to determine perceived intensity and valence. Intensities for positive and neutral odors decreased with age. Valences of positive and negative odors changed with age, indicating higher variability in odor valences in children. This suggests that intensity and valence of retronasal odor perception differ between children and adults and can potentially be modified by experience. Ongoing work

focuses on the relationships between retronasal odor perception and individual differences in eating behavior within children.

213 **Egr4 Is Critical For Development Of The Peripheral Gustatory System**

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Early Growth Response 4 (EGR4) belongs to the EGR family of zinc-finger transcription factors and has a critical role in the development of several cell types such as spermatogonia and dorsal root ganglia (DRG) neurons. During our investigation of novel genes important for the development of geniculate ganglion neurons, EGR4 was identified as a gene enriched in Phox2b-positive oral sensory neurons. Its function in the gustatory system is currently unknown. We observed severe loss of Phox2b expression in oralsensory neurons of the geniculate ganglion, and a reduction in the chemosensory innervation of taste buds in both fungiform and circumvallate papillae in the *Egr4*$^{-/-}$ mice. Chorda tympani nerve recordings also demonstrated that *Egr4*$^{-/-}$ mice exhibit deficits in responses to some taste stimuli. To understand the downstream mechanism of EGR4 function, we performed RNA-seq on the geniculate ganglia from *Egr4*$^{+/+}$ and *Egr4*$^{-/-}$ mice. We found that axon guidance genes such as PlexinB3, Robo2, and Draxin were significantly downregulated in *Egr4*$^{-/-}$ mice. On further investigation, these proteins were also significantly reduced in the axon terminals innervating taste buds in both fungiform and circumvallate papillae. These results indicate that EGR4 plays an integral role in cell fate determination of the oralsensory neurons in the geniculate ganglion and controls the expression of the axon guidance molecules required for the proper neuronal innervation, and/or synapse formation in taste buds.

214 **The Effectiveness Of Structured Exposition To Odors On The Nociceptive And Olfactory Function In Children And Adolescents**

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Objective: In previous studies the influence of short-term odor presentation on the pain perception threshold was shown for adults. We wanted to investigate the effects of smell training on the nociception and olfactory function in healthy children. *Methods:* Seventy participants aged 6 to 17 years attended 2 appointments, 3 months apart. Between those appointments the test group trained twice daily with four odors and the control group trained with odorless “Sniffin’ Sticks”. We performed quantitative sensory testing and transcutaneous electrical nerve stimulation to establish the detection threshold, pain threshold and pain sensitivity. Additionally, we conducted extensive olfactory assessment using the “Sniffin’ Sticks” tests. *Results:* Neither the mechanical perception nor the electric pain perception was significantly influenced by the smell training. In the “U-Sniff” test we found a significant interaction between training and time of measurement (p=.036). The post-hoc test revealed a significant decrease in odor identification ability by means of the “U-Sniff” test from the first (M=11.17 SEM=.21) to the second appointment (M=10.5 SEM=.21) in the control group (p=.008). In addition, the “U-Sniff” test results differed significantly between the test (M=11.18 SEM=.02) and control group (M=10.50 SEM=.21) at the second appointment (p=.022). *Conclusions:* In this study we were not able to verify an association between training with odors and modulation of nociception in healthy children. However, we conducted a feasibility study for further research in a pediatric research field. The decrease in odor identification ability in the control group is possibly caused by a loss in confidence in one`s own olfactory ability during 3 months of training with odorless “Sniffin’ Sticks”.

215 **The Activating And Repressive Activities Of The Transcription Factor Tfap2E/Ap-2E Define The Cellular Identity Of Vomeronasal Sensory Neurons.**

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The cellular identity of neurons defines their ability to detect, process, and transmit specific signals to defined targets. Transcription factors (TFs) determine cellular identity via direct modulation of gene transcription and recruiting chromatin modifiers. However, our understanding of the mechanisms that define neuronal identity and their magnitude remains a critical barrier to elucidating the etiology of congenital and neurodegenerative disorders. The rodent vomeronasal organ provides a unique system to examine in detail the molecular mechanisms underlying the differentiation and maturation of chemosensory neurons. The cellular identity of neurons, once established, has, for long been assumed to be immutable. Here we show, by using a series on new

mouse models, that the identity of postmitotic/maturing VSNs and vomeronasal dependent behaviors can be reprogrammed through 1) the rescued expression of the transcription factor AP-2ε in the AP-2εNull mice, and 2) by inducing ectopic AP-2ε expression in mature apical VSNs. By analyzing single-cell sequencing of control and mutant mice we found that the transcription factor AP-2ε can reprogram both maturing and mature VSNs bypassing cellular plasticity restrictions. These changes translate into both morphological and behavioral alterations. After performing Cut&Run and sequencing we also found that the TF AP-2ε directly binds and controls the expression of batteries of vomeronasal genes. In conclusion, we found that AP-2ε acts as a terminal selector transcription factor able to activate and repress specific genetic programs enriched in different types of vomeronasal sensory neurons.

216 **Olfactory And Cognitive Effects Of Olfactory Training In Children After Mild Traumatic Brain Injury**

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Head trauma may lead to olfactory dysfunction and impairment of cognitive functions. Presented study aimed to verify whether repeated exposure to odors, i.e., olfactory training (OT), improves functioning in olfactory and cognitive domains in children after mild traumatic brain injury (TBI). Study sample included 159 children (M_{age}=9.68±2.78 years, 107 males) – 77 after TBI and 82 healthy controls. Participants were randomly assigned to one of two training regimens: (1) OT with odors in low concentration, and (2) OT with odors in high concentration. OT was performed twice a day for 12 weeks with set of four odors (rose, lemon, eucalyptus, cloves). Olfactory functions (threshold and identification, measured with Sniffin' Sticks and U-Sniff), fluid intelligence (measured with Raven's Progressive Matrices), and executive functions (measured with Tower of Hanoi and Tower of Lonond tasks) were assessed before and after the training. We found that OT with odors in low concentration related to increased olfactory sensitivity in children after TBI (pre-OT M=6.77±.63, post-OT M=9.58±.63). Children after TBI and healthy controls who underwent OT with low-concentrated odors scored higher in Raven's Progressive Matrices at post-training measurement (pre-OT M=50.71±3.18, post-OT M=60.31±3.18) whereas score of children performing OT with high-concentrated odors did not change significantly over time. Participants who performed OT with high-concentrated odors obtained greater scores in Tower of Hanoi task after OT as compared with their baseline score (pre-OT M=68.7±4.01, post-OT M=47.76±4.01), whereas scores in the low-concentration group remained unchanged. Our study suggests that systematic OT might have both olfactory and cognitive effects but the role of odor concentration needs further investigation.

217 **Dose Dependent And Cell Autonmous Effect Of Gli3 On Oec Development And Gnrh-1 Neuronal Migration**

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Gonadotropin releasing hormone-1 (GnRH-1) is the master regulator hormone of sexual development and pubertal onset of mammals. This hormone is released by a subset of neurons known as the GnRH-1 neurons (GnRH-1ns), which controls the hormonal axis between the hypothalamus, pituitary gland, and the gonads. The GnRH-1ns reside within the hypothalamus but originate from the olfactory placode in the nose. During embryonic development the GnRH-1ns migrate with a neural crest derived glial cell type known as olfactory ensheathing cells (OECs) along the axons of the terminal nerve to get to the brain and eventually to their final positions within the hypothalamus. Perturbations in the development and/or migration of the GnRH-1ns or their ability to release GnRH-1 in humans leads to a disorder known as hypogonadotropic hypogonadism (HH), characterized with delayed or absent puberty. Previous studies have shown that OECs are crucial for GnRH-1ns migration. We recently demonstrated that loss of transcription factor Gli3 in mouse caused a loss of OECs leading to GnRH-1 migratory defects. Whether cell autonomous loss of Gli3 in the OECs is the cause of this defect is still unknown. To test the cell autonomous role Gli3 in OEC development, and GnRH-1 migration, we utilized two mouse models 1) $Gli3^{Pdn/Pdn}$ which is a hypomorphic Gli3 model and will elucidate if Gli3 has a dose dependent effect and 2) Neural crest specific Sox10Cre/$Gli3^{Flx}$ Gli3 conditional knockout. Our data suggest that Gli3 has dose dependent and cell autonomous effect on OECs development and GnRH-1 migration.

218 **The Olfactory Epithelium Of Adult Gad65-Gfp Mice Expresses Glutamic Acid Decarboxylase (Gad), The Enzyme For Synthesizing The Neurotransmitter, Gaba**

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During embryonic development, the neurotransmitter GABA influences the migration of maturing cortical neurons and their formation of functional synapses, activity of which deters apoptosis. Synaptic activity is also required for survival of olfactory sensory neurons (OSNs), which unlike cortical neurons, undergo turnover throughout life. Thus, it seems reasonable that, due to its lifelong regenerative capacity, the adult olfactory

epithelium (OE) might express GAD, the synthetic enzyme for synthesizing GABA from L-glutamic acid. Here, we have examined expression of the 65 and 67 KDa isoforms of GAD (GAD2 and GAD1, respectively) in the OE and olfactory bulbs (OBs) of GAD65-GFP mice (P ≥25) in which the promoter of the *GAD2* gene drives GFP expression. The OE of these mice has high densities of immature GFP+ OSNs and progenitor cells, suggesting it might express GAD, and the OBs express both GAD isoforms. The OE and OBs were separately harvested and homogenized in TRI Reagent, the RNA was purified, residual genomic DNA was eliminated, and the RNA was quantified and converted to first strand cDNA. RT-PCR was performed using primers for the two GADs and for OMP, which is expressed by mature OSNs in the OE and their axons in the OBs. The results were analyzed by gel electrophoresis and showed that the OE of our adult GAD65-GFP mice expresses both GAD isoforms as well as OMP. A previous study using mice from the same GAD65-GFP founder strain as our mice showed that by P21, most GFP+ cortical cells were immunoreactive for GAD65 and GABA. Expression of GAD65 and GABA in GFP+ OSNs and/or progenitor cells of the adult OE would suggest that GABA might play a role, e.g., in OSN turnover, axonal outgrowth, and/or formation of new synapses with OB neurons.

219 **Can Earthworms Detect Glutamic Acid In Soil?**

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Earthworms are detritivores that derive their nutrients from decaying organic matter as they move through the soil. While many ecological studies have suggested that earthworms are attracted to soil containing decaying organic material, few studies have attempted to establish molecules that are appetitive to earthworms. Many animals detect glutamic acid (Glu), which may indicate a source of protein, and respond to the presence of Glu with appetitive behaviors. Therefore, in this study, we mined an *Dendrobaena veneta* (European nightcrawler, formally *Eisenia hortensis*) transcriptome for evidence of metabotropic Glu receptors and developed a feeding assay to determine if earthworms found soil containing additional Glu more appetitive than control soil. The transcriptome contained 4 sequences which share homology to metabotropic Glu receptors and were predicted to contain protein domain PF00003.21, which is associated with ligand binding in Tas1Rs. We have confirmed the identity of 3 of these sequences through RT-PCR and Sanger sequencing. To test if earthworms consumed soil containing Glu at a higher rate, we first starved earthworms on agar for 48 hours to empty their gastrointestinal (GI) tract. Then, we placed worms in soil with or without Glu for 60 mins and measured the faction of their GI tract that was filled with soil. We tested 0, 5, 10, 50 μM L-Glu (n = 10 to 57) and found that it significantly (ANOVA ($F(1,96) = 18.148$, $p < 0.001$) increased the rate the earthworms consumed soil. Taken together, these results suggest that earthworms respond to the presence of Glu in their subterranean environment by increasing their rate of feeding and provides a possible molecular mechanism by which Glu could be detected by these organisms.

220 **Identifying Humans From The Smell Of Their Ear**

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Mammals can identify conspecifics, and humans, by their body-odor. This has driven an effort to develop machines that can do the same. However, these machines, also referred to as electronic noses, or eNoses, have seen only limited success at this task. A critical step for body-odor based identification is selection of the body-odor to be smelled. Most studies have concentrated on mouth and armpit. These body regions, however, pose specific complications: the mouth has high humidity that complicates real-time sampling, and the armpit is often covered in cosmetics. By contrast, the ear may pose an attractive target for this task. The inside of the ear is 1. accessible, 2. typically not washed or doused in cosmetics, 3. it is a bodily cavity without excessive humidity, and 4. It contains cerumen, a known source of body-VOCs. To test the hypothesis that humans can be identified by the smell of their ear, we used a PEN3 eNose equipped with a custom-tip made of Teflon. We initially sampled 10 individuals across 10 days. Each sample entailed 5 repetitions, each lasting 50 seconds. Using a Fine KNN classifier, we found that within a sampling day, we achieved 82% accuracy, which is significantly better than the 10% chance in this task. In other words, consistent with our hypothesis, humans can be identified in real time by the smell emanating from their ear. However, due to drift, this level of performance drops to 12% when comparing across days. Here we will present this basic result, its comparison to other body-regions in the same body-odor donors, and our efforts to deal with drift.

221 **Neural Network Dynamics Of Retronasal Olfactory Discrimination**

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An odor can enter the nasal cavity through the mouth, referred to as retronasal olfactory route and generate olfactory percepts integrated to flavor learning. Our study aims to examine the neural network evoked by retronasal inputs as they relate to gustatory, reward, and learning circuits. Rats were trained to perform retronasal olfactory discrimination tasks with multiple odor sets, and we recorded local field potentials in ipsilateral olfactory bulb, olfactory tubercle, anterior piriform cortex, gustatory cortex and hippocampus. Fast neural oscillations in the olfactory systems demonstrates context-specific characteristics over stages of learning. Results show a significant decrease in the gamma (40 - 110Hz) band during retronasal odor sampling in the olfactory system and a cue-specific effect in the beta (15 - 35Hz) range. In addition to neural signals, we monitored

breathing with nasal thermocouple electrodes and licking with jaw muscle movements and showed both sensorimotor inputs drive electrophysiological responses in the olfactory system. In summary, we are able to portray a comprehensive picture of the dynamical patterns for retronasal olfaction with coherence and causality analysis in relation to reinforcement type, learning phase and interaction with breathing and licking.

222 **Taste Perception Of Prebiotic Oligosaccharides**

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Prebiotic oligosaccharides are naturally occurring non-digestible carbohydrate oligomers with demonstrated health benefits. Given their natural presence in foods and popularity as a commercial prebiotic product, a relevant question is whether these compounds are detected by the human gustatory system, and if so, by what mechanism are they detected. While the taste of commercial prebiotic preparations is often described as mildly sweet, these preparations contain a mixture of oligosaccharides with varying degree of polymerization and their substituent sugars (e.g., glucose, fructose, and sucrose). Here we measured the gustatory detection of highly pure prebiotic oligosaccharides [fructooligosaccharides (FOS), galactooligosaccharides (GOS), and xylooligosaccharides (XOS)]. Each of these classes of prebiotic oligosaccharides differs in the type of glycosyl residue (glucose, fructose, galactose, or xylose), and the position and type of bond between those residues. During each trial, subjects were asked to discriminate a target stimulus prepared at 75 mM from two water blanks (i.e., triangle test). Tests were conducted either in the presence or absence of lactisole, a sweet receptor antagonist. We found that all compounds tested were detectable at a significant level ($p<0.05$). Study findings will be discussed in terms of how molecular structures could impact taste perception of oligosaccharides.

223 **Predicting Odor Mixture Similarity Leveraging Natural Language Percepts**

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Language is often thought as being poorly adapted to objectively describe or quantify smell and in particular olfactory attributes. In this work, we show that semantic descriptors of odors can be implemented in a model to successfully predict for odor mixtures the two olfactory attributes of similarity and discriminability. We achieve this by taking advantage of the structure to percept model we previously developed using chemical descriptors to predict pleasantness, intensity and 19 semantic descriptors (fish, cold, burnt, garlic, fruity, sweet.) describing monomolecular odorants. We first used this model to predict perceptual values of olfactory mixtures through linear averaging and then implemented a metric learning using lasso regression to predict from these percepts the discriminability between any two odor mixtures. Using 3 different publicly available datasets (Snitz, Ravia, Bushdid) to train and test our approach, we show that our method is robust and generalizable and it clearly outperformed state of the art methods predicting odor mixture discriminability directly from chemical descriptors. This not only meets the need of rapidly obtaining interpretable predictions of odor mixture similarities, as only 10 of the percepts were used to predict discriminability (fish, cold, sour, bakery, grass, burnt, garlic, sweet. intensity and chemical) but also shows that language can be used to establish a metric of odor similarity and discriminability in the everyday olfactory space.

224 **Taste Processing In The Gustatory Cortex In A Mouse Model Of Frontotemporal Dementia (Ftd)**

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Frontotemporal dementia (FTD) is the second most prevalent form of presenile dementia. Patients with FTD show a pathological sweet tooth and decreased ability to identify flavors. Taste perception depends on neural processing in chemosensory regions such as the insular cortex - a brain region that also contains the primary taste cortex, gustatory cortex (GC). The chemosensory deficits in FTD may be related to GC damage as insular cortex is one of the primary targets in FTD disease progression. Little is known on how circuitry changes related to FTD lead to abnormal activation of GC and to deficits in taste processing and taste-odor association in FTD. The goal of this project is to test the hypothesis that the chemosensory deficits in a mouse model of FTD are related to abnormal patterns of neural activity in GC. TDP-43 inclusions are a significant pathological feature in 50% of FTD cases, thus we use a transgenic mouse model overexpressing human transactivating response region (TAR) DNA binding protein (TDP-43) with a Q331K mutation. To assess chemosensory deficits, we relied on a taste-based two alternative forced choice (2AFC) task probing the ability to discriminate sucrose/NaCl mixtures. Analysis of psychometric functions of mutant and control mice revealed that TDP-43 Q331K mice make more mistakes and show significant deficits in the mixture discrimination 2AFC task. To monitor neural activity, we relied on electrophysiological recordings using chronically implanted tetrodes in alert mutant and control mice. Activity in GC was probed as mice licked at different concentrations of sucrose. We observed a larger number of taste-evoked excitatory responses in TDP-43 Q331K mice compared to control mice, suggesting the possibility that GC may be abnormally excitable in TDP-43 Q331K mice.

226 **Taste Dysfunction In Covid-19: Specificity For Umami?**

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COVID-19 continues to impact the lives of most people throughout the world. The influences of this disease on quantitative taste tests remain enigmatic. We compared taste test scores of three groups on the 27-item version of

the Waterless Empirical Taste Test (WETT®): 22 COVID-19 patients in the acute phase of the disease; 40 who had recovered from COVID-19 for 5 or more months; and 194 healthy controls who had never contracted COVID-19. An 8-item olfactory test was also administered. The overall taste test scores were equivalent among the three groups [ANOVA p = 0.33; respective means (95% CIs) = 14.9 (12.3-17.5); 16.1 (14.2-18.0); 16.7 (16.0-17.4)]. However, unlike the sweet, sour, bitter, and salty tasting stimuli, the acute subjects underperformed the other two groups in identifying umami [ANOVA p = 0.003; respective means (95% CIs) = 0.6 (0.1-1.1); 1.2 (0.7-1.6); 1.5 (1.4-5.7)]. The olfactory scores of the acute group were significantly lower than those of the other two groups, which did not differ significantly [ANOVA p <0.0001; respective means (95% CIs) = 5.8 (4.8-6.8); 6.4 (5.9-6.9); 7.2 (7.1-7.3)]. These findings are in accord with earlier studies suggesting that, on average, taste-bud mediated taste sensations of the four classic taste qualities are not meaningfully impacted by COVID-19, unlike olfactory sensations. However, the fifth taste quality, umami, appears to be impacted by this disease. Although there may be individual cases where true taste dysfunction occurs for all taste qualities, they appear to be rare or very transient. As in the general population, most complaints of taste loss in COVID-19 patients likely reflect loss of flavor sensations secondary to retronasal stimulation of the olfactory receptors during deglutition.

227 **Application Of The Mandibular Function Impairment Questionnaire As Diagnostic Aid In Elderly Bms Patients**

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Elderly patients with burning mouth pain with taste/sensory alterations (BMS) can have difficulty pinpoint the origin of the discomfort. The Mandibular Function Impairment Questionnaire (MFIQ), created by Stegenga et al (1993) and validated to measure jaw impairment, is a 17 item questionnaire rated on a Likert scale (0-4). Here we use the MFIQ to see if it is can help differentiate BMS from pain originating in the temporomandibular disorder (TMD) to help better target examination. A retrospective study was conducted at a private oral medicine clinic. 96 patients filled out the MFIQ prior to examination; 27 were diagnosed with BMS and 69 were diagnosed with TMD (age 58.7±11.2; 33.9±14.3; p<0.000). 7 BMS patients also had clinical signs of TMD. TMD patients had significantly more difficulty with taking a large bite (2.23±1.214; BMS 0.52±1.05; p<0.001), chew hard foods (2.23±1.274; BMS 1.04±1.53; P<0.001), chew resist foods (2.07±1.35; BMS 0.89±1.50; P<0.001), yawning (1.68±1.14; BMS 0.44±1.01; P<0.001), kissing(0.86±1.19; BMS 0.30± 0.8; P=0.028), eating meat(1.96±1.18; BMS 0.067±1.30; P<0.001), carrot (1.80±1.34; BMS 0.81±1.41; P=0.001), baguette(1.67±1.35; BMS 0.96±1.53; P=0.029), and apple (1.74±1.207; BMS 0.81±1.52; P=0.002). The MFIQ score was significantly higher in TMD (1.70±0.671; BMS 1.26±0.656; P=0.005). BMS patients reported significantly less impairment in jaw function, even in those with clinical signs of TMD, despite their report of severe facial/oral pain. The MFIQ may aid diagnosis of BMS in elderly patients.

228 **Role Of The Tas2R-Mediated Signaling Pathway In Gingival Inflammation And Periodontal Bone Loss.**

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Solitary Chemosensory Cells (SCCs) in the airways, gut and urogenital tract are involved in innate immune responses. We recently reported SCCs expressing a T2R-mediated signaling transduction cascade in mouse gingival tissue. Gingival SCCs play a role in oral microbiome regulation and protection against periodontitis--affecting 64 million in the US alone (Zheng et al. 2019). Here, we conducted ligature induced periodontitis model on 112 littermate-controlled cohoused mice, including WT (wild-type), $Gnat3^{(-/-)}$, $TrpM5^{(-/-)}$, and $Skn1a^{(-/-)}$ mice. Using the software Dragonfly (v2020.2), a deep learning computer model was developed for quantifying alveolar bone loss from micro-CT data. Ligatured WT mice lost 29.56% of the alveolar bone volume compared to the un-ligatured baseline, while $Gnat3^{(-/-)}$ mice lost 34.32% (P<0.01), and $Skn1a^{(-/-)}$ mice lost 37.46% (P<0.05). The bone density was also lower in ligatured $Gnat3^{(-/-)}$ and $Skn1a^{(-/-)}$ mice compared to ligatured WT mice (P<0.05). No significant differences in alveolar bone volume and density were found in $TrpM5^{(-/-)}$ mice or the three heterozygotes ($Gnat3^{(+/-)}$, $Skn1a^{(+/-)}$, $TrpM5^{(+/-)}$) compared to WT mice. RNA-seq data revealed that the bone loss observed in the Gnat3-KO and Skn1a-KO was consistent with the changes in inflammatory marker genes. Also, the P38/MAPK pathway was downregulated in Gnat3-KO and Skn1a-KO mice, but upregulated in the WT and TrpM5-KO mice. The results demonstrate that Gnat3 but not TrpM5 provides protection against periodontitis. Moreover, similarly to the $Gnat3^{(-/-)}$ mouse, the $Skn1a^{(-/-)}$ shows severe inflammation and comparable alveolar bone loss. This T2R-mediated protective role in periodontitis represents a critical defense mechanism against bacterial infection and provides novel targets for treating periodontitis.

230 **Improving Diet Quality And Health Behaviors Through Sensory Based Tailored Messaging: A Study Protocol For An Online Multicomponent Intervention**

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Precision nutrition recognizes multiple, synergistic levels of influence on phenotype for personalizing dietary interventions. Paramount is sensory nutrition, including taste-related likes/dislikes as driving food choice. Initial findings from our laboratory show that health promotion messages tailored to liking phenotype via an online platform are acceptable and useful to adults to motivate diet quality improvements. Adults (n=229) reported ease of survey completion; usability in behavioral reflection; informative and helpful tailored messages; and willingness to try healthier behaviors in response to the messages. Here we report a feasibility trial to assess acceptability and utility of an online intervention to increase knowledge, motivation, and confidence for diet quality improvements. Guided by the Information, Motivation and Behavioral Skills framework, the intervention includes an online nutrition and health behavior survey that delivers tailored messages to inform a virtual motivational interviewing (MI) session for goal setting, 9-weeks of goal-specific tailored messages, and support from a private Facebook group. Adults receive messages tailored to their food likes and learning style to motivate healthier diet quality and address barriers to behavior change. Primary outcomes include: feasibility and usefulness of the survey and tailored messages, ability of the messages to inform the MI session and goal setting; and intervention fidelity. Qualitative analysis of the MI session will inform additional message content on sensory nutrition and barriers to healthy eating. Secondary outcomes are increases in knowledge of and confidence in diet behaviors and diet quality improvements. This study will address gaps in understanding the role of sensory nutrition in behavioral interventions.

231 **Changes In Gut Bacteria, Olfactory Neuron Abundance, And Neuroinflammatory Signaling Following Fatty Diet Consumption That Is Not Obesogenic.**

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It is well documented that a fatty diet causes a shift in gut microbiota, systemic inflammation, and neuroinflammation. Mice fed a moderately high-fat (MHF, 32.5% kcal from fat) diet display a loss of olfactory sensory neurons (OSNs) and have deficits in olfactory discrimination. We hypothesized that excess fat in the diet can induce a state of dysbiosis of the gut microbiome, which can lead to neuroinflammation and subsequent OSN loss. To explore our hypothesis, M72*tau*LacZ mice were maintained for 5 months on the following diets: CF *ad libitum* (CF), MHF *ad libitum* (MHF adlib), or MHF isocalorically matched to CF mice (MHF iso). Fecal samples were collected from a subset of mice at 3 months into the dietary treatment. Preliminary analysis of fecal bacteria revealed a significant dietary effect on the *Firmicutes* to *Bacteroidetes* ratio (F/B ratio; CF=1.24 (n=9), MHF iso=2.79 (n=9), MHF adlib=4.13 (n=8), males and females combined; Kruskal-Wallis p=0.0004). The MHF adlib and MHF iso mice had significantly higher F/B ratios than CF (p=0.0005; p=0.0151), without differing from each other (p=0.8555; Dunn's post-hoc). Olfr160+ OSNs were counted throughout the entire epithelium to quantify neuronal loss. MHF adlib and MHF iso mice had fewer OSNs than CF mice (1-way ANOVA, Males: $F(2, 22)=14.71$, $p<0.0001$; Females: $F(2, 20)=8.286$, p=0.0024, Tukey post-hoc). Serum tumor necrosis factor alpha (TNFα) was measured by ELISA to determine inflammatory status. MHF adlib and MHF iso mice had significantly elevated serum TNFα compared to that of CF mice (1-way ANOVA, Males: $F(2, 21)=6.772$, p=0.0054; Females: $F(2, 21)=7.450$, p=0.0036, Tukey post-hoc). We conclude that excess fat in the diet causes inflammation, OSN loss, and an increased F/B ratio for mice that are isocalorically yoked to prevent obesity.

232 **Variations In Taste-Related Salivary Proteins Are Differently Associated With Gender And Prop Taster Status In Normal Weight And Obese Subjects**

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Individual variations in taste perception, 6-n-propylthiouracil (PROP) bitter taste phenotype, and gender are known to affect food preferences, nutritional status and obesity. Some data suggest that the salivary proteins, belonging to basic proline rich proteins (bPRPs) and histatin (Hist) families, play a role in altering human taste perception. This study investigated variations in the profile of salivary proteins, using HPLC-low resolution-ESI-IT-MS analysis, between 61 normal weigh (NW) subjects and 57 subjects with obesity (OB), characterized by gender and PROP taster status. Results showed variations in taste-related salivary proteins between NW and OB, which were differently associated with gender and PROP classification. In general, levels of Ps-1 protein were higher in OB than that in NW individuals ($p = 0.00079$; Fisher's test LSD). Higher levels of several bPRPs including Ps-1, II-2 and IB-1 as well as PRP-1 protein belonging to the acid proline rich family were found in OB males ($p \leq 0.038$; Fisher's test LSD), who showed a lower body mass index (BMI) than OB females ($p \leq 0.017$; Fisher's test LSD). With respect to PROP status, higher levels of Ps-1 protein and Cystatin SN (Cyst SN) were found in OB non-tasters ($p \leq 0.032$; Fisher's test LSD), who had lower BMI than OB super-tasters ($p \leq 0.002$; Fisher's test LSD). These results demonstrate that specific salivary proteins, previously associated with higher taste perception, may be associated with body weight excess in females or those with higher PROP sensitivity . Further study of the role of these proteins in individual taste responses and eating behavior as they relate to obesity is warranted.

233 **Use Of C-Peptide As A Measure Of A Taste-Induced Cephalic Phase Insulin Release In Humans**

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Cephalic phase insulin release (CPIR) is a small, transient spike of insulin secreted within minutes of food-related sensory stimulation. This brief release of insulin is primarily thought to aid in reducing postprandial hyperglycemia to maintain glucose homeostasis. However, the degree of CPIR in humans appears to be highly variable across studies and between subjects. There are several factors contributing to this variability. One may be the use of peripherally measured insulin as an indicator of secreted insulin, since a substantial portion of insulin is metabolized by the liver before delivery to peripheral circulation. Here, we investigated the use of c-peptide, which is co-secreted in equimolar amounts to insulin from pancreatic beta cells, as an index of insulin secretion. We tested subjects (N=18) over two repeated sessions where blood samples were taken during the cephalic phase period (0-8 minutes) after oral stimulation with a sucrose-containing gelatin stimulus. Changes in plasma c-peptide and insulin were monitored. As expected, we found a significant rise in both c-peptide and insulin from baseline 2-4 minutes post-stimulation. Notably, when c-peptide and insulin concentrations were compared across sessions, we found that changes in c-peptide were significantly correlated at the 2 minute (r = 0.50, p = 0.03) and 4 minute (r = 0.65, p = 0.003) time points, as well as when subjects' peak values of c-peptide over 0-8 minutes were considered (r = 0.64, p = 0.004). In contrast, no significant correlations were observed for changes in insulin measured from the sessions (r = -0.06-0.35, p <0.05). Study findings will be discussed in terms of how the measure of c-peptide could be used as a more reliable index of insulin secretion when investigating taste-induced insulin responses.

234 **Active Olfactomotor Movements In Head-Fixed Mice**

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Olfactomotor responses are respiratory, orofacial, and locomotive movements for sampling and reacting to odors (Rabell et al. 2017, Kurnikova, Deschênes, and Kleinfeld 2019, Findley et al. 2020, Johnson et al 2003, Wesson et al 2008, Jones and Urban 2018). Altered sensory sampling behaviors, such as eye movement, temperature insensitivity, and excessive sniffing, have been identified in individuals with Autism Spectrum Disorder (ASD). In addition, Rozenkrantz et al. (2015) showed that olfactomotor behavior is affected in children with ASD. These children do not modulate sniffing behavior to aversive odors despite correctly identifying odors as unpleasant, suggesting an altered unconscious motor response. To investigate the neural mechanisms underlying olfactomotor sampling, we investigated respiratory and orofacial responses to odor using wildtype mice. Wildtype mice are exposed to 2-phenylethanol (attractive odor), 2-methylbutyric acid (aversive odor), alpha-pinene (neutral odor), or clear air in the course of a behavioral session. We record respiration with an intranasal thermistor, and track orofacial movements using DeepLabCut. Our preliminary results in wildtype mice (n=2) suggest that mice alter their sniffing and nose movement in response to odor stimuli. This work will shed light on active olfaction and help us understand more about naturalistic olfactomotor behaviors.

235 **Axially Independent Optical Control And Readout Of Neuronal Circuit Activity In The Olfactory Bulb**

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To investigate the odor response properties of sister mitral and tufted cells, which share input from the same parent glomerulus, we coupled one-photon patterned optogenetic stimulation with quasi-simultaneous readout of activity using multiphoton microscopy. We used a digital micro-mirror device (DMD) to create patterned illumination profiles. Axial separation of stimulation and readout planes was implemented by conjugating a motorized translatable holographic diffuser with the desired photo-stimulation plane, thus achieving widefield axial optical sectioning (15 μm x-y, 30 μm z-resolution, across 1.5 x 1.5 mm, calibrated up to a 500 μm axial shift). We controlled the activity of single glomeruli by stimulating OSN terminals in OMP-Cre x ReaChR mice on the surface of the main olfactory bulb. In parallel, we monitored responses (GCaMP6) of the output neurons (mitral and tufted cells) across different optical planes. We sampled mitral and tufted cell responses either within the photo-stimulation plane (glomerular dendritic tufts) or at 150-250 μm (cell bodies) below the photo-stimulation plane across a range of photo-stimulation intensities. Glomerular stimulation of OSN terminals selectively evoked responses in the targeted glomerulus and in the cell bodies of sister mitral and tufted cells receiving input from the photo-stimulated glomerulus and, further, revealed lateral inhibitory interactions between glomeruli. In ongoing experiments, we are analyzing the response properties of the identified sister cells as a function of brain state and experience.

236 **Quantitative Odor Intensity From Qualitative Comparisons**

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The perceived intensity of an odorant depends both on the molecules that compose it and the concentration at which they are delivered. Traditionally, these relationships have been estimated by asking subjects to provide numerical intensity ratings for each odorant at each of a series of concentrations, and then fitting these ratings to a theoretical curve whose parameters describe the concentration-intensity relationship for that odorant. However, numerical ratings are not always ideal: the meaning of a numerical value may drift across time, or due to anchoring effects; some subjects may be innumerate or otherwise unreliable with numbers; or available

information about intensity may be non-quantitative. To overcome these concerns, we validated a proprietary statistical model provided by an industrial company for translating qualitative information about the relative intensity of pairs of odorants into quantitative information about intensity. This model uses relative, categorical information about odorant pairs (e.g. “A is stronger than B”, “A is much weaker than B”, “A and B are about equal”) to estimate intensities at all concentrations for each odorant. To validate this approach, we collected both qualitative intensity comparisons and quantitative numerical ratings for 6 odorants (and solvent controls) across 5 orders of magnitude in concentration in the lab using a system that blinded subjects to odorant identity or concentration. We fit this statistical model to the qualitative data and obtained good fits to theoretical curves that distinguished between the odorants. This model may thus be applicable in other settings where qualitative, comparative judgments of odor intensity are available.

237 **Habituation Of Piriform Cortex From Passive Odor Exposure In Awake Freely Moving Mice**

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Piriform cortex (PC) has long been established as a critical processing point for olfactory information in the mammalian brain. Prior anesthetized studies have demonstrated that PC odor responses do not maintain the same spatial organization of the olfactory bulb but reliably encode odor identity. Recent studies have demonstrated less reliability of PC odor responses over longer periods of time in awake animals, highlighting the need to further explore the role of PC in processing odors in a more naturalistic setting. To do this, we have collected PC calcium recordings of awake, freely-moving mice receiving passive presentations of a panel of six chemically different odorants. Animals received ten randomized trials of each odorant for five consecutive days, allowing for tracking of odor response fidelity within a single day and across multiple days of experience. On a population level, we find that the percent of PC cells responding to odor presentations decreases during each session, but not across days, while strength of response, conversely, decreases across days, but not during each session. Individual animals respond preferentially to different odors, matching prior anesthetized and awake studies. We find that the reliability of responses, however, varies both across days and within single recording sessions. Trial-to-trial response correlations are low on the first imaging sessions but show modest increases after multiple days of exposure. Overall, our findings demonstrate increased spontaneous activity in PC recordings of awake animals alongside genuine responses to odor presentations. Current work focuses on correlating this observed spontaneous activity with the behavioral state of the animal during each presentation and in the absence of olfactory stimuli.

238 **Models Of Inhibition In The Accessory Olfactory Bulb**

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Olfaction is not a structured sensory space, therefore the rules governing the topological organization of the olfactory bulb are still largely unknown. Investigating the logic behind olfactory inhibitory wiring could be the key to decoding odor maps and also shed light on how inhibitory interneurons participate in non-topographical neural coding in general. In the accessory olfactory bulb (AOB), we had previously observed that specific types of odorant-selective glomeruli are often juxtaposed in spatial clusters. When we delivered odors to GCaMP-labelled vomeronasal sensory neurons and imaged in the AOB for the responses in their axon termini, glomeruli fluoresced less if their neighbors were also activated, unless downstream glutamatergic signaling was blocked by D-AP5. This suggests that the observed clustering may be a feature of odor map organization, possibly to facilitate lateral inhibition. To test this hypothesis, we evaluated computational models for biologically plausible patterns in interglomerular inhibition. For each experimental recording, we modelled glomerular activity as excitatory nodes connected by various matrices of inhibitory weights, fitting the inhibition to account for the difference between control and glutamate blockade conditions. When we modelled inhibition as a function of interglomerular distance, we found little evidence of distance dependent decay, suggesting globally uniform rather than lateral inhibition. However, when we utilized a different model architecture that grouped glomeruli by their odor identities, we found some preliminary evidence of stereotypical inhibitory relationships which may suggest preferential inhibition between clustered glomeruli.

239 **Molecular And Cellular Mechanisms Of Vomeronasal Stimulus Uptake**

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In rodents and most other mammals, the vomeronasal organ (VNO) is important for the detection of semiochemicals, which trigger a wide range of innate behaviors. The VNO is a cylindrical structure with a blind ending mucus-filled lumen. Studies on vomeronasal stimulus uptake are scarce and the mechanism(s) underlying vomeronasal pumping remain elusive. Here, we investigate the molecular and cellular mechanisms that control stimulus uptake into the mouse VNO. Using both antibody staining and genetic cell labeling, we demonstrate that the lateral non-sensory VNO tissue is largely built by smooth muscle cells (SMCs), which are innervated by cholinergic and / or adrenergic fibers. By combining time-lapse imaging in acute coronal VNO tissue slices, custom movement analysis code, and Ca^{2+} imaging in samples from mice conditionally expressing GCaMP6f in SMCs, we show that (*i*) SMCs display rather uncoordinated spontaneous activity likely to maintain a constant

muscle tone, that (*ii*) SMC activation by noradrenaline mediates tissue contractions, that (*iii*) contractions are sensitive to isoform-specific inhibition of α-adrenergic receptors, and that (*iv*) noradrenaline triggers broad SMC responses in the lateral VNO, whereas acetylcholine signals are restricted to a subset of SMCs located close to the lumen. In addition, superresolution and fluorescence lifetime imaging microscopy reveal essentially perpendicular orientation axes of the two SMC populations that respond to noradrenaline *versus* acetylcholine. Together, these morphological and functional data suggest that vomeronasal pumping is mediated by antagonist groups of SMCs that are each activated by noradrenaline or acetylcholine, respectively.

240 **Deepnose: Using Artificial Neural Networks To Represent The Space Of Odorants.**

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The olfactory system employs an ensemble of odorant receptors (ORs) to sense molecules and to derive olfactory percepts. We hypothesized that ORs may be considered 3D spatial filters that extract molecular features relevant to the olfactory system, similar to the spatial filters employed in other modalities. If so, the composition of OR ensemble can be understood by training such filters using conventional artificial intelligence methods and large-scale databases of 3D molecular structures. We trained artificial neural networks to represent the chemical space of odorants and used this representation to predict human olfactory percepts. First, we trained an autoencoder called DeepNose to deduce a compressed representation of odorant molecules based on their 3D spatial structure. Next, we tested the ability of DeepNose features in predicting human odorant percepts based on 3D molecular structures alone. Finally, we finetuned the DeepNose network to better represent perceptual properties of odorants defined as semantic descriptors. We found that, despite the lack of human expertise, DeepNose features led to perceptual predictions of comparable or higher accuracy to molecular descriptors often used in computational chemistry. We propose that DeepNose network can use 3D molecular structures to yield high-quality predictions of human olfactory percepts and can help understand the factors influencing the composition of ORs ensemble.

241 **Using Neuropixels Probes To Examine Plume Following At The Cellular Level In The Main Olfactory Bulb Of Awake Mice**

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As mice navigate olfactory environments, they must interpret the intermittent and stochastic cues of olfactory plumes. Spatiotemporal patterns of odor concentration within plumes convey information regarding odor sources and their locations. New research suggests that the mitral and tufted cells (MT cells) of the olfactory bulb (OB) track dynamic sensory signals, and are perhaps capable of following plume dynamics. In wide-field recordings paired with passive, miniature odor sensors, our group recently showed that glomerular networks of MT cells follow plume dynamics (Lewis et al., 2021). Since our wide-field recordings lacked the cellular level resolution needed to investigate the responses of individual cells, we utilized the enhanced spatiotemporal resolution of electrophysiological recording using Neuropixels probes to understand how the activity of individual neurons in the OB contributes to its sensitivity to odor concentration dynamics. We recorded from an awake mouse in a wind tunnel as it experienced a variety of naturalistic plume presentations. We changed the spatiotemporal dynamics of plumes by changing the location of the odor source within the wind tunnel while keeping the location of the mouse constant. We also recorded sniffing behavior alongside electrophysiological activity to understand how odor dynamics affected sniff locked cell firing. We used machine learning approaches to analyze plume following at both the individual cell level as well as at the larger population level. Our results suggest that populations of MT cells in the first stage of olfactory processing accurately follow plume dynamics.

242 **Effect Of Temporal And Sequential Stimulus Presentations On Logistic Models From Sniff Olfactometer (So) Data**

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An unanswered question in smell perception is “How does the brain represent a stimulus intensity?”. A recent study (Ni, et al. preparation) showed that when human subjects are presented odorant puffs in a sequentially increasing or decreasing order, as would be the case in the usual “stair-case method” (e.g.,1-2-3 and then 4-5-6), the data produced questionable models with enormous confidences intervals (CIs). However, when the concentrations were interlaced (e.g.,1-3-5 and then 2-4-6), the models were more robust and reproducible with CIs <10% of the stimulus intensity. One consequence of interlacing is that the difference between adjacent odorants is much greater. We hypothesized that the brain is “recording” the difference between adjacent pairs, rather than their difference from a fixed standard. If true, this may have two consequences; 1) A noticeable difference is required for the brain to relate two stimuli, 2) The interstimulus difference (ISD) should affect the CIs. The experiments reported here were designed to determine the effect of varying the ISDs between stimuli on the CI of the resulting logistic functions. Six concentrations ranging across the subject's threshold were grouped in two triads, and the probability of perception of an odor was used to model a threshold function from the six concentrations. The ISDs were increased from 1minute to 1 day, 2 days and 7 days. Results indicate that increasing the ISDs did not affect the robustness, reproducibility or CIs.

243 **Don Tucker Finalist: High-Precision Mapping Reveals The Structure Of Odor Coding In The Human Brain**

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Odor perception is inherently subjective. Previous work has shown that odorous molecules evoke distributed activity patterns in olfactory cortices, but how these patterns map onto individualized odor percepts has remained an open question. Addressing this question requires mapping neural responses to a large number of odors. Here we examine high-resolution functional magnetic resonance imaging responses to 160 monomolecular odors from individual subjects (18 h of fMRI hours per subject, N=3) to reveal the neural coding scheme underlying odor perception. We first show that detailed odor percepts beyond odor category are represented in piriform cortex, amygdala, and orbitofrontal cortex (OFC). This detailed neural representation of odor percepts beyond a simple odor category representation is most prominent in the OFC. We then show that computational encoding models can predict odor-evoked fMRI responses based on multidimensional perceptual spaces and that the dimensionality of the encoded spaces increases from the primary olfactory cortex to OFC. Importantly, whereas encoding of lower dimensions generalizes across subjects, higher dimensions reflect the subjective nature of odor percepts. These results indicate that detailed and idiosyncratic olfactory experiences reside in OFC in form of multi-dimensional representations of our olfactory environment.

244 **Tumor Necrosis Factor (Tnf) Receptor Signaling Is Required For Taste Bud Regeneration And Neurophysiological Recovery From Chorda Tympani Nerve Sectioning**

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Mechanisms responsible for taste bud regeneration are not fully understood, hindering strategies to restore normal taste sensation after trauma, cancer treatment and viral infection. As long recognized, taste buds depend on innervation to maintain their structure and function. Several factors mediating taste bud and axonal regeneration after injury have been identified including signaling by the master proinflammatory cytokine, interleukin (IL)-1 through its receptor. Following CT nerve (CT) sectioning most ipsilateral, anterior taste buds fail to regenerate and neural responses to tastants remain negligible in *Il1r* knockout (KO) mice. IL-1 and TNF-α each promote the recovery of neural function in other severed nerves. We tested whether TNF-α signaling is also needed for the restoration of taste-elicited CT responses. The CT nerve was unilaterally sectioned or sham-sectioned in mice lacking both major forms of the TNF-α receptor (*Tnfr1/2* KO) or C57BL/6J wild-type mice. CT responses to salt, umami, sweet, bitter and acid stimuli were similar in sham-sectioned *Tnfr1/2* KO and wild-type mice, demonstrating normal taste function in the absence of injury. In contrast, <45% of taste buds regenerated and minimal CT responses to tastants were recorded from injured vs. sham-sectioned *Tnfr1/2* KO mice 8 weeks after axotomy. Taste buds that did regenerate in the absence of *Tnfr1/2* were of similar size and composed of similar numbers of taste receptor cells but insufficient to support the recovery of neurophysiological taste responses. Thus, similar to IL-1, injury reveals a requirement for TNF-α receptor signaling in taste bud regeneration and functional recovery from CT nerve injury. Whether these two proinflammatory cytokine pathways promote taste bud regeneration by shared molecular and immunological mechanisms is under active study. These results indicate that TNF-α has a pro-regenerative role in recovery from injury distinct from detrimental effects on the peripheral taste system during inflammation.

403 **Targeted Qrt-Pcr And Real-Time Cell Calcium And Atp Secretion Assays Validate Primary Human Taste Bud Tissue-Derived (Htbec) Cell Cultures As A Viable *In Vitro* Human Cell Model For Taste Modulator Research**

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There remains need for human taste bud tissue-derived (hTBEC) cell cultures as an *in vitro* human cell model of human taste transduction pathways. Our collaboration has realized primary hTBEC cell cultures derived from individual human donors where the taste behavior and genetics of the donor are also known. This effort was successful in 9 of 10 donors, yielding 1 to 11 primary cultures per donor with methods refinement across the donors. A particular donor H, highly sensitive to bitter and other taste stimuli, yielded 11 hTBEC cultures from 12 possible wells. These cultures became the focus of the creation of a bitter-responsive hTBEC platform. Responsiveness to multiple and different bitter stimuli (bitter medicines, bitter industry standard molecules) and candidate bitter blockers were documented in dual real-time ATP secretion detection and cell calcium assays. Targeted, quantitative, real-time PCR (qRT-PCR) analysis showed mRNA expression of the bitter taste receptors (TAS2R1, TAS2R8, TAS2R10, TAS2R14, TAS2R39), transient receptor potential (TRP) channels (TRPC1, TRPV1, TRPM5, TRPML3), phospholipase C beta (PLCbeta2,3,4), and the ATP release channel CALHM1. TAS2R38 and gustducin (GNAT3) remain at the limit of detection, with additional primer pairs being designed and re-tested. Additional targets are being assessed at present. TRPA1, a known irritant receptor, was not expressed well in donor H cells, although it was expressed in a panel of cell cultures from another donor J that were not useful in taste cell assays. Taken together, we continue to use an integrated approach of human sensory, genetic, and functional analyses, assays and methods to realize robust *in vitro* human cell models for taste modulator research, such as medium-throughput screening for bitter blocker molecules.

Friday, April 22, 2022

7:30 - 9:00 AM	Estero Foyer
Continental Breakfast - Breakfast with Industry	

8:00 - 10:00 AM	Estero Ballroom
Poster Session III	

300 **Role Of Taste Receptors In Marine Mammal Physiology: Identification Of Solitary Chemosensory Cells In The Florida Manatee, *Trichechus Manatus Latirostris*, Using Archived Tissue Samples**

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The use of gustation by marine mammals is not well understood, with likely a reduction or complete loss of the sense of taste in many species. However, genomic analysis indicates most marine mammals maintain small numbers of in-tact sweet and bitter taste receptors. This project explores the potential role for those receptors in the immune system of marine mammals in the form of solitary chemosensory cells (SCCs). SCCs in mammals are found in respiratory epithelium of the upper respiratory tract and utilize bitter taste receptors to respond to signals given off by pathogens to mount an innate immune response. From 2003 through 2014 samples of Florida manatee, *Trichechus manatus latirostris*, tracheal and nasal tissue were collected and stored as paraffin embedded blocks in an archive. This provides a relatively large sample size and comparison between sex, age, and cause of death. For the first time ever in a marine mammal, SCCs have been identified in this tissue using immunohistochemistry against gustducin and TRPM5 antigens. It is expected that there will be no difference in the number of SCCs when comparing sex or age. However, it may be that animals that died from a respiratory illness such as red tide would have fewer SCCs than those that died healthy such as after a sudden boat strike. Through a description of these cells in manatees and comparison to well-studied species including humans, we can develop an understanding of how manatees respond to respiratory infections such as red tide. Recommendations can then be made on treatment of respiratory illness as well as inform management decisions related to the threatened Florida manatee.

301 **Micellar Casein From Milk Reduces The Oral Burn From Capsaicin In A Dose Dependent Manner**

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The hydrophobic chemical capsaicin from chili peppers causes oral burning in the mouth via activation of the TRPV1 receptor. Folk wisdom and psychophysical experiments each suggest fluid milk is the best means to reduce burn after consumption, but specific mechanism(s) remain unknown. It is widely assumed this reduction arises from partitioning of the hydrophobic agonist away from the receptor into the lipid phase, but data from Nolden et al. show full fat milk is no better than skim milk at reducing the burn, leading to speculation this may be due to sequestration by milk protein rather than hydrophobicity. Here, we selected micellar casein (a phosphoprotein that makes up the bulk of protein in milk) as a model protein and tested its ability to (a) bind with capsaicin in vitro, and (b) reduce oral burn in a convenience sample of untrained moderate chili consumers. In vitro, micellar casein is capable of binding capsaicin in a dose dependent manner. In vivo, 92 adults rated the burn of 4 stimuli for 120 seconds each using discrete interval time intensity scaling on a general Labelled Magnitude Scale, with appropriate breaks between stimuli. The four stimuli contained: no protein (0% casein / 5 ppm capsaicin), low protein (2% / 5 ppm), high protein (5% / 5 ppm), and vehicle only (5% / 0 ppm). Burn ratings for all samples with capsaicin peaked at 10 sec before decaying over 2 min; burn of the protein only vehicle was below weak on all trials. As hypothesized, the no protein stimulus had the greatest peak burn (near moderate), while peak burn for the low protein and high protein stimuli were reduced by ~21% and ~42%, respectively. Further studies with whey protein are underway. Collectively, these data support the hypothesis that micellar casein reduces oral burn and this may be due to protein binding.

302 **Optogenetic Activation Of Somatosensory Cgrp-Containing Fibers Modulates Taste Responses**

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In the tongue, the anatomical close proximity of taste buds and somatosensory fibers represents a privileged location for interactions. Somatosensory fibers contain and release neuropeptides such as substance P and

calcitonin gene-related peptide (CGRP) upon stimulation. However, their role in taste bud function and mechanism of action are unclear and have not been studied at a systems level. We used a transgenic mouse model that expresses channel rhodopsin under the CGRP promotor (CGRP-Cre/ChR) to specifically stimulate CGRP-expressing fibers with optogenetics. Immunohistochemistry confirmed that CGRP is exclusively expressed in somatosensory nerve fibers that do not co-express P2X2, a marker of gustatory fibers. Chorda tympani nerve recordings show that when blue light is applied on the tongue of CGRP-Cre/ChR mice, no direct response on the nerve was observed. However, when tastants were simultaneously applied, gustatory responses were significantly decreased. The inhibitory effect of CGRP on taste responses is blocked by the i.p injection of a CGRP antagonist (BIBN4096BS) suggesting that CGRP release modulates taste responses. To clarify the mechanism involved in this modulation, slices of taste tissue and isolated taste cells were stimulated with CGRP. A few Type III taste cells responded to CGRP (150nM) suggesting that Type III cell may express CGRP receptors. Further studies will be required to identify the CGRP receptors and their cellular expression pattern.

303 **Can People Discriminate Between Everyday Odors In The Absence Of Contextual Cues?**

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How accurate are people at discriminating between odors in the absence of contextual cues? Though rarely highlighted, average performance on published 3-alternative forced-choice odor discrimination tests is typically only ~75% correct. In the present study, we measured odor discrimination using a novel presentation technique and compared performance between children and adults. In Exp. 1, odors were presented using Snap & Sniff® wands and discriminations were made between all combinations of pairs of phenyl ethanol, amyl acetate, anethole, and guaiacol on a 22-trial, 3-alternative forced-choice (AFC) odd-ball task. Participants were 41 adults (mean 59.4 yrs; 23 women). Consistent with previous studies, performance only reached 75% correct. In Exp. 2, we compared the odor discrimination performance of 25 children (mean 8.2 years, 17 girls) to that of 22 young adults (mean 19.3 years, 17 women) on a 16-trial, 2-AFC discrimination task using the odor pairs lemon/orange, lilac/lavender, cinnamon/chocolate and two uncommon odorants (diethyl malonate/vigaflor) presented in glass jars. In Exp. 3, 36 college students (19 female) were tested with these and two additional pairs of odors, bubblegum/banana and Brut aftershave/Ivory soap. In Exps. 2 and 3, overall performance was 71% and 78% correct, respectively, with some discriminations being close to chance (e.g., lemon/orange) and others approaching 95% (chocolate/cinnamon). We observed no sex differences. Children as young as 6 years of age performed as well as adults. Although the ability to discriminate between odors appears to be as developed in children as in adults, odor discrimination may not be as good as is commonly thought, at least in the absence of contextual cues. The physiological basis of this curious phenomenon needs to be explored.

304 **Changes In Olfactory Bulb Gaba(B) Receptor Signaling Induced By Fear Learning**

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Olfactory-cued fear conditioning induces plasticity throughout the brain's olfactory system. Learning-induced, stimulus-specific plasticity has been observed as early as the olfactory sensory neurons (OSNs), which exhibit greatly enhanced odor-evoked synaptic output following olfactory fear conditioning. A candidate mechanism for this plasticity is the GABA(B) receptor, which presynaptically modulates glutamate release from the OSNs and whose expression is downregulated in OSNs following conditioning (Bhattarai et al. 2020). To evaluate the role of GABA(B) receptors in olfactory fear learning on olfactory bulb circuits, we used *in vivo* calcium imaging to observe the effect of local GABA(B) receptor blockade on population-level calcium dynamic in OSNs, periglomerular cells, and mitral cells from anesthetized mice that had undergone odor-cued conditioning or control exposures. In control mice, blockade of GABA(B) receptors increased the odor-evoked response amplitudes in all three cell types and for all odors tested, reflecting a global increase in odor-evoked activity in the OSNs and downstream neurons. However, in the animals that received odor-shock fear training, GABA(B) receptor blockade caused a significantly smaller increase in the neural responses evoked by the shock-predictive odor than for control odors in all three populations of neurons. GABA(B) receptor blockade also induced changes in the kinetics of the bulbar response over the course of an odor presentation, and these kinetics were also altered in fear conditioned mice. These data support the idea that changes in GABA(B) receptor-mediated inhibitory signaling underpin associative learning throughout the olfactory bulb circuit.

305 **Odor Modulation Of Drug Cue-Reactivity In Alcohol-Dependent Adults.**

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Drug cue-reactivity, the array of physiological, cognitive, and behavioral effects elicited by drug-related stimuli, is an established methodology to investigate drug craving and dependence. Previous functional neuroimaging (fMRI) studies of drug cue-reactivity (i.e. brain activation in response to drug-related versus neutral visual cues) indicate disrupted limbic-prefrontal circuits in the pathophysiology of drug dependence. Given the ability of odors to engage limbic-prefrontal circuits, we sought to explore how a neutral odor influenced drug cue-elicited activation and striatal-prefrontal connectivity. 27 normosmic, non-treatment seeking, alcohol-dependent, adults (23 M, 4 F; mean age 27.1, range 21-39) underwent a validated fMRI task during which either lavender (LAV) or odorless propylene glycol (PG) was delivered within blocks of alcohol (ALC) or neutral (NEU) visual cues. While ALC>NEU+PG demonstrated a pattern consistent with previous studies (i.e. increased activation in bilateral striatum (bS) and medial prefrontal cortex (mPFC); clusters determined by $Z>2.3$, corrected cluster threshold $p<.05$), region of interest analyses indicated a significant picture x odor interaction in mPFC ($p<.05$). LAV normalized drug cue-reactivity in mPFC, reducing activation during ALC to levels measured during NEU. Moreover, mPFC was functionally connected to bS during ALC+LAV, but not ALC+PG. A trend toward a

reduction in subjective craving was also noted during LAV compared to PG (p=.083). These findings suggest that engagement of limbic-prefrontal brain circuits via odors may have a role in restoring dysregulated prefrontal circuits and processing of drug cues. Other odors and the relationship between them and brain activation in response to drug cues and craving requires more exploration.

306 **Sequential Odor Learning In The Newborn Rabbit**

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Newborn rabbits are blind and deaf but extremely efficient to respond to odor cues. In particular, they can learn new odorants or mixtures of odorants very rapidly, by single and brief pairing with a natural reinforcer, the mammary pheromone (MP). The MP, emitted by rabbit mothers in their milk and strongly active as a releaser of sucking-related behaviors, functions indeed as an unconditioned stimulus (US) able to promote the response to a new odorant (representing a conditioned stimulus CS1) through first-order pavlovian conditioning. Moreover, newborn rabbits can learn additional novel odors (CS2) through higher-order conditioning, e.g. second-order conditioning, based on CS1-US pairing followed by CS2-CS1 association, or sensory preconditioning, when CS2-CS1 pairing takes place before CS1-US. In these different procedures, we have previously shown that simultaneous presentation of stimuli (either CS1 and US or CS2 and CS1) allows efficient conditioning, while a short - 1 min - delay between stimuli presentation abolishes CS responses. Here, we will present new results regarding the boundary conditions of serial stimuli presentations during higher-order conditioning, a topic that remains weakly investigated early in life. We will present original data indicating that 1) a 10-sec delay between CS1 and US impedes CS1 responding, whereas CS2-CS1 association supports 2) a 10-sec, but not a 30-sec delay during preconditioning and 3) a 10- and 30-sec, but not a 60-sec delay during second-order conditioning. This demonstrates that rabbit pups can perform sequential association of odor stimuli during higher-order, but not first-order conditioning. These results challenge the idea that, contrary to adults, non-human and human newborns are not able to perform trace learning.

308 **Salient Feature Selection In An Odor-Guided Discrimination Task**

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In a complex odor environment, how do animals identify salient stimuli? In this study, we investigate which stimulus features are relevant for solving an odor discrimination task. Briefly, the mice were trained to discriminate between overlapping multi-component odor mixtures in a digging task. Once the mice reached criterion performance, they were challenged to perform the task with single components of the mixtures on 10% of trials, hereafter referred to as “pop-outs.” We find that mice are able to use a single salient feature of the rewarded mixture to perform the task. However, only features that differed in identity between two mixtures were salient. When presented with the sole component that differed between the mixtures (acetophenone) mice successfully dug in the correct pot. However, when mice were presented with the structurally similar and vapor pressure matched component (methylbenzoate) that differed only in concentration between two mixtures, the mice either correctly aborted the trials or continued searching for the rewarded odors. Our findings suggests that when mixtures that differ by two features are presented, identity or concentration, mice prioritize using the identity of a single component over concentration differences, or the mixture as a whole. This finding begs the questions of how or even if mixtures are encoded in discrimination tasks and how does the olfactory system filter salient versus irrelevant features of an odor mix?

309 **Interaction Of Trigeminal, Somatosensory, Olfactory, And Respiratory Signaling In The Mouse Olfactory Bulb**

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Odor-cued aversive conditioning induces odor-specific plasticity throughout the olfactory system. However, it remains unclear whether information about an aversive, non-olfactory stimulus converges with olfactory signals in the olfactory bulb. We used *in vivo* calcium imaging of population-level neural activity in mitral cells and olfactory sensory neuron (OSN) terminals in the olfactory bulb to test whether aversive trigeminal or somatosensory stimulation could impact olfactory bulb signaling and whether the evoked activity interacted with ongoing respiration- and odor-evoked activity. Stimulation of the trigeminal nerve via CO_2 presentation or direct electrical stimulation evoked robust bilateral neural activity in the olfactory bulb’s mitral cells in anesthetized mice. No such response was observed in the OSN terminals. Naris occlusion eliminated both spontaneous respiration-locked and trigeminal nerve shock-evoked mitral cell activity ipsilateral to the occlusion, but contralateral responses persisted. Similar results were observed using electrical stimulation of the tail, which evoked bilateral, respiration-coupled bursts of population activity in olfactory bulb mitral cells that was eliminated when intranasal airflow was prevented. These findings illustrate that aversive non-olfactory stimuli, including those used in olfactory fear conditioning paradigms, can evoke strong, presumably centrifugal modulation of early olfactory signaling. This convergence of odor- and shock-related activity suggests that the early olfactory circuit could be a locus for associative learning. The apparent respiratory gating of bulbar signals driven by aversive sensory stimulation suggests that the olfactory bulb may be an unexpected intermediary between respiration and pain perception (Iwabe et al. 2014). Keywords: trigeminal, plasticity, coding

310 **Hippocampal-Prefrontal Theta Coupling Develops As Mice Become Proficient In Associative Odorant Discrimination Learning**

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Learning and memory requires coordinated activity between different regions of the brain. Here we studied the interaction between medial prefrontal cortex (mPFC) and hippocampal dorsal CA1 during associative odorant discrimination learning in the mouse. We found that as the animal learns to discriminate odorants in a go-no go task the coupling of high frequency neural oscillations to the phase of theta oscillations (phase-amplitude coupling or PAC) changes in a manner that results in divergence between rewarded and unrewarded odorant-elicited changes in the theta-phase referenced power (tPRP) for beta and gamma oscillations. In addition, in the proficient animal there was a decrease in the coordinated oscillatory activity between CA1 and mPFC in the presence of the unrewarded odorant. Furthermore, the changes in PAC resulted in a marked increase in the accuracy for decoding odorant identity from tPRP when the animal became proficient. Finally, we studied the role of Ca^{2+}/calmodulin-dependent protein kinase II α (CaMKIIα), a protein involved in learning and memory, in oscillatory neural processing in this task. We find that the accuracy for decoding the odorant identity from tPRP decreases in CaMKIIα knockout mice and that this accuracy correlates with behavioral performance. These results implicate a role for PAC and CaMKIIα in olfactory go-no go associative learning in the hippocampal-prefrontal circuit.

311 **Changes In Patterns Of Gustatory Cortex Responses After Conditioned Taste Aversion**

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The role of gustatory cortex in the processing sensory input and generation of behavior associated with taste is still not fully understood. Existing findings in the field suggest that the gustatory insula contributes to taste-based learning, and specifically the learning of conditioned taste aversions. There is some additional suggestion that after aversion learning, the neural representation of previously palatable tastes is altered, potentially becoming more similar to innately aversive tastes. We attempted to investigate the representation of conditioned taste aversion in the gustatory cortex of mice. Using calcium imaging via head-mounted “miniscopes”, we recorded the activity of a consistent population of 871 cells from 7 awake, behaving mice through a conditioned taste aversion paradigm, permitting an unprecedented investigation of neural correlates of behavior and learning in real time. Over the course of several days, animals were first presented with a panel of several tastes, and after conditioning an aversion to one previously accepted taste solution, the panel was presented again. We found that responses to different tastes were initially uncorrelated, but responses to accepted tastes began to intercorrelate over successive exposures. After conditioning an aversion to sodium chloride, however, responses to that taste reverted to their previously uncorrelated state. This pattern of change appears related to, but not fully explained by, quantity of ingestion. Minimally, the conditioned aversive taste seems to become less similar to accepted tastes, though it may not become more similar to taste solutions that are innately rejected. Current work focuses on identifying changes in single cells and clusters of cells to more fully characterize the broad changes following aversion conditioning.

312 **Fear Generalization And Extinction Learning Alter Primary Sensory Input To The Brain**

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Generalization of learned fear is typically thought to occur in brain networks far removed from the sensory periphery, such as the amygdala and prefrontal cortex. However, work from our lab and others has demonstrated that odor-cued conditioning paradigms inducing generalizing fear, where mice become afraid of odors beyond those experienced during training, boost neural responding these odors in the periglomerular and mitral cells of the olfactory bulb. Do these generalization-related changes demonstrate learned changes in those neuronal populations, or do they reflect upstream plasticity in the input from the olfactory periphery? If so, how does that plasticity relate to the mouse’s beliefs about the odors? To answer these questions, we used single-odor fear conditioning in mice that generalized to be afraid of multiple odors, as demonstrated on an olfactory avoidance task. *In vivo o*ptical imaging of exocytosis from populations of olfactory sensory neuron (OSN) terminals in the olfactory bulb revealed a large increase in OSN output evoked by not only the shock-predictive odor (the CS) but also by other odorants to which the mouse had generalized its behavioral fear response. Extinction training, in which the fear conditioned mouse was subsequently taught that the original CS no longer predicted a shock, reversed the learning-induced changes in odor-evoked OSN output for all odors. “Novelty-based refinement training”, in which fear conditioned mice were exposed to the alternate odors to which they had generalize their fear but not exposed to the CS, narrowed the range of odors that evoked fear and exhibited facilitated OSN output. The ability to narrow fear, at the sensory and behavioral levels, to just the stimuli that are appropriately supposed to cause fear, has clinical implications.

313 **Evidence For A Glucose-Specific Taste Pathway That Enhances Insulin Secretion In Mice**

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In humans and rats, orosensory input from food activates the dorsal motor vagal nucleus in the brainstem, which

causes parasympathetic stimulation of pancreatic beta cells. This parasympathetic stimulation enhances glucose-stimulated insulin secretion from beta cells and lowers postprandial hyperglycemia. In humans, orosensory input can also trigger secretion of glucagon (a counterregulatory hormone) from pancreatic alpha cells. Here, we examined these cephalic-phase responses in C57BL/6 mice. In Experiment 1, mice took 200 licks for solutions containing saccharin (S), glucose (G) or glucose + saccharin (G+S). We measured plasma levels of insulin, C-peptide (co-secreted with insulin) and glucagon, both before and 5 min after the mice initiated licking. G and G+S enhanced plasma insulin and C-peptide levels but not glucagon levels; while S had no effect on plasma insulin, C-peptide or glucagon levels. These results show that active licking for glucose enhances insulin, but not glucagon, secretion in mice. In Experiment 2, we asked whether taste alone is sufficient to enhance insulin secretion. After performing a tracheotomy and esophageal ligation, we infused the oral cavity of anesthetized mice for 3-min with a glucose or fructose solution. Glucose alone enhanced insulin secretion within 5 min. Because mouthfeel and olfaction were minimized, the predominant sensory input was taste. In Experiment 3, we examined the necessity of parasympathetic stimulation to cephalic-phase insulin release (CPIR) by treating mice with atropine, an antagonist of the acetylcholine receptor. Atropine treatment eliminated CPIR, establishing the necessity of parasympathetic stimulation. We propose that mice possess a glucose-specific taste pathway, which, when activated, enhances insulin secretion and glucose homeostasis.

314 **Aav-Php.S-Mediated Delivery Of Reporters To Chemosensory Ganglia**

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Adeno-associated viruses (AAVs) are essential tools for defining central circuits and functional neural interactions. A synthetic serotype, AAV-PHP.S, was reported to target peripheral nervous system neurons in dorsal root ganglia, and enteric neurons. We sought to expand the use of this virus to cranial ganglia, particularly for chemosensation. Fluorescent neurons were seen in the dorsal root, nodose, petrosal, trigeminal, and geniculate ganglia. A time course of expression in the geniculate ganglion showed that GFP was detected by 2 days post-injection, and reaching the maximum of labeled neurons (≈70%) by 7 days. By 7 days post-injection, GFP-labeled nerve fibers could be detected in peripheral projections such as within the circumvallate taste buds, while GFP fibers were evident in fungiform taste buds only by 14 days. Similarly, in the medulla and spinal cord, GFP-labeled sensory afferent fibers (gustatory, trigeminal) were present, allowing us to image in detail the projections to resident CNS neurons. Further, we did not detect any GFP positive neuronal somata in the CNS. To label cell-type selective neurons, we injected a Cre-dependent GCaMP6s in AAV.PHP.S into Mafb-mCherry-Cre mice. In the geniculate ganglion, ~90% of Mafb-expressing neurons (auricular and T2 types) were fluorescent while other neuron types remained unlabeled. We also tested neuronal responses by imaging GCaMP6s-expressing neurons in anesthetized mice while stimulating the pinna. This constitutes the first demonstration of mechanosensitivity in these auricular neurons (which were so named based only on neuroanatomy). Our study expands the use of AAV to many peripheral somatic and visceral sensory neurons, and studies of their sensitivities, receptive fields and circuits, that until now have been difficult to assess.

315 **Chemogenetic Inhibition Of Somatostatin Neurons In The Nucleus Of The Solitary Tract Differentially Modulates Bitter And Sweet Taste Signals**

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The rostral nucleus of the solitary tract (rNST) is the first central taste circuit and houses a network of heterogeneous neurons. A recent study (Jin et al., 2021) proposed that one genetically distinct neuron type, comprised of rNST neurons expressing somatostatin (Sst), exclusively processes bitter taste. However, in the caudal NST, Sst neurons are a mixed population of excitatory glutamatergic and inhibitory GABAergic cells, suggesting that rNST Sst neurons might be multifunctional. To explore this, we made injections of a cre-dependent AAV virus expressing an inhibitory DREADD (hM4Di) into the rNST of Sst-cre mice and then tested licking in response to concentration series of bitter (quinine) and sweet (sucrose) stimuli after I.P. injections of CNO or saline. Consistent with the previous report, inhibition of Sst neurons increased quinine licking (taste: water ratio, P=0.0004, N=6), suggesting that bitter signaling was suppressed. More surprisingly, sucrose licking also increased (taste-water licks, P=0.04, N=5), similar to our previous observations using DREADDs to suppress rNST GABA signaling. To explore the possibility that effects on bitter and sweet-driven behaviors might arise from glutamatergic Sst versus GABAergic Sst neurons, we performed fluorescent in situ hybridization for Sst, VGLUT2, and VGAT in the rNST. Preliminary data based on counting 249 Sst-positive cells (2 mice, 4 rNST sections) show that ~2X as many Sst neurons are GABAergic (N=162) than glutamatergic (N=81). Thus, rNST Sst neurons are heterogeneous and have the potential for multiple functions. Ongoing optogenetic neurophysiological studies in mice expressing ChR2 in Sst neurons are directly assessing the gustatory response profiles of these cells and their ability to modulate taste activity in other neurons.

316 **Analysis Of *Lhx5*-, Lhx6, And *Lhx2A*-Driven Gfp In Zebrafish Reveals Extended (Olfactory) Amygdala**

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Zebrafish is an important genetic model system for neural mechanisms of olfaction; however, its extended (olfactory) amygdala (EA) has remained anatomically ill-understood. To gain insights into the evolution and development of the EA, we analyzed the distribution of calretinin, lhx6, otpa-protein, and GABA in both

developing and adult zebrafish of the transgenic lines *Tg(lhx5:GFP), Tg(lhx6QF;QUAS:GFP), Tg(vGlut2a:GFP),* and *Tg(lhx2a:GAP-YFP)*. In the latter GFP olfactory bulb neurons axons form the lateral olfactory tract (lot) that projects into olfactory pallial territories. In zebrafish, the lot specifically innervates the posterior ("Dp") and ventral most lateral aspects of the dorsolateral zone ("Dlv"). Comparing the GFP distribution in *Tg(lhx5:GFP)* with Tg(lhx6QF;QUAS:GFP) and *Tg(lhx2a:GFP),* our analyses reveal that the projection sites are more complexly organized than previously thought. In line with our previously published framework of the zebrafish amygdala, we redefine both territories often labeled as "Dp" and "Dlv." The differential expression of *lhx5*-driven GFP confirms that these territories most likely correspond to the nucleus of the lateral olfactory tract (nLOT), whereas one previously overlooked territory should be considered homologous to the lateral pallium (mammalian entorhinal cortex). Altogether, our results explain the extended amygdala in zebrafish and thereby drastically facilitating its usability for spectrum autism disorders know to affect the sense of smell and social behavior.

317 **Trmp5 Knockout Mice Express Fewer Taste-Elicited Fos-Immunoreactive Neurons In The Waist Area Of The Parabrachial Nucleus Than B6 Mice**

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The parabrachial nucleus (PBN) is a viscerosensory nucleus in rodents that includes taste-responsive neurons primarily within the waist area. Physiological studies show many of these neurons receive convergent visceral or somatosensory input, and the extent to which their orosensory activation is dependent on intact peripheral taste processing is unclear. The transient receptor potential cation channel Trmp5 is present in taste receptor cells and necessary for normal behavioral and physiological responses to tastants (Damak et al., *Chem. Senses 31:* 253-264, 2006). In the current study, Trmp5 knockout (KO) mice were used to assess the role of this channel in neural activity in the PBN elicited by intra-oral infusion (0.1 ml/min, 15 min) of 1.0 M sucrose (S), 3 mM quinine hydrochloride (Q), 0.1 M monopotassium glutamate + 0.01 M inositol monophosphate (U) as well as distilled water (W). Immunohistochemistry for the Fos protein was used to identify active neurons in the PBN of KO and wild type (B6) mice. In the PBN overall, as well as in the waist area and dorsomedial (DM) subnucleus, fewer Fos-immunoreactive (Fos-IR) neurons were present in KO mice than in B6 mice that received U (N's=5, P's<0.0125). When the number of Fos-IR neurons in each PBN subdivision was expressed as a percent of the total counted in each mouse, there was a smaller percentage of Fos-IR neurons in the waist areas of KO mice compared to B6 mice following infusion of S, Q, and U but not W (N's=9 or 10 for S, Q, and W). Consistent with previous studies and indicating the importance of taste input for neural activation in the PBN, the current results show that the Trmp5 cation channel is necessary for normal activation of neurons in the waist area following intra-oral infusion of sweet, bitter and umami tastants.

318 **Comparative Anatomical Analysis Of The Main And Accessory Olfactory System**

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The rodent olfactory system comprises at least two complementary central pathways: the main and the accessory olfactory pathway. Chemosensory information detected by olfactory sensory neurons is relayed to the main olfactory bulb. Many semiochemicals and other social chemosignals, however, are detected by vomeronasal sensory neurons that project to the accessory olfactory bulb. As principal projection neurons, both main and accessory olfactory bulb mitral cells integrate chemosensory input and route this information to distinct downstream target regions. Despite separate anatomical investigations of either the main or accessory olfactory circuitry, parallel comparative analysis of individual projection paths using modern tracing techniques and largely intact brain samples is lacking. Here, we provide a detailed anatomical description of both main and accessory olfactory bulb mitral cell projections, implementing state-of-the-art viral tracing and imaging methods. Using transgenic mice selectively expressing Cre recombinase in olfactory bulb mitral/tufted cells under the t-box protein 21 (tbx21) promoter, Cre-dependent expression of adeno-associated viral genomes enabled mitral cell-specific optical tracing. Whole-brain slice preparations allow for parallel three-dimensional reconstruction of olfactory bulb principal neuron projections. Together, this study provides detailed anatomical insight into both unique and common target areas along each olfactory pathway, thus, laying a solid foundation for future investigations into parallel olfactory information processing by the main and accessory olfactory system.

320 **Optogenetic Stimulation Gad65$^+$ And Pkd2L1$^+$ Taste Bud Cells Are Sufficient For Driving Neural And Behavioral Responses Through Benzamil-Sensitive And Benzamil-Insensitive Salt Taste Pathways**

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Taste bud cells (TBCs) transduce salt through at least two mechanisms, and communicate gustatory sensations to the Nucleus Tractus Solitarius (NTS) through peripheral afferents. Gustatory neurons within the NTS respond to salts through an amiloride/benzamil-sensitive mechanism or an amiloride/benzamil-insensitive mechanism. We used an optogenetic approach to selectively stimulate Type I GAD65$^+$ or Type III PKD2L1$^+$ TBCs in fungiform papillae and determine their respective contributions to salt taste physiology and behavior. Stimulation of GAD65$^+$ TBCs evoked robust neural activity in "N neurons," which responded best to NaCl through a benzamil-sensitive mechanism. In contrast, stimulation of PKD2L1$^+$ TBCs evoked robust neural activity in benzamil-insensitive "A neurons," which responded best to NH_4Cl, but also responded well to NaCl and citric acid.

Stimulation of GAD65$^+$ TBCs was significantly correlated with NaCl responses, whereas stimulation of PKD2L1$^+$ TBCs was significantly correlated with NH_4Cl and citric acid responses. Stimulation of fungiform GAD65$^+$ or PKD2L1$^+$ TBCs recapitulated stereotypical amiloride-sensitive and amiloride-insensitive salt taste behaviors, respectively. Indeed, stimulation of GAD65$^+$ TBCs was sufficient for driving appetitive licking behaviors in Na^+ deprived mice and drove a conditioned taste aversion (CTA) that generalized specifically to NaCl. Stimulation of PKD2L1$^+$ TBCs, however, was insufficient for driving appetitive licking behaviors in Na^+ deprived mice but was marginally sufficient for driving a CTA that generalized to NaCl and NH_4Cl. These data demonstrate that GAD65$^+$ TBCs are sufficient for driving NaCl taste through the benzamil-sensitive pathway, whereas PKD2L1$^+$ TBCs may be sufficient for driving the cation generalist benzamil-insensitive pathway.

321 **Meta-Analysis Identifies Decreased Odor Identification In Pregnancy**

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The sense of smell is vital for environmental hazard detection and may influence eating behavior and nutrition. Therefore, changes in olfactory perception during pregnancy can affect the health of the mother and fetus. However, data on objective olfactory function are limited in number and sample size, with a resultant lack of consensus on pregnancy-related chemosensory changes. Therefore, we aimed to verify whether olfaction changes during pregnancy. We performed a comprehensive literature search (PubMed, MEDLINE, Scopus, Web of Science, and Google Scholar databases) that included studies of healthy, pregnant subjects ≥ 18 years of age with at least one smell outcome of interest (odor identification, threshold, or discrimination) measured with an objective olfaction instrument. A total of 9 studies met inclusion criteria for meta-analysis: 8 measured odor identification (ID), 5 measured odor threshold (OT) and 4 measured odor discrimination (OD). Meta-analysis of the subset of odor ID studies that were peer reviewed (n = 6) revealed worse odor ID in pregnant women compared to healthy controls with a moderate effect size and low heterogeneity (k = 6, SMD, -0.42 [95% CI, -0.62 to -0.22]; p <0.001; I^2 = 28.42%). Subgroup analysis revealed that this decreased performance was observed during all trimesters of pregnancy but not post-partum. No significant differences were detected for OD (k = 5, SMD, -0.09 [95% CI, -0.26 to 0.09]; p = 0.32; I^2 = 0.00%) nor OT (k = 4, SMD, -0.11 [95% CI, -0.30 to 0.08; p = 0.26; I^2 = 0.00%) between pregnant women and non-pregnant controls. These findings upend conventional wisdom and suggest that some measures of olfaction (i.e., odor identification) may be impaired during pregnancy.

322 **Does Olfactory Viral Infection Accelerate Alzheimer&Rsquo;S Disease?**

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A critical barrier in treating Alzheimer’s disease (AD) is the years- to decades-long lag from disease onset to the clinical diagnosis of dementia, when reversal of brain pathology may, at best, slow cognitive decline. Thus, it is essential to identify contributory pathological processes in early disease to prevent progression to clinical dementia, disability and death. An early process in AD prior to clinical dementia is a deficit in the sense of smell accompanied by olfactory sensory neuron (OSN) dysfunction. However, little is known about changes in the cellular processes of the proximal brain areas of the olfactory system, the olfactory bulb (OB) that receives olfactory input from the OSNs, and the olfactory tract (OT) that carries information to downstream brain processing areas including the hippocampus. Here we use Temp-O-seq, a novel technique optimized to perform reliable transcriptomic analysis in post-mortem human brain, to characterize the transcriptome of the OB and OT in familial AD subjects vs. controls. We study the transcriptome in presenilin 1 (PSEN1) E280A mutation carriers from the world’s largest autosomal dominant familial AD kindred. E280A carriers develop mild cognitive impairment and dementia at the median ages of 44 and 49 years. Interestingly, we find a marked increase in viral responsive gene expression in the OB and in the transcriptome involved in inflammatory processes in the OT. Infection of undifferentiated human OE cultures by herpes simplex virus 1 (HSV1) and varicella zoster virus (VZV) leads to a sharp increase in β amyloid and CNGA2, a marker of mature OSNs. Our study raises the question whether viral infection of the proximal olfactory system accelerates Alzheimer’s disease.

323 **Decreased Chemosensory Sensitivity In 366 Healthcare Workers 11 Months After Covid-19**

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Presentation of COVID-19-induced acute olfactory and gustatory dysfunction are now widely reported. In many individuals, these chemosensory dysfunctions persist and only few studies have described the effects of COVID-19 on chemosensory function past 6 months. The main objective of this study was to understand the prevalence of subjective olfactory, gustatory, and trigeminal dysfunction in patients with COVID-19 11 months after infection. We designed a longitudinal study with an online questionnaire sent to healthcare workers 5 and 11 months after PCR-confirmed COVID-19. To assess whether each sensory modality had worsened or improved compared to baseline levels, participants were asked to separately rate their chemosensory functions on a 10-point visual analogue scale (VAS). Participants also completed the Chemosensory Perception Test (CPT), an at home olfactory test. At 10.6 (SD:0.7) months after infection, 366 participants (mean age: 44.8 (11.7)) completed the follow-up questionnaire. During acute COVID-19, at 5 and 11 months, 84%, 56% and 50% of participants reported decreased olfactory sensitivity (gustatory: 82%, 45% and 45%; trigeminal: 53%, 36% and 24%). Participants' mean CPT scores significantly improved from 5 to 11 months, however, prevalence of parosmia increased from 11% to 57 % among those with decreased olfactory sensitivity. Our findings reveal persistent subjective chemosensory dysfunctions at 11 months after COVID-19 in half of participants. Increased prevalence of parosmia could indicate ongoing changes in the olfactory system. Possible overrepresentation of individuals with more severe impairments at follow-up is a limitation. More studies on the physiopathology of post-COVID-19 are necessary to develop efficient treatments for patients with long-term impairments.

324 **Identifying Candidate Genes Underlying Isolated Congenital Anosmia**

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An estimated 1 in 10,000 people are born without the ability to smell. Despite the importance of olfaction for our quality of life, the genetic basis for these cases of congenital anosmia (CA) remains largely unknown: only two genes to date have been identified for isolated, or non-syndromic, congenital anosmia (ICA). In contrast, the genetic basis of other inherited sensory deficits is well investigated, with almost 100 genes implicated in congenital deafness and over 200 genes implicated in congenital blindness. We conducted whole exome sequencing (WES) in ten families to find variants that associated with inherited patterns of ICA. 368 genes had variants that were rare (dominant inheritance MAF<0.005; recessive inheritance MAF<0.07) or novel, were predicted to disrupt the protein, and had the correct inheritance pattern. 11 of these genes occurred in at least two families, and 23 of these genes had protein-truncating mutations in at least one family. FLG stood out as having 3 variants in two families, one of which is a protein-truncating mutation. We conducted additional WES in 141 individuals with ICA to search for other variants that are rare in the general population, but occur frequently in our cohort. Seven genes were either significantly enriched in our population compared to the general population (ZNF574 50-fold; CNOT6L 11-fold; WHSC1 8-fold), or were never seen in the 1000 Genomes Project or ALFA frequency databases (ITGB1; GRM7, EVI5). Historically, identification of genes related to other sensory disorders has provided a gateway to better understanding of those senses. Given how few genes have been implicated in olfactory disorders, we have the opportunity to uncover a plethora of new avenues for which to better understand basic olfactory function.

325 **Human *Apoe-E3* And *Apoe-E4* Alleles Have Differential Effects On Mouse Olfactory Epithelium**

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Alzheimer's disease (AD) is a progressive age-dependent disorder whose risk is determined by genetic factors. Better models for investigating early effects of highly penetrant risk factors such as apolipoprotein E (*APOE)* genotype are needed. To determine whether *APOE* genotype produces neuropathologies in an AD-susceptible neural system we compared effects of human *APOE-ε3* (E3) and *APOE-ε4* (E4) alleles on the mouse olfactory epithelium. E3 and E4 olfactory mucosae show 121 differentially abundant mRNAs at age 6 months by RNA-seq. These mRNAs do not indicate differences in cell type proportions, but effects on 17 odorant receptor mRNAs suggest small differences in tissue development. Ten mRNAs encoding oxidoreductases or related proteins important for cellular metabolism and mitochondria are less abundant in E4 olfactory mucosae. This does not translate into differences in cellular respiration as measured by Seahorse Mito Stress Test but E4 olfactory mucosae show lower glucose uptake, characteristic of susceptibility to AD. This is consistent with greater amounts of mRNA from the glucose-sensitive gene, *Asns*, in E4 olfactory mucosae. Olfactory sensory neuron apoptosis, detected by immunohistochemistry for the active fragment of Casp3, does not differ at age 6 months but is greater in E4 mice at 10 months. Effects of human *APOE* alleles on mouse olfactory epithelium phenotype are apparent in early adulthood, and neuronal loss begins to increase by middle age (10 months). The olfactory epithelium is an appropriate model for the ability of human *APOE* alleles to modulate age-dependent effects associated with the progression of AD.

326 **The Effects Of Vaping On Olfactory Function**

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Vaping using electronic nicotine delivery systems (ENDS) exposes the nasal epithelium and olfactory system to a complex array of potential toxicants while simultaneously stimulating the olfactory sensory neurons with high concentrations of odorants. The surge in vaping popularity motivates the assessment of potential olfactory dysfunction induced by vape exposure to assess potential public health consequences and inform government regulation. Olfactory function in over 100 participants was tested via Snap & Sniff ® kits to assess detection, discrimination, and identification performance. Subjects also underwent rhinomanometry testing to measure the relationship between pressure and airflow in the nose and answered a questionnaire about their vaping habits. Preliminary analysis of the data indicates that subjects who reported regular vaping of nicotine-containing products exhibited significantly worse olfactory discrimination performance than non-vaping control subjects. This impairment was no worse in subjects who had vaped shortly before assessment then those who had not, suggesting that it was not caused by short-term adaptation. It also did not obviously correlate with the nasal resistance to airflow, suggesting that it is not caused by conduction block. Approximately half of vapers indicated that they exhale vape aerosol through the nose when vaping between “sometimes” and “all the time,” demonstrating a previously underappreciated route of intranasal vape exposure. Additional analyses explore the role of nicotine concentration, effects of vaping device type, and a hypothesized interaction of vaping-associated olfactory impairment with COVID-19 history. Further research is needed to understand the mechanism of olfactory toxicity in vaping. Keywords: olfactory, vaping, nicotine, COVID-19

327 **Field Assessment Of Olfactory Function In An Age Diverse Cohort Of Adults Using The Adaptive Olfactory Measure Of Threshold (Aroma-T)**

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Quantitative olfactory disorders such as anosmia or hyposmia are typically assessed via objective psychophysical tests. However, commonly used tests have limitations that create barriers to broad adoption outside lab settings – e.g., odor identification tests may confound sensory performance with memory recall or verbal ability. Tests based on odor detection may have an advantage as they avoid such issues. However, the time, equipment, and/or staff needed to conduct many smell tests has made them impractical for use as part of a short wellness visit or in field studies. Here we tested the performance of the Adaptive Olfactory Measure of Threshold (ArOMa-T)– a novel odor detection threshold test that employs an adaptive Bayesian algorithm implemented via web app along with a card-based odor delivery device–in 534 adults (mean age: 39 ± 16 yrs) at the 2021 Twins Day Festival in Twinsburg, OH. Participants successfully completed the test in under 3 min with a false alarm rate of 9.6%. A broad range of odor detection thresholds were observed, suggestive of olfactory function ranging from normosmic to functionally anosmic. There were significant differences in odor detection thresholds between sexes and between age groups, consistent with published population-level findings. In a small exploratory analysis in this convenience sample, we failed to find evidence of differing detection thresholds between participants with a positive COVID-19 history and matched controls without. We did find evidence of heritability of odor detection thresholds via analysis of twin concordance. These results indicate the ArOMa-T can determine odor detection thresholds in the field and may be valuable in settings where rapid and portable assessment of olfactory function is needed.

328 **Cgrp Neurons In The Parabrachial Nucleus Mediate Avoidance Of Bitter Taste Stimuli In Mice.**

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Our prior neural data show that the bitter taste stimulus quinine excites taste-trigeminal integrative neurons in the lateral parabrachial (PB) area. Lateral PB neurons can express the Calca gene encoding calcitonin gene-related peptide (CGRP). PB-CGRP neurons are implicated in protective behaviors, although their role in taste behavior is unclear. Here, we used Cre-directed chemogenetic silencing to inhibit PB-CGRP neurons in mice during brief-access fluid licking tests with quinine. Under anesthesia, heterozygote Calca-Cre mice received bilateral PB injections of a Cre-dependent AAV virus encoding either an inhibitory designer receptor exclusively activated by designer drugs (hM4Di) or a control element (mCherry). Following recovery, water-restricted hM4Di and mCherry mice entered brief-access tests where, prior to daily testing, they all received the hM4Di ligand clozapine-N-oxide (5 mg/kg, i.p.), providing between-subjects drug side effect control. Mice were proffered multiple 10 sec licking access trials with quinine solutions (0 [water], 0.1, 0.3, and 1 mM) during test sessions over 4 days. Data were collected blind to mouse conditions. Licking responses were computed as the ratio of quinine to water licks. Preliminary results show that over test days, hM4Di mice with silenced PB-CGRP

neurons show a loss of orosensory avoidance of normally avoided concentrations of quinine. On test days 3 and 4, hM4Di mice lick 0.1 mM quinine nearly like water (median lick ratio [mlr] = 0.87) and do not decrease (Wilcoxon test, p = 0.3) their licks to 0.3 mM quinine (mlr = 0.83). In contrast, control mCherry mice decrease (p = 0.01) licking to quinine when concentration is increased from 0.1 (mlr = 0.82) to 0.3 (mlr = 0.49) mM. These data uncover a critical role for PB-CGRP neurons in bitter taste avoidance.

329 **Cross-Cultural Comparison Of Waterless Empirical Taste Test (Wett&Reg;) Scores: Chinese Vs. American Subjects**

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Objectives: To determine whether scores on a novel taste test that requires no water differ between American and Chinese adults, and whether the test scores are influenced by gender and age. Methods: The 53-trial Waterless Empirical Taste Test (WETT®) was administered to 113 Chinese and 214 Americans. The subjects orally sampled monomer cellulose pads containing one of four concentrations of sucrose, citric acid, NaCl, caffeine, and monosodium glutamate and indicated whether a sweet, sour, bitter, salty, brothy, or no taste sensation was perceived. Separate gender by culture analyses of covariance with age as the covariate were performed on the total score and the scores for each taste stimulus. Results: No difference between American and Chinese subjects was found for the total WETT® score (p=0.129) or for sucrose (p=0.129) or NaCl (p=0.368). However, for monosodium glutamate, the scores were 28.40% higher for the Chinese than for the American subjects (p=0.024), and for citric acid and caffeine the scores were 24.12% and 21.79% higher for the American subjects (p=0.001 and 0.029). For all test qualities, women outperformed men and test scores declined with age. Conclusions: Although total scores on the WETT did not differ significantly between healthy adult Chinese and American subjects, individual taste qualities did differ, with better Chinese performance for monosodium glutamate and better American performance for citric acid and caffeine. Both age- and gender-related differences were noted. Future work is needed to determine the cause of these differences and whether the findings generalize to other Chinese and American samples.

330 **Biological And Environmental Influences On Individual Differences In Flavor Perception**

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Flavor perception is a multisensory phenomenon that mainly uses gustation and olfaction. Previous work from our lab and others has shown that inputs from these two systems interact to determine flavor preferences. However, there is considerable individual variation in flavor preference. Here, we used a series of consumption tasks in rats to measure the influence of environmental and biological variables on learning and decision-making in the context of multisensory flavor consumption. Environmental variables were experimentally manipulated by feeding one group of rats a wide variety of real foods. Real foods included fruits, vegetables, fish, meat, seeds, and nuts and were added to the cage daily starting at birth. A second group was only fed standard chow. Sex served as a biological variable. First, the magnitude of taste-odor association learning was measured using sucrose and quinine as unconditioned stimuli. Second, instantaneous preferences for taste-smell mixtures were measured to determine the weight each sensory component contributed. Concerning the learning task, the results yielded a significant Experience x Taste interaction, suggesting that food-raised animals formed stronger appetitive associations, whereas chow-raised animals formed stronger aversive associations. For instantaneous decision making, the results showed a significant effect of Experience, suggesting that food-raised animals rely stronger on the odor component of taste-smell mixtures than chow-raised animals. Finally, there was a significant Experience x Taste x Sex interaction. Overall, our results demonstrate that both environmental and biological factors contribute to the individuality of flavor perception. Further research will address the neurobiological underpinnings of individual differences in flavor preferences.

331 **Rat And Human Avidity For 'Ideal' Mixtures Of Sweetness To Orally-Detected Calories**

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Introduction: We seek to understand motivating signals from orally sweet and caloric foods that also influence appetite. Rats show a very strong preference for mixtures of saccharin and glucose in solution and drink their body-weight of this mixture daily in excess of either component alone. Less is known of the short-term, oral controls of this appetitive synergy and whether it is relevant for humans. Hypothesis: We hypothesize that the ideal oral solution will signal both sweetness and calories rather than either alone. Methods: Female Wistar rats were given a concentration range of Na-saccharin (sweetness) and glucose (calories and very low sweetness) and their binary mixtures in a series of 10 second presentations via a Davis-rig gustometer. In parallel, humans were given a binary concentration matrix of sucralose and glucose solutions and asked to rate and rank them for liking after tasting and expectorating. All tests were repeated. Results: Rats doubled their number of licks for the 6.8 mM saccharin + 89 mM glucose compared to either 12 mM saccharin or 160 mM glucose, p<0.05. Human participants also strongly liked mixtures of sucralose and glucose to solutions of majority sucralose or glucose. Conclusions: Rats lick the most for a mixture of oral glucose and saccharin, which is mirrored in human ratings

and rankings of binary mixtures of glucose and sucralose. Rats and humans appear to have ideal ratios of oral calories to sweetness when restricted to brief exposures. In the future, we will investigate the interactions of dual signaling from sweet taste and orally detected calories by studying related motivated behaviors and neural activity. *Supported by NIH R01 DC014286 and Busch Biomedical Grant 604095 to PASB and NIH R01 AT008933 to NTB*.

332 **Trpv1-Lineage Fibers Differentially Mediate Orosensory Avoidance Of Bitter And Somatosensory Stimuli In Mice**

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The ion-channel TRPV1 is an embryonic marker of trigeminal nociceptors that interact with central bitter taste neurons. Yet the influence of TRPV1-lineage afferents in oral sensory behavior is unstudied. We generated TRPV1-DTA mice, where TRPV1-lineage cells were ablated, by breeding TRPV1-cre mice (JAX #017769) with ROSA26-eGFP-DTA mice (JAX #032087), which have Cre-inducible diphtheria toxin A subunit (DTA) gene. We measured orosensory responses to different taste and oral somatosensory stimuli in 10 TRPV1-DTA and 10 wild-type mice in a multi-day brief-access paradigm (10 s trials). Mice were tested with quinine (QUI [0.01, 0.03, 0.1, 0.3, 1.0 and 3.0 mM]), cycloheximide (CHX [0.1, 0.3, 1, 3 and 10 μM]) in standard lickometers, and different water temperatures (<5°, 15°, 35°C) in a custom thermo-lickometer. Mouse lines were confirmed by brief-access tests with capsaicin (0.003, 0.03, 0.03 and 0.1 mM). TRPV1-DTA mice were insensitive to oral capsaicin, which elicited strong aversion in wild-types. Both TRPV1-DTA and wild-type mice avoided but developed tolerance for bitter stimuli over test days. The concentration of QUI that inhibited licks to 50% of the maximum lick rate (IC50) increased from 0.5 to 1.5 mM over 7 test sessions ($P = 0.003$) with no effect of genotype. A similar trend emerged in CHX tests, where the IC50 increased from 3 to 6 μM over 11 test days ($P = 0.013$) with no genotype effect. For temperatures, wild-type mice preferred cooled water (15°C) over warm (35°C; $P = 0.003$) whereas TRPV1-DTA mice showed no significant oral thermal preferences averaged across days ($P = 0.06$), albeit some avoidance of warming on initial tests. Thus, orosensory responses to bitter taste stimuli remain in the absence of TRPV1-lineage fibers whereas oral temperature preferences are disrupted.

333 **The Bitterness Of Antibiotics Is Associated With Genetic Variability In *Tas2Rs***

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For drugs to deliver their full benefits and have maximum efficacy, patients need to follow the recommended dosage and frequency. Unfortunately, the taste of drugs, specifically bitterness, can reduce patient compliance. Due to genetic differences in bitter taste receptor genes (*TAS2Rs*), some individuals may be at greater risk for low compliance due to heightened bitterness compared to others. Here we report on the sensory attributes of two antibiotics (ofloxacin and chloramphenicol) and investigate whether bitterness perception is associated with genetic variability in *TAS2Rs*. Participants (n=149) reported the intensity of suprathreshold concentrations of ofloxacin and chloramphenicol on the general Labeled Magnitude Scale. The dominant sensation reported from ofloxacin and chloramphenicol was bitterness, followed by drying. The mean bitterness from ofloxacin and chloramphenicol was 14.06 (±1.17) and 16.47 (±1.16), respectively, falling just below 'moderate.' For *TAS2R38*, A49P was significantly associated with the bitterness of chloramphenicol but not ofloxacin. The bitterness of ofloxacin was associated with SNP Val187Ala in *TAS2R9*, which confirms previous *in vitro* studies (Dotson et al., 2008). In summary, we observed individual differences in the bitterness perception of two bitter antibiotics, which are associated with genetic variability in *TAS2Rs*. Data on bitterness perception from drugs may help reformulate or innovate the delivery system, ultimately increasing compliance. Furthermore, a growing body of literature supports functional roles of T2Rs outside of the oral cavity; thus, relationships between T2R genotypes and taste perception may provide insight into efficacy or incidence of unintended side effects through interaction with extra-oral taste receptors.

334 **How Sweet Is It? Sweetness Perception In Habitual And Non-Habitual Consumers Of Low-Calorie Sweetners**

Stephanie Okoye[1], Sara Petty[2], Clara Salame[1], Yanina Pepino[1,2,3]
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It is estimated that close to 50% of adults in the US consume products with low-calorie sweeteners (LCS), commonly to reduce sugar or calorie consumption. However, it is unclear if regular exposure to LCS could blunt sweet taste perception, thus leading to a higher demand for sugar to achieve the same sweetness level. We hypothesize that habitual LCS consumers will experience a blunted sweet taste perception, prefer higher concentrations of sweetness, and adapt to a greater degree or more quickly when repetitively tasting sweet taste stimuli. To test these hypotheses, we assessed taste perception of various concentrations of a sugar (glucose and fructose) and one LCS (sucralose) alone or in blend in 17 LCS habitual consumers and 25 non-habitual consumers (i.e., consumed >5 or <1 diet soda or LCS equivalent product per week, respectively). Taste intensity was assessed by using the general labeled magnitude scale and sweetness preferences by using the Monell 2-series, forced choice tracking procedure. We also examined sweetness adaptation to sucralose alone and also when mixed with glucose or fructose. Preliminary analyses show that compared to non-habitual consumers, habitual consumers perceived sweetness of sugars as less intense and prefer higher concentrations of sucralose than non-habitual consumers (both P<0.05). There were no differences in the degree of adaptation to sweetness

between groups, but overall adaptation to the sweetness of sucralose was reduced in the presence of a small addition of sugar (111 mM glucose or 45 mM fructose). Frequent consumption of LCS might blunt sweet taste perception, but the results are preliminary, and more research is warranted.

335 **The Contribution Of Olfaction To Fat Perception: A Systematic Literature Review**

Matjaz Pirc, Shuo Mu, Gino Frissen, Markus Stieger, Sanne Boesveldt
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The olfactory system's involvement in fat perception has been studied less extensively than those of somatosensation and gustation. To identify and summarize evidence on the contribution of olfaction to fat perception and highlight knowledge gaps, we carried out a systematic review, utilising the PRISMA protocol. Articles were included if they reported an investigation of olfactory exposure (ortho- / retronasal) to fat via foods, beverages, oils and/or fatty acids in humans or rodents. Studies on added aromas/flavourings were included as well if their addition impacted relevant fat-related attributes. Searches from Scopus, Web of Science and PubMed resulted in the identification of 2596 relevant articles (1704 after deduplication). After screening, 86 full texts remained and were assessed against the eligibility criteria. Ultimately, 5 rodent and 25 human studies were included, the quality of which was assessed using the Cochrane Association Risk of Bias methodology. Human studies show that fat can be detected, discriminated, and identified even when embedded within complex food matrices, either retro- or orthonasally. Studies show that odours can enhance other fat-related sensations. However, the relevance of fat-related odours for food preference and eating behaviour in humans is less clear. Rodent studies are aligned with human studies in showing that olfactory cues are relevant for the detection of fat, but also suggest that olfaction might mediate preference for fat-containing foods. As the first systematic review on the topic, our work concludes that olfaction plays a major role in the detection, recognition and perception of fat in foods. Its contribution, in combination with other sensory modalities, to eating behaviour, food choice and preference needs to be explored further.

336 **Diversity And Natural Selection In Human Tas2R Receptors: Unraveling The Missense Of Bitterness**

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The bitter receptor family is rich in polymorphisms which give rise to amino acid substitutions. These changes have the potential to significantly modify the resulting gene product and contribute to phenotypic variation. Our analysis examines these changes using in silico tools to predict the impact of such variants on the gene product in order to prioritize potentially high impact variants in the bitter receptor genes that may give rise to distinct phenotypes. In order to examine the population structure and diversity of this variation we compute population genetic statistics S, F_{ST}, π, and Tajima's D in the 1000 genomes phase III population, which is composed of 2504 unrelated individuals from 26 global populations. To test deviation from neutrality, and thus the presence of selective pressures, we compare these statistics to the empirical distributions of all sites in the genome for the population, with the assumption that most of the human genome is under neutral conditions. Statistics ranged from both extremes for all statistics, with *TAS2R20* and *TAS2R42* demonstrating >95th percentile for F_{ST}, S, and Tajima's D. As a demonstrating example, predictions of functional effects in agreement by two tools returned *rs139960283* (MAF ~2% in African population) and *rs1669412* (MAF ~22% in Global population) in *TAS2R42* which are relatively common and have potentially high impact on protein function function.

337 **Experience-Dependent Plasticity Of Gustatory Insular Cortex Circuits And Taste Preferences**

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Taste preferences are critical for survival as they direct consumption towards the most nourishing food and away from dangerous substances. As animals wean and transition from mother's milk to foraging for food, they experience an abundance of new tastes and acquire information associated with consuming them. Although evidence suggests that early experiences shape taste preferences, it is not known whether gustatory cortical regions show plasticity in critical periods or remain plastic throughout life. Here we report, in mice, that taste experience at weaning, but not in adulthood, affects sucrose preference later in life. Using a brief access test, we determined that sucrose preference is enhanced following exposure to a variety of tastes at weaning compared to naïve mice exposed only to water and chow. The same exposure at 8 weeks of age did not affect sucrose preference. The change in sucrose preference did not depend on familiarity with sucrose. Early exposure also modulated neural function. Calcium imaging in mice engaged in licking for sucrose revealed that exposure affected the activation of cortical neurons and sharpened neural coding in response to sucrose. Within the gustatory cortex (GC), early exposure accelerated the association of parvalbumin (PV^+) neurons with perineuronal nets (PNNs) and increased inhibitory synaptic transmission onto pyramidal neurons. Degrading PNNs with intra-GC infusions of chondroitinase ABC restored sensitivity to taste exposure in adults. These results point to the presence of a critical period when taste experience induces long-term changes in taste preference. This experience-dependent modulation of taste preference relies on inhibitory control of GC, specifically, PV^+ neurons and their association with PNNs.

338 **Diet-Induced Taste Plasticity Occurs Via Changes In Synaptic Morphology**

Hayeon Sung, Anoumid Vaziri, Monica Dus
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Previous studies have shown that diet composition can induce profound changes in chemosensory plasticity, but the cellular mechanisms through which this occurs are still under investigation. We previously reported that in

the fly *D. melanogaster* high levels of dietary sugar irreversibly blunt sweet taste by lowering the responses of the taste cells to sweet stimuli. These changes were caused by the action of a nutriepigenetic pathway composed by the metabolic enzyme O-GlcNAc Transferase (OGT) and the epigenetic regulator Polycomb Repressive Complex 2.1 (PRC2.1), which supresses genes involved in synaptic physiology and plasticity. Here we investigated the function of a handful of these genes and pathways and showed that their repression by dietary sugar results in alterations in synaptic number and physiology that decrease the output of the sweet gustatory neurons. Together our findings suggest that in chemosensory neurons transcriptional programs that underlie synaptic plasticity are sensitive to interoceptive mechanisms that regulate the responses of cells to the environment.

339 **The Effects Of Breast Versus Infant Formula Feeding On Adult Glutamate (Msg) Taste Perception And Preference**

Sarah Sywanycz[1], Alexandria Stellwag[1], Noor Shah[1], Paul Breslin[1,2]

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Introduction: Flavor imprinting during the early stages of development has effects on future food preferences as an adult. Glutamate, MSG, is largely responsible for the savory taste quality in food. Human breast milk contains a large concentration of free amino acids, particularly rich in free glutamate (~1.5 - 2.0 mM). Infant formula, however, has markedly less free glutamate, which may result in reduced savory taste compared to breast milk. We hypothesize that infants exposed to the high glutamate levels in breast milk may have altered umami taste perception and preferences as adults compared with formula fed subjects. Methods: Adult human subjects who were breast or formula fed (confirmed with mothers) were recruited. They completed a food preference survey of 7 glutamate-rich foods and were asked to taste and rank seven soups varying in added MSG in order from least to most liked. They were also asked to rate savory taste intensity of soups on a Labeled Magnitude Scale (LMS). Subjects were also tested for differential sensitivity to MSG in soups, and Weber's Fractions were calculated. Soups with added quinine served as negative controls. Results: Breast fed subjects rated perceived intensity of MSG as stronger, $p<0.01$, end tended to have lower Weber's Fractions for MSG, compared with formula fed subjects. Additionally, breast fed women, but not men, reported eating savory foods more frequently and gave higher ratings to MSG soups $p<0.05$. Quinine showed no effects as a function of infant feeding group. Conclusion: The difference in free glutamate concentration in human breast milk vs infant formula appears to enhance adult MSG sensitivity and perceived intensity, as well as increase liking of MSG-rich foods. Future research will investigate the gender effect of flavor imprinting with MSG.

340 **Effects Of Cranberry Polyphenol Extract (Cpe) Supplementation On Astringency And Flavor Perception As A Function Of Prop Taster Status And Other Individual Factors**

Neeta Y. Yousaf[1], Mariano Mastinu[2], Iole Tomassini Barbarossa[2], Beverly J. Tepper[1]

[1]Department of Food Science & Center for Sensory Sciences & Innovation, Rutgers University, New Brunswick, NJ, United States, [2]Department of Biomedical Sciences, University of Cagliari, Monserrato, *, Italy

We investigated if PROP taster status, age, gender, ethnicity, and BMI are markers of variation in perception of astringency and other flavor attributes of cranberry juice. Participants (n=125) evaluated cranberry juice cocktail samples supplemented with 0, 0.3, 0.5 and 0.75 g/L cranberry-derived polyphenol extract (CPE; Ocean Spray), and two controls (unsweetened cranberry juice and an aqueous solution of 0.75g/L CPE). Subjects evaluated the samples for sweetness, sourness, thickness, bitterness, astringency, cranberry flavor, overall flavor and liking using 15-cm end-anchored line scales. The data were analyzed using ANCOVA and machine learning tools (regression trees & random forest modeling) to examine if the latter approach would extract additional insights. ANCOVA revealed robust stimulus effects but no effect of PROP status on astringency perception. Instead, PROP status influenced cranberry flavor perception and liking, where super-tasters perceived more flavor and liked the samples less than non-tasters. Caucasian subjects generally perceived more bitterness and astringency from the samples and liked them less compared to Asian subjects. The visualized framework of regression trees showed that each sensory attribute was influenced by a different set of independent variables. Random forest modeling showed that each independent variable had a different explanatory power for each sensory attribute. These data show that PROP taster status did not specifically influence astringency perception when CPE was added to an ecologically relevant cranberry beverage but affected other key attributes. This study underscores the need to explore more complex stimuli that mimic real-world foods and to include personal factors, particularly ethnicity, which are important in shaping perceptual experiences.

341 **Effects Of Controlled Cooling Of Taste Solutions On Orosensory Avoidance Of Bitter Quinine In Mice**

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University of Oklahoma, Norman, OK, United States

Temperature modifies gustatory neural activity in rodent models. However, data on temperature effects on taste preferences in rodents are scarce, with the role of oral thermoreceptor mechanisms in thermal-taste behaviors unknown. Here, we studied how controlled cooling of taste solutions impacts mouse orosensory aversion of bitter taste stimuli in brief-access fluid licking tests. We hypothesized cooling suppresses bitter avoidance, as cooling can serve as a counter stimulus to aversive stimuli in other settings. Wild-type mice (C57BL/6J) and mice deficient for the transient receptor potential melastatin 8 (TRPM8) ion channel, a cold receptor on trigeminal fibers, were tested to study TRPM8 involvement in thermal-taste behavior. Thirst-motivated wild-type ($n = 8$) and TRPM8 gene deficient (TRPM8-/-; $n = 6$) mice were proffered solutions of 1 mM quinine cooled to 20° and 15°C in a contact lickometer modified to independently control the temperature of fluids in separate sipper tubes to within 0.3°C SD. A second squad (wild-type, $n = 3$; TRPM8-/-, $n = 3$) was tested with quinine at

28°, 20°, and 15°C. Mice received several 10 sec presentations of 1 mM quinine at different temperatures in random sequence during daily tests. Data were collected blind to genotype. Licking responses were computed as the ratio of quinine to room temperature water licks, with a ratio of 1 indicating indifference. Preliminary trends imply that at 28°C, 1 mM quinine elicits comparably low licks in wild-type (median lick ratio [mlr] = 0.24) and TRPM8-/- (mlr = 0.09) mice. However, wild-type mice show greater (Wilcoxon test, p = 0.04) licks to 1 mM quinine at 20°C (mlr = 0.71) than TRPM8-/- mice (mlr = 0.15). These data suggest oral sensations of cooling mediated by TRPM8 may counter orosensory avoidance of bitter tastants.

342 **Gaba Influences Taste Nerve Fibers And Responses In The Periphery**

Gennady Dvoryanchikov[1], Andoni I. Asencor[1], Stephen D. Roper[1,2], Nirupa Chaudhari[1,2]

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ATP is the main afferent transmitter from taste buds to gustatory nerves, but there is also evidence for other transmitters, e.g. GABA. Taste bud cells synthesize and release GABA, and geniculate ganglion neurons which innervate them express $GABA_A$ receptors. We found that the most abundant subunit, $GABA_{A\alpha1}$, is located on peripheral (but *not* central axons) of the ganglion neurons, implicating a role for GABA release from taste bud cells. In taste buds stained for both P2X2/3 (ATP receptors) and $GABA_{A\alpha1}$, we quantified where these receptors are located and found they were distributed significantly differently (Kolmogorov-Smirnov, K-S, test $p<0.0001$; 15 buds each): P2X2/3 was along fibers throughout the taste bud whereas $GABA_{A\alpha1}$ was predominant at the base. $GABA_A$ receptors were proposed to bind and stabilize ("chaperone") P2X receptors until the latter are stimulated by ATP (Shrivastava et al 2011). Intriguingly, in taste buds of *Plcb2*KO mice, which lack taste-evoked ATP secretion, $GABA_{A\alpha1}$ is distributed throughout the bud, similar to P2X2/3 (K-S test n.s.; 15 buds each). Thus, in wild-type taste buds, $GABA_A$ receptors may be lost from supra-basal regions as released ATP activates P2X2/X3. We also asked whether GABA modulates taste-evoked responses in afferent neurons. We i.v. administered saline or $GABA_A$ agonist, muscimol (1mg/kg), while confocally imaging responses of geniculate ganglion neurons to oral tastants in *Pirt*-GCaMP3 mice. Responses to 250mM NaCl, 10mM citric acid and 1μM cycloheximide+0.3mM quinine were decreased following muscimol (2-way ANOVA; 3 mice; $p=0.0002$, $p<0.0001$, $p=0.0021$ respectively). Responses to 300mM sucrose were not ($p=0.6916$). We surmise that GABA may influence the maturation of afferent gustatory fibers as well as modulate neurotransmission from taste buds.

343 **Comparison Of Unipolar And Bipolar Electrogustometric Threshold Measures For Clinical Applications**

Toshi Matsuda[1], Pavana Mysore Ganesh[1], Robert Brown[1], Vince Grosso[1], Richard L Doty[2]

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Electrogustometry has proven useful in clinical taste testing. Extant electrogustometers typically employ unipolar electrodes which require current movement between the tongue and distal body locations, such as the hand, forearm, or neck. While constant current circuits account for varied resistances due to different sectors of the body, concerns arise regarding the impact of even low electric current on non-taste tissues (e.g., the heart). Moreover, bipolar electrodes are more practical and patient compliant, since an indifferent electrode does not have to be held in the hand or attached to the skin, minimizing the likelihood of creating an open or discontinuous circuit. In this study of 16 healthy subjects, we compared bipolar and unipolar electrode detection thresholds, as measured by current density and a forced-choice staircase threshold paradigm. Threshold values were equivalent for bipolar and unipolar electrodes. Unipolar anodal thresholds were lower (greater sensitivity) than unipolar cathode thresholds ($p <0.05$). Age was inversely related to all threshold scores, and women outperformed men (all ps <0.001). No left:right tongue differences were apparent. This research indicates that taste testing using bipolar electrodes produces threshold scores equivalent to those using unipolar electrodes, validating the utility of such electrodes for clinical evaluations of the taste system.

344 **Sweet Desensitization Mediated By Glia-Like Taste Cells On The Tongue**

Gha Yeon Park[1,2], Hyeyeong Hwang[1,2], Pyonggang Choi[1,2], Jisoo Han[2], Myunghwan Choi[1,2]

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Due to the lack of taste receptors, type-1 taste cells, also known as glia-like taste cells, have long been regarded merely as a supporter or a bystander in the central pathway of taste transduction. Although a recent *ex vivo* study demonstrated that type-1 taste cells can receive purinergic input from bitter-sensing type-2 taste cells, their functional role in transducing taste signals is yet speculative. Here, we pursue investigating the molecular-level functional crosstalk between glia-like type-1 and chemosensory type-2 cells during taste sensation by harnessing an *in vivo* functional imaging platform (μTongue) on subtype-specific taste cells. We found that sweet taste-elicited activation of type-2 taste cells gave rise to an increase in functional activity of adjacent type-1 taste cells, which was mediated by ATP released from type-2 taste cells. Synthetic activation or inhibition of type-1 taste cells led to modulation of central processing of sweet taste signals, from calcium activity in type-2 taste cells to neural activity in gustatory afferent nerves. We further investigated molecular mediators and receptor subtypes using functional screening and *in situ* hybridization. Taken together, our results propose that a functional interplay between type-1 and type-2 taste cells contributes to modulating sweet taste perception.

345

Umami Peptide Suppresses Intracellular Ca^{2+} Signaling In Sixteen Tas2Rs Via A Competitive Or Non-Competitive Mechanism

Mee-Ra Rhyu[1], Yiseul Kim[1], Takumi Misaka[2]

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Receptor binding studies using cells expressing TAS2R16 provide evidence for direct binding of umami substances to the antagonist binding sites on the receptor, resulting in the suppression of bitterness. This study was designed to investigate whether these findings would be replicated in other members of TAS2Rs. We monitored temporal changes in intracellular Ca^{2+} in HEK293T cells expressing sixteen TAS2Rs, respectively in respond to ligand by Glu-Glu (EE), and L-glutamate (L-Glu) using Ca^{2+} signaling assay. Pretreatment of EE elicited a concentration-dependent reduction of intracellular Ca^{2+} signal evoked by each ligand in all TAS2Rs. While L-Glu was not recognized by TAS2R3, TAS2R31, TAS2R38, TAS2R39, TAS2R40, and TAS2R46 at fixed concentration of each bitter ligand. The half maximal inhibitory concentration of EE ranged between 0.8 and 3.5 mM and that of L-Glu ranged between 15 and 30 mM. This indicates that the inhibitory potency of EE on bitter ligand binding to the receptor is roughly 3 to 30 times stronger than that of L-Glu. EE and L-Glu are classified as a partial non-competitive and a competitive antagonist at the TAS2R16, respectively in our previous study. We then investigated the putative pattern of antagonism of ligand concentration response using a fixed concentration of EE and L-Glu in other TAS2Rs. Both EE and L-Glu can act as either competitive or non-competitive antagonists in members of TAS2Rs. These observations indicate that umami peptide, in particular, targets most of TAS2Rs via a competitive or a non-competitive mechanism, which varies from receptor to receptor

10:00 - 12:00 PM	Calusa ABCD
RECENT SURPRISES IN OLFACTION	

Chair(s): Lisa Stowers

10:00 **Recent Surprises In Olfaction**

Lisa Stowers
Scripps Research, La Jolla, CA, United States

Neuroscience textbooks neatly describe how the sense of olfaction occurs; from ligands to receptors and the activation of sensory circuits. However, the study of the sense of smell is still young which leaves room for scientific surprises. This symposia features outstanding young scientists who will highlight some of the most exciting, impactful, and unexpected recent studies that may upset the established paradigm.

10:01 **Sensory Experience Alters Transcriptional Profile Of Olfactory Sensory Neurons**

Tatsuya Tsukahara[1], David H Brann[1], Stan L Pashkovski[1], Grigori Guitchounts[1], Thomas Bozza[2], Sandeep Robert Datta[1]

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Animals traversing different environments encounter both stable background stimuli and novel cues, which are thought to be detected by primary sensory neurons and then distinguished by downstream brain circuits. We recently find that each of the ~1000 mouse olfactory sensory neuron (OSN) subtypes, as defined by the expressed odorant receptors (ORs), harbors a distinct transcriptome that is precisely determined by interactions between its odorant receptor and the environment. This transcriptional variation is systematically organized to support sensory adaptation: expression levels of more than 70 functional genes relevant to transforming odors into spikes continuously vary across OSN subtypes, dynamically adjust to new environments over hours, and accurately predict acute OSN-specific odor responses in each environment. When an OSN is highly active in one environment, genes that can attenuate odor response are upregulated while ones that can amplify odor response are downregulated, and thus odor responses are attenuated. The response of the same neuron is boosted when it is inactive in another environment, and we observed the opposite trend in the expression of these functional genes. Furthermore, in vivo calcium imaging of OSN axons revealed that functional odor response and relationship between odors are altered across environments. This result indicates that sensory codes accessible from the brain are flexibly modulated in an environment-specific manner. The sensory periphery therefore separates salient signals from predictable background via a transcriptional rheostat whose moment-to-moment state reflects the past and constrains the future; these findings suggest a general model in which structured transcriptional variation within a cell type reflects individual experience.

10:30 **Non-Canonical Odor Coding In The Mosquito**

Meg A Younger[1,2,3], Margo Herre[2,3], Olivia V Goldman[2,3], Tzu-Chiao Lu[5], Gabriela Caballero-Vidal[6], Yanyan Qi[5], Zachary N Gilbert[2], Zhongyan Gong[2], Takeshi Morita[2,4], Saher Rahiel[2], Majid Ghaninia[6], Rickard Ignell[6], Benjamin J Matthews[2,4], Hongjie Li[5], Leslie B Vosshall[2,3,4]

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Female *Aedes aegypti* mosquitoes are a persistent human foe, transmitting arboviruses including dengue and yellow fever when they bite us to obtain a blood meal. Mosquitoes are intensely attracted to human-emitted body odor, heat, and carbon dioxide, which they detect using three different large multi-gene families encoding odor-gated ion channels. Genetic mutations that cause profound disruptions to the olfactory system have modest effects on human attraction, suggesting significant redundancy in odor coding. The canonical view is that olfactory sensory neurons each express a single chemosensory receptor that defines its ligand selectivity. Using immunostaining, RNA *in situ* hybridization, and single nucleus RNA sequencing, we discovered that *Aedes aegypti* uses an entirely different organizational principle, with many neurons co-expressing multiple chemosensory receptor genes. *In vivo* electrophysiology demonstrates that the broad ligand sensitivity of mosquito olfactory neurons is due to this non-canonical co-expression. The redundancy afforded by an olfactory system in which many neurons co-express multiple receptors with different chemical sensitivity may greatly increase the robustness of the mosquito olfactory system and explain our longstanding inability to engineer new compounds that disrupt the detection of human body odor by mosquitoes.

11:00 **Neural Correlates Of State And Place In The Olfactory Bulb Of Freely-Moving Mice**

Matt Smear[1], Morgan Brown[1], Teresa Findley[1], Aldis Weible[1], James Murray[1], Scott Sterrett[2], Adrienne Fairhall[2]

[1]University of Oregon, Eugene, OR, United States, [2]University of Washington, Seattle, WA, United States

Odors carry useful navigational and episodic information, but no matter how many receptor genes are in an

animal's genome, there is no receptor for time or place. To optimally orient by olfactory information, brains must unify odor-driven activity with contextual representations of self-movement and -location. Studies in other sensory modalities demonstrate that motor- and location-related signals are common in primary sensory areas. Motivated by these findings, and given the reciprocal connection between olfactory system and hippocampus, we hypothesized that the olfactory bulb encodes contextual information. To test this hypothesis, we captured the sniffing and movement of mice while recording spiking in olfactory bulb (OB), in the absence of experimenter-applied stimuli or tasks. Breathing and spiking differ between head-fixed and freely-moving states. During head restraint, sniffing is arrhythmic. In contrast, during free movement respiration is rhythmically organized into discrete states lasting minutes. This discrete organization is likewise apparent in the spontaneous activity of the olfactory bulb – many individual neurons fire selectively during particular rhythmic states. In addition to these state-selective signals, we also found that allocentric position can be decoded from neuronal ensembles in OB, with comparable decoding performance to hippocampal ensembles recorded under the same conditions. Thus, even during uninstructed behavior and ambient stimuli, contextual information about state and place can be read out from the activity of the olfactory bulb. We propose that these contextual signals facilitate the incorporation of olfactory information into cognitive maps of environment and self.

11:30 **Representational Drift In Primary Olfactory Cortex**

Carl E. Schoonover[1], Sarah N. Ohashi[2], Richard Axel[1], Andrew J.P. Fink[1]

[1]Howard Hughes Medical Institute, Mortimer B. Zuckerman Mind Brain Behavior Institute, Department of Neuroscience, Columbia University, New York, NY, United States, [2]Immunobiology Graduate Program, Yale School of Medicine, New Haven, CT, United States

We have discovered that in the rodent primary olfactory cortex (piriform) the pattern of neural activity evoked by a smell changes with the passage of time. These changes, which unfold absent a task or learning paradigm, accumulate to such an extent that after just a few weeks odor responses bear little resemblance to their original form. The piriform has been traditionally hypothesized to establish the identity of odorants. Our observations have forced us to radically reconsider the role of this vast brain region in olfactory perception. We propose that the piriform operates instead as a flexible learning system, a 'scratch pad' that continually learns and continually overwrites itself. This poses the problem of how transient memory traces can subsequently be stored over long timescales.

10:00 - 12:00 PM	Calusa FGH
THE GESTALT OF FAT PERCEPTION: MORE THAN TASTE AND SMELL	

Chair(s): Linda Flammer & Nancy Rawson & Paul Breslin

10:00 **How Do We Sense Fats In The Mouth?**

Paul A.S. Breslin[1,2], Linda J. Flammer[2], Nancy E. Rawson[2]
[1]Rutgers University, New Brunswick, NJ, United States, [2]Monell Chemical Senses Center, Philadelphia, PA, United States

The oral sensation of fat is foundationally mechanosensory. As our scientific community explores how we taste and smell fats in the mouth, we wish to incorporate into our thinking the mechanosensory side of fatty mouthfeel. Furthermore, the mechanoreception experience of fats and fatty acids modulates taste and olfaction, and vice versa. This is a new and evolving research area that deepens our understanding of taste and smell because the oral experience is not a series of isolated sensations, but rather a Gestalt, where the whole is greater than the sum of its parts. Together the inputs of these sensory modalities are cognitively integrated. Translationally, mechanoreception is of paramount importance in governing sensory appreciation and the pleasurable experience of consuming fatty foods and beverages. This symposium brings together several different disciplines both within and outside the AChemS community including physiology, chemical engineering, physics, psychophysics, and psychology in an integrated manner to deepen our understanding of the oral experience of fats. Using dynamic tribology, we have learned that the sensory perception of texture and mouthfeel is the result of the friction created between oral surfaces such as the tongue, hard and soft palate, and teeth, as well as the mixture of stimuli with saliva. This provides a level of understanding oral experiences that enables us to design healthier foods, create sustainable alternatives without compromising mouthfeel, and tailor new foods, such as for an aging population or to generate better plant-based meat substitutes. How taste, olfaction, and fatty mouthfeel integrate to generate the experience of oral fatty foods will be a subject of our panel discussion. The organizers claim no financial conflict of interest.

10:05 **The Cells And Neurons That Underlie Oral Mechanosensation**

Yalda Moayedi
Columbia University, NEW YORK, NY, United States

Oral mechanosensation subserves a myriad of functions in feeding including flavor perception, bolus preparation, and swallow. During feeding, food contacts oral and upper airway sites, initiating mechanotransduction. Each site has a unique complement and organization of mechanosensory cells and neurons embedded in its mucosa that transduce features of food texture and contribute to flavor construction and feeding mechanics. These tissues also have unique structural and biomechanical properties that influence the responses of mechanosensory neurons to touch. Understanding the diversity of mechanosensory organs in oral tissues, their environment, and their response properties allows us to build hypotheses about how they contribute to flavor construction. Recent work describes the diversity of oral mechanoreceptors and their response features. The organization of mechanoreceptors in the hard palate is homologous to that of the fingertips with Merkel-cell neurite complexes at the base of epithelial pegs and Meissner's corpuscles between pegs. This arrangement confers the palate with high tactile acuity that gives it the ability to detect moving and static stimuli. The tongue, on the other hand, has unique end organs whose functional properties are not well known. We investigated the functional properties of trigeminal mechanoreceptors innervating the tongue and found five distinct functional groups, each with varying response thresholds and kinetics. Collectively, the proportion of mechanosensory units suggests that the tongue is best equipped for sensing moving stimuli, like that felt when actively feeding, drinking or speaking. We use this information to develop hypotheses on the roles of mechanoreceptors in detecting fat-associated textures like oiliness, mouthcoating, and viscosity.

10:35 **Rheology And Tribology Are Not The Same: Insights Into The Dynamics Of Oral Lubrication And Mouthfeel**

Jason R Stokes
School of Chemical Engineering, The University of Queensland, Brisbane, *, Australia

Rheology describes how fluids and materials flow and deform, and is a key design parameter for foods (including beverages) due its direct impact on processing, stability, and organoleptic properties. However, small changes in composition and/or structure of a food can have a major effect on texture and mouthfeel without significant differences in rheology. To explore and quantify why, we turn to the field of tribology; the study of friction, lubrication and wear between interacting surfaces. While rheology applies shear at a fixed gap, soft-tribology involves shear between deformable substrates under load and narrow gaps, which more closely captures oral lubrication processes. For oil or fat containing multiphase fluids and soft solids with the same rheology, their tribological response can be completely different. This arises because in the narrow gaps experienced in tribology, the oil droplets or fat particles can undergo confinement and spread in the contact zone if they are sufficiently large enough to bridge the gap between surfaces or via adsorption to the substrates. It is also noted that emulsifying and stabilising agents, including proteins and surfactants, may also adsorb to surfaces to alter their wetting characteristics and, if a mucosal fluid layer is present, can cause this layer to desorb from surfaces to dramatically affect measured friction. Tribological studies on a range of model and complex multiphase fluids and soft solids show the physics governing how fat/oil behave in lubrication, which provides testable hypothesises on factors governing the somatosensory qualities of fat. The talk aims to highlight our latest research bridging tribology, rheology and other measurement techniques with sensory perception towards enabling rational design of healthier and/or sustainable products food and beverage products.

11:05 **Thinking Beyond The Tongue: The Importance Of A &Ldquo;Whole Mouth&Rdquo; Approach To Assessing Texture Perception**

Brittany L. Miles
The Ohio State University, Columbus, OH, United States

The human oral cavity is comprised of a variety of mechanosensitive structures likely involved in food texture perception. However, studies aiming to relate oral tactile acuity to food perception are often limited to the tongue, which often misses relevant contributions coming from other oral tissues. It is thus critical to evaluate perception across the soft tissues of the oral cavity to generate a more-complete picture of how signals may be integrated during the texture evaluation process. To highlight the importance of this "whole mouth" approach we will explore the percept of solution viscosity. Previously, we elucidated a potential mechanism for the perception of high-viscosity solutions ($\eta \leq 1000cP$) on the tongue related to the directional deformation of the non-tasting, filiform papillae. Average papillary length and density significantly predicted discrimination acuity. When relating evaluations completed only on the tongue to those completed with the entire oral cavity, however, there was a notable decrease in acuity, suggesting other tissues – primarily the hard palate – might be involved. Further investigation into the hard palate indicated that the palate may be similarly sensitive to differences in viscosity, with perception ability linked to palatal rugal attributes of compressibility and proportion of the total palate area. Moreover, sensitivity of the tongue and palate within a given individual were not correlated, suggesting that individuals may compensate for a lack of sensitivity of one tissue with sensitivity of the other. As such, both tissues likely also serve as active perceptual surfaces related to fat perception in food systems.

11:30 ***Weaving Together Multiple Sensations To Produce The Oral Perception Of Fats***

John Prescott[1,2]

[1]TasteMatters Research & Consulting, Sydney, *, Australia, [2]Dept. DAGRI, Università degli Studi di Firenze, Florence, *, Italy

Although detection of oral fat may partially rely on a taste receptor mechanism, the perception of fat in foods is primarily via its impact on the food's sensory characteristics, principally texture. A wide variety of oral sensations related to foods can be described by such qualities as *viscosity, body, smoothness,* and *mouthcoating,* which to a great extent, are determined by fat content, and based on the somatosensory effects mediated by fat. Importantly, such perceptions are multimodal, reflecting the fact that fat contributes to flavour in combination with tastes, odours, and other somatosensory stimuli. Thus, the perception of creaminess relies on both mouthfeel characteristics as well as odour, which together can be modified by tastes. Some of these flavour interactions arise from mutual suppression and enhancement effects that occur within foods. Others are based on learned associations between fat and other sensory qualities, in particular, odours, analogous to the experience-based odour-taste interactions that form the basis of food flavours.

12:00 - 1:00 PM	Lunch On Own
Lunch On Own	

1:00 - 2:00 PM	Calusa ABCD
Business Meeting	

Get involved! Join us for reports from the society leadership on the state of the association All members are welcome and encouraged to attend.

2:00 - 4:00 PM	Calusa ABCD
TRANSFORMING THE CHEMICAL SENSES: GRAND CHALLENGES AND NEW PARADIGMS	

Chair(s): John McGann

2:00 **Focusing Research Efforts On Developing Treatments For Chemosensory Restoration**
Bradley Goldstein
Duke University

2:12 **Strategies For Reckoning With The Action In Olfaction**
Matt Smear
University of Oregon

2:24 **The Olfactory Bulb As A Major Player In Brain Health And Disease**
Leslie Kay[1], Daniel Wesson[2]
[1]University of Chicago, [2]University of Florida

2:36 **Investigating The Impacts Of Somatosensory Integration With The Chemical Senses**
Yalda Moayedi
Columbia University

2:48 **Fostering The Development Of Chemosensory Medicine: Broader Perspectives On Disease And Healthcare Delivery**
Paule Joseph
National Institutes of Health

3:00
Diego Restrepo
University of Colorado Denver

3:12 **Taste Isn'T Just Perception. Taste Intersects With Nutrition, Metabolism, Aging.**
Nirupa Chaudhari
University of Miami

3:24 **Panel Discussion**

3:45 - 4:15 PM	Calusa Foyer
Coffee Break	

4:00 - 5:30 PM	Calusa ABCD
DEIB Distinguished Lecture	

Chair(s): Paul Breslin

4:00

Tyrone Porter
The University of Texas at Austin

5:30 - 6:30 PM	Estero Terrace
Diversity Networking Reception (Invite Only)	

7:00 - 9:00 PM	Calusa ABCD
Career Award Lectures	

Chair(s): Danielle Reed

7:00 **Achems Young Investigator Awardee**

7:30 **Iff Awardee**

8:00 **Ajinomoto Awardee**

8:30 **Max Mozell Awardee**

9:00 - 11:00 PM	Estero Ballroom
Poster Session IV	

400 **Comprehensive Functional Characterization Of Type-1 Taste Cells In Vivo**

Pyonggang Choi[1,2], Jisoo Han[2], Myunghwan Choi[1,2]

[1]School of Biological Sciences, Seoul National University, Seoul, *, Korea, [2]The Institute of Molecular Biology and Genetics, Seoul National University, Seoul, *, Korea

Taste cells can be classified into several subtypes according to their morphological and functional characteristics. Among those, type-1 taste cells are the most numerous cell type comprising nearly half of cells in the taste bud, but their functional role in taste information processing remains largely veiled. Using our recently developed in vivo functional screening platform (μTongue), we performed the comprehensive functional characterization of type-1 taste cells in response to the five basic taste qualities. Compared to the other type-2/3 taste cells, type-1 taste cells were predominantly responsive to sweet, bitter, and salty taste qualities. Notably, the responses of type-1 taste cells showed distinctive temporal kinetics: the response onset was significantly delayed and stimuli-response relationship showed greater nonlinearly. Collectively, our results suggest that type-1 taste cells may contribute to more complex processing of peripheral taste information.

401 **Birth And Death Of Taste Cells In Circumvallate Papillae Of Mice**

Thomas E. Finger, Rubaio Yang, Yannick Dzowo, Robert S. Lasher, John C. Kinnamon, Courtney E. Wilson
Univ. Colorado Sch. Medicine, Aurora, CO, United States

Cells in taste buds have a limited lifespan and undergo continual replacement by proliferative basal cells situated along the basal lamina (BL). Using serial blockface EM sections though circumvallate taste buds in mice, we have identified the progression of taste cells from their origins along the BL to ultimate senescence and death within the taste bud. Dividing cells, recognized by lack of nuclear envelope and rearrangement of chromatin, have an irregular, ragged appearance and always lie outside of the nominal boundaries of the taste bud. Apparent immature cells lie in the basal region of the taste bud with shapes ranging from irregular and roundish to vertically elongate, often with apical extensions but reaching only part way up in the taste bud. At this stage, many immature cells begin to exhibit features consistent with a Type II cell morphology, including rounded nucleus and appearance of organelles. Elongate, not fully mature Type II cells have an apical process not reaching the taste pore but may form specialized contacts with nerve fibers including atypical mitochondria that typify a channel synapse. Mature Type II cells have a single apical microvillous process extending into the taste pore and prominent synapses with nerve fibers. Senescent Type II cells exhibit numerous cytoplasmic vacuoles while retaining synaptic contacts and an apex still reaching the taste pore. Still older Type II cells withdraw from the taste pore although maintaining synaptic contacts, and begin fragmentation while apparently being engulfed by Type I cells. At terminal stages, dying cells with fragmented nuclei have a dense cytoplasmic matrix and are fully engulfed by Type I cells. We see no obvious examples of dying Type III cells, which may relate to their lower overall numbers and relative longevity.

402 **Taste Nerve Arbor Morphology Is Not Determined By Taste Bud Size Or Cellular Composition**

Lama Hanbali, Lisa Ohman, Robin Krimm
University of Louisville School of Medicine, Louisville, KY, United States

On the tongue, taste buds are localized to three classes of papillae, which differ in size and cellular composition. How do these differences in taste bud size and cellular make up influence the portion of the taste nerve fibers which innervate the taste bud (arbors)? To address this question, we compared the morphology of arbors in circumvallate (CV) and fungiform (FF) taste buds and their proximity to receptor cells. Taste-transducing cells were immunohistochemically stained and nerve fibers were labeled with sparse cell genetics and images were captured using confocal microscopy. Locations where nerve fibers and receptor cells were overlapping (contacts) were identified using the Imaris software and branching structures of individual nerve arbors were traced using Neurolucida. We confirmed that CV taste buds are larger than FF taste buds ($p<0.0001$). However, the size of arbors in CV taste buds compared to FF taste buds is not different as measured by convex hull. The arbors from CV taste bud were less complex in that they had fewer terminal branches than arbors from FF taste buds ($p<0.05$). This indicates tastes bud size does not regulate arbor size or branching. Although CV arbors were less complex than FF arbors, they contacted the same number of taste-transducing cells (CV=1.51, FF=1. 87). We found that CV taste buds have a larger number of Car4 cells per taste bud ($p<0.0001$), but have the same number of PLCB2 cells, as compared to FF taste buds. However, the percent of arbors contacting only PLCB2, only Car4, or both cell types was similar between arbors in CV and FF taste buds. This suggests that contacts between different cell types is similar between arbors entering FF and CV tastebuds. Taken together, neither taste bud size, nor cellular make-up influences taste nerve arbor structure.

404 **A Nutriepigenetic Pathway Links Nutrient Information To Sensory Plasticity**

Anoumid Vaziri[1], Carrie Ferrario[2], Peter Freddolino[3], Monica Dus[1]

[1]University of Michigan, Department of Molecular, Cellular, and Developmental Biology, Ann Arbor, MI, United States, [2]University of Michigan, Department of Pharmacology, Ann Arbor, MI, United States, [3]University of Michigan, Department of Biological Chemistry and Department of Computational Medicine and Bioinformatics, Ann Arbor, MI, United States

Diet composition has a profound influence on brain physiology and behavior, but the mechanisms through which nutrient information is transmuted into neural changes remain elusive. Here we uncover how the metabolic enzyme O-GlcNAc Transferase (OGT) transforms information about the dietary environment into taste adaptations. We show that in the fly *D. melanogaster*, OGT decorates the chromatin of the sweet taste neurons and provides the nutrient context to drive changes in chromatin accessibility in response to high dietary sugar. Specifically, we found that OGT cooperates with the epigenetic silencer Polycomb Repressive Complex 2.1 (PRC2.1) to promote nutrient-sensitive variations in chromatin openness; these chromatin dynamics result in changes in gene expression and taste plasticity that are dependent on the catalytic activity of OGT. Parallel nutrigenomic signatures were also observed in the lingual epithelium of rats exposed to high dietary sugar, suggesting that this conserved metabolic-epigenetic pathway may also underlie diet-dependent taste changes in mammals. Together our findings reveal a novel role for nutriepigenetic signaling in the brain: amplifying nutrient perturbations into robust changes in chromatin accessibility and transcriptional output that shape neural and behavioral plasticity.

405 **The Taste Of Water: Evidence For Contributions Of Type Iii Taste Cells And The Sour Receptor Otop1**

Courtney E Wilson[1,3], Emily Liman[2], Sue C Kinnamon[1,3]

[1]University of Colorado School of Medicine Department of Otolaryngology, Aurora, CO, United States, [2]University of Southern California, Los Angeles, CA, United States, [3]Rocky Mountain Taste and Smell Center, Aurora, CO, United States

Taste buds, the end organs of the gustatory system, contain at least two types of taste-transducing cells. Type II cells transduce bitter, sweet, or umami stimuli, while Type III cells transduce sour and some salty stimuli. Type III cells detect sour stimuli via the recently identified sour receptor, OTOP1. Besides their involvement in sour and salty transduction, Type III cells have also been implicated in sensing water. To gain insights into the cellular and molecular basis for water sensing, we recorded from the chorda tympani nerve of mice, which innervates the fungiform taste buds. Chorda tympani recordings from wild type mice reveal a large response to ddH_2O when presented immediately following artificial saliva, a solution containing the ionic components of natural saliva. In Skn1a knockout mice, which lack mature Type II cells, the chorda tympani response to water is preserved, confirming that this response depends on Type III and not Type II taste cells. Interestingly, the nerve response to water is drastically reduced in recordings from OTOP1 knockout mice. Further studies will be required to determine how OTOP1 contributes to the water response.

406 **Don Tucker Finalist: Gustatory Cortical Plasticity Underlying Gustatory Perceptual Learning In Behaving Mice**

Joshua F Kogan[1,2,3], Alfredo Fontanini[3]

[1]Medical Scientist Training Program, Stony Brook, NY, United States, [2]Program in Neuroscience, Stony Brook, NY, United States, [3]Department of Neurobiology and Behavior, Stony Brook, NY, United States

To adapt and survive, animals must learn to discriminate overlapping sensory stimuli predicting different outcomes. This phenomenon, known as perceptual learning (PL), has been well described in the visual, auditory, somatosensory and olfactory systems. However, little is known about gustatory PL and the associated patterns of plasticity in the gustatory cortex (GC). Here we present results addressing this significant gap in the literature. The experiments are based on a novel taste PL paradigm, which relies on a two alternative forced choice task. Mice first learn a sucrose (100mM) vs NaCl (100mM) discrimination, in which sucrose presentation at a central spout is associated with reward at one lateral spout and NaCl with reward at the other. They are then trained to discriminate between increasingly similar pairs of mixtures: 75/25 vs 25/75, 65/35 vs 35/65 and 60/40 vs 40/60 (%sucrose/%NaCl). Before and after learning, mice are tested on a battery of mixtures to establish psychometric curves. After learning, performance increases for all mixture pairs, indicating an improvement in discrimination (6 mice, two way ANOVA, effect of learning $p<0.001$). To assess GC plasticity, we monitor ensemble activity with two photon calcium imaging (Pre-learning: 2337 neurons; Post-learning: 2658 neurons) After learning, we observe several changes including an increased proportion of responsive neurons (Pre - 18%, Post – 23% X^2 $p<0.001$) and an increased proportion of neurons discriminating the 60/40 mixture pair (Pre – 7%, Post 18%, X^2 $p<0.001$). These experiments further elucidate the GC plasticity associated with PL and provide novel information about taste processing in gustatory circuits.

407 **Identification And Functional Role Of Glucagon-Like Peptide-1 Receptors In The Gustatory Cortex**

Milayna M Kokoska[1], Aishwarya S Kulkarni[1,2], Daniel W Wesson[1,2], Amanda M Dossat[1,2]

[1]Department of Pharmacology and Therapeutics, Gainesville, FL, United States, [2]Center for Smell and Taste, Gainesville, FL, United States

Taste perception results from interplay between peripheral sensing and central processing of orosensory information. The gustatory cortex (GC) is a brain region wherein tastant information is processed in manners critical for taste-guided behaviors, yet the cellular mechanisms that afford the GC with such robust influence are unclear. Internal state (*e.g.,* hunger) effects GC activity, and human imaging studies have identified the glucagon-like peptide-1 (GLP-1) system as an effector of GC activity. Central GLP-1 is produced by preproglucagon neurons in the nucleus of the solitary tract, which project to GLP-1 receptor expressing (GLP-1R+) brain regions. What underlies the ability for the GLP-1 system to affect the GC has remained unknown. We performed RNAscope on mouse GC tissue and identified GLP-1R mRNA expression upon neurons in GC layers ii/iii. Expression in GC was comparable to the nucleus accumbens, which is a known GLP-1-sensitive region. We next investigated the functional role of GC GLP-1R+ neurons and infused Exendin-4 (GLP-1R agonist; 0, 0.01, 0.03,

0.1μg) 30min prior to dark cycle onset. GC GLP-1R activation dose-dependently reduced dark cycle chow intake, with the greatest effects at 2h (One-Way ANOVA: $F(2.6, 23.4=5.176$, $p<0.009$). These data suggest that GC GLP-1Rs influence food intake. Ongoing work includes pharmacological manipulation of GC GLP-1Rs to test their possible role in taste-guided behavior. We also are investigating the function and circuitry of GC GLP-1R+ neurons. The outcomes of this project will contribute to a better appreciation of GC cellular heterogeneity and of the mechanisms whereby peripheral and central processing of orosensory information influences taste.

408 **Discrete Coding States During Taste-Guided Decision-Making In Mouse Gustatory Cortex**

Liam Lang[1,2,3], Giancarlo La Camera[1,2,3], Alfredo Fontanini[1,2,3]

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Recent evidence suggests mouse gustatory cortex (GC) is involved in taste-guided decision-making in addition to sensory processing. Rodent GC also exhibits metastable ensemble dynamics during ongoing and taste-evoked activity, suggesting an unexplored, potential link between metastability and decision-making in GC. To uncover this link, we employed analytical and modeling approaches to study neural dynamics in mouse GC during a taste-guided decision-making task where 4 tastants cue correct licking directions according to: Sucrose or Quinine → Lick left; Maltose or Sucrose Octaacetate → Lick right. We applied Hidden Markov Model (HMM) analysis to extract metastable states from GC ensemble spiking activity in task-performing mice. HMM states were categorized according to differential occurrence frequencies between trials of opposite type (sweet cue vs. bitter cue, left cue vs. right cue, left action vs. right action). Within-trial state onset times revealed that taste-related states preceded cue-related states, which preceded action-related states, suggesting GC metastability in decision-making supports sequential encoding of sensory, cue, and decision information over time. In parallel, we developed a spiking neural network (SNN) model of GC to investigate the mechanisms underlying metastability in the task. SNN data yielded HMM state onset time distributions similar to those yielded by experimental data, suggesting our model captured GC's essential decision-making dynamics and that key model features (clustered E/I connectivity, partial cluster overlap, a preparatory gating signal) may have neural correlates crucial for coding via metastability.

409 **Respiratory-Linked Sleep Spindles In Human Olfactory Cortex**

Justin Morgenthaler, Andrew Sheriff, Chris Cyr, Navid Shadlou, Julia Jamka, Josh Rosenow, Stephan Schuele, Greg Lane, Christina Zelano
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Nasal breathing drives neural oscillations in the olfactory epithelium, olfactory bulb, and piriform cortex, and other limbic and neocortical areas. Recent findings in rodents suggest respiration coordinates UP/DOWN state transitions during sleep, which are linked to hippocampal sharp-wave ripples important for memory consolidation. Depolarizing UP states are grouped by slow oscillations and drive thalamocortical spindles and sharp-wave ripples in the hippocampus. Human sleep spindles (12-15 Hz) occur during stage 2 sleep. Could breathing rhythms function to group neuronal activity in piriform cortex in depolarizing UP and hyperpolarizing DOWN states, given the lack of thalamic relay in between the periphery and piriform cortex? If spindle activity is correlated to nasal breathing, then it should have a skewed distribution across respiratory cycles during stage 2 sleep, such that more spindles would occur during inhalations. Here we used intracranial EEG to test this hypothesis by analyzing human piriform cortical oscillations during sleep. Preliminary results from one subject show significantly increased spindle activity during inhales in human piriform cortex during stage 2 sleep ($p<0.05$, FDR corrected for multiple comparisons).

410 **Multiple Ephrinas And Ephas Are Broadly Distributed In Embryonic Lingual Epithelium, Differentially Expressed In Geniculate Ganglion Neuronal Populations, And Repel Geniculate Neurites**

Metin Aksu[1], Albert J. George[2], Kajol H. Doshi[3], David Cho[4], Matthew Russo[5], Jason S. Hwang[6], Randall W. Treffy[7], John Wong[1], Syuzanna Darbinyan[1], Marissa P.L. Pilon[8], M William Rochlin[1]

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We are investigating the role of Ephs and ephrins, cell surface proteins that act as ligands and receptors for one another, in the guidance of embryonic taste axons. EphrinAs are lipid-linked and interact primarily with EphAs, whereas ephrinBs are transmembrane and interact primarily with EphBs. In embryonic day 14.5 (E14.5) mouse tongue, when gustatory afferents have just entered fungiform papillae (FP) epithelium, *in situ* hybridization and immunostaining revealed that ephrinA1, A3, A4, and A5 are broadly distributed in the dorsal epithelium. At E16.5, epithelial ephrinA mRNA levels are higher than at E14.5. The FP epithelium and the papilla core tissue traversed by afferents exhibited lower levels of ephrinAs than surrounding epithelium at both stages. A variety of EphAs are also expressed in the epithelium at these stages, e.g., at E16.5, EphA1 and EphA2 are distributed similarly to ephrinA1 and ephrinA3, respectively. In E14.5 geniculate ganglia, EphA/ephrinA expression varies in intensity and localization, with some (e.g., EphA5, A6, and A7) restricted to Phox2B+ (oral, mostly gustatory) neurons and others (ephrinA2, A3, and A5) concentrated in Prrx1l+ neurons (aural somatosensory) neurons. In vitro, ephrinA-Fc's and some EphA-Fc's repel rat geniculate and trigeminal neurites dose-dependently, demonstrating that both forward signaling (via axonal EphAs) and reverse signaling (via axonal ephrinAs) can occur. We are examining Phox2b-Cre::tdTomato mice to determine if triple knockout mice lacking ephrinA1,

A3, and A4 exhibit aberrant innervation within lingual FP. Our data also raise the possibilities that Eph/ephrin signaling contributes to the divergence of oral and aural geniculate axons, and influences the migration and organization of dorsal lingual epithelial cells.

411 **&Shy;Dll4-Notch1 Signaling Determines Apical Vs Basal Neuronal Cell-Fate Determination In The Vomeronasal Organ Of Rodents**

Raghu Ram Katreddi[1,2,3], Ed Zandro M Taroc[1,2,3], Sawyer M Hicks[1,2], Jennifer M Lin[1,2,3], Paolo E Forni[1,2,3]
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The Vomeronasal organ (VNO) is a part of accessory olfactory system (AOS) that plays a primary role in the detection of pheromones that trigger a spectrum of sexual and social behaviors. Most of the mammals have uniform AOS system with only one type of vomeronasal neurons (VSNs). However, rodents have two main classes of VSNs – 1) VSNs in the apical zone of the vomeronasal epithelium express V1R receptors, Gai2 G-protein subunit, Meis2 transcription factor (TF) and project their axons to the anterior portion of the accessory olfactory bulb (AOB) and 2) VSNs in the basal zone contain V2R receptors, Gao subunit, Tfap2e TF and project to the posterior portion of the AOB. These two VSN cell types form from a common pool of stem cells. Notably, the mechanisms underlying their cell fate determination are not fully understood. To address this question, we performed single cell RNA sequencing of whole VNOs from adult C57B6 wildtype males. Single cell clustering analysis identified non-symmetric expression of the Notch1 receptor and Dll4 ligand among Neurog1+ precursors along the Meis2- and Meis2+ differentiation trajectories respectively. In-vivo conditional Notch1 receptor loss of function experiments, at Ascl1+ progenitor stage, shifted VSN differentiation towards the apical cell fate. Interestingly, inducing Notch Intracellular domain (NICD) gain of function at Ascl1+ progenitor stage redirected the cells towards non-neuronal Sustentacular cell fate, whereas NICD induction at later Neurog1+ precursor stage shifted neurogenesis towards the basal VSN fate. Overall, our research demonstrated that Dll4-Notch1 signaling controls the apical Vs basal VSN cell fate determination in rodents.

412 **What Determines Selective Vulnerability And Circuit Integration Of Olfactory Bulb Dopaminergic Neurons?**

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Selective loss of specific sub-populations of neurons is a hallmark of neurodegenerative disease. Around 40% of dopaminergic (DA) neurons in the mouse olfactory bulb (OB) undergo cell death after a month of sensory input blockade but the remaining 60% are resilient. Furthermore, OB DA neurons are continuously generated throughout life, enabling them to fully repopulate after reversal of naris occlusion. OB DA neurons therefore provide an ideal model system in which to understand selective vulnerability and how newborn neurons can functionally integrate to replace previously lost neurons. Systemically injecting the olfactotoxin methimazole enables us to see the impact of rapid elimination followed by gradual restoration of sensory input to the OB. We used chronic *in vivo* 2-photon imaging in DAT-cre;Ai9;Ai162 mice that express both a red fluorescent protein and a green genetically encoded calcium indicator to track the survival and integration, as well as the odor response properties, of individual DA neurons over weeks. We found that loss of DA neurons was significantly elevated during the first week after methimazole treatment but then returned to baseline. We are analyzing the vulnerability of the previously described large embryonically generated vs. small postnatally generated DA neurons and whether there are differences in the odor response characteristics between neurons that are vulnerable or resilient to loss of sensory input. We are also quantifying the rate of newborn DA neuron integration before and after sensory disruption. Determining what distinguishes vulnerable neurons from their resilient neighbors and whether stem cell-derived neurons can restore circuit function may inform novel targeted therapeutic strategies for the treatment of neurodegenerative diseases.

413 **Function Of R-Spondin - Rnf43/Znrf3 In Taste Tissue Homeostasis**

Chanyi Lu[1], Xiaoli Lin[1], Ichiro Matsumoto[1], Minliang Zhou[1], Ranhui Xi[1], Yali Zhang[1], Hong Wang[1], Robert F. Margolskee[1], Bon-Kyoung Koo[2], Hans Clevers[3], Peihua Jiang[1]
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Taste bud cells turn over continuously throughout life. To maintain taste tissue homeostasis, adult taste stem cells generate new taste cells to replace senescent ones. This process depends strictly on innervation. Previously, we and others demonstrated that Lgr5 marks adult taste stem/progenitor cells in posterior tongue. Furthermore, we demonstrated that R-spondin can substitute for neuronal input for taste cell generation. R-spondin is the ligand of the Lgr5 receptor and its analogs Lgr4/6 as well as two stem cell-expressed E3 ubiquitin ligases Rnf43 and Znrf3, which act as either positive (Lgr4/5/6) or negative (Rnf43/Znrf3) regulators of Wnt signaling in certain tissues. R-spondin 2 is expressed in gustatory neurons, indicating that R-spondin 2 may be the long-sought neuronal factor that regulates taste tissue homeostasis. To determine whether the activity of R-spondin is mediated by Rnf43/Znrf3, we took a loss-of-function approach by ablating Rnf43/Znrf3 in Krt5-expressing epithelial stem/progenitor cells. Mice deficient for Rnf43/Znrf3 displayed taste cell hyperplasia, which mirrors the effect of exogenous R-spondin. More importantly, taste tissue renewal becomes independent of gustatory innervation in this ablation model, suggesting that Rnf43/Znrf3 acts as a brake for taste cell regeneration, whereas neuron-produced R-spondin activates taste stem cells to generate taste cells by removing this brake

function. Surprisingly, we also found that deletion of Rnf43/Znrf3 resulted in degeneration of dorsal lingual epithelial tissues (i.e., filliform papillae), indicating the context-dependent effects of Rnf43/Znrf3. However, in Rnf43 or Znrf3 single-knockout mice, only mild degeneration of the dorsal lingual epithelium was observed, and there was no apparent change detected in taste tissues.

414 **Don Tucker Finalist: Not So Sweet: Investigating The Role Of C-Kit In Sweet Cell Homeostasis**

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Gustation is mediated by taste buds that each house ~100 short-lived taste receptor cells (TRCs) comprising type I glial-like cells, type II cells that detect sweet, bitter or umami, and type III cells that detect sour. TRCs are rapidly renewed by basal progenitors, and this turnover makes the taste system prone to disruption by cancer drugs. Metastatic renal cell carcinoma (mRCC) patients treated with tyrosine kinase inhibitors (TKIs) intended to target VEGFR and PDGFRß often experience taste dysfunction, or dysgeusia. These TKIs also inhibit many off-target receptor tyrosine kinases (RTKs) including c-Kit, Met, Ret, and PDGFRα. Our single-cell RNA sequencing data reveal that VEGFR and PDGFRß are not present in taste tissue, while c-Kit and other listed receptors are expressed in subsets of progenitors and differentiated TRCs, suggesting that inhibition of off-target RTKs impedes taste homeostasis. To test this, we cultured organoids derived from mouse lingual taste progenitors with common TKIs used to treat mRCC (axitinib, cabozantinib, sunitinib). Each TKI inhibits different combinations of RTKs expressed in taste epithelium. We found that these TKIs did not affect progenitor cell proliferation/survival but instead impacted TRC differentiation. Specifically, all three drugs decreased expression of *Tas1r2*, which marks sweet type II TRCs. Importantly, the only taste RTK predicted to be inhibited by all three drugs is c-Kit, which via HCR in-situ hybridization we found co-expressed with *Tas1r2* in sweet-sensing TRCs. These data implicate c-Kit in sweet cell homeostasis and suggest that TKI-induced dysgeusia is due to loss of sweet-sensing TRCs. We are now evaluating if c-Kit is specifically inhibited by our panel of TKIs, and if SCF (the c-Kit ligand) augments sweet TRC production in organoids.

415 **Plasticity Of Taste Progenitor Cells In Taste Epithelial Homeostasis**

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In mice, the circumvallate taste papilla (CVP) contains hundreds of taste buds. Each taste bud comprises type I, II and III taste receptor cells (TRCs) that renew continuously from progenitor cells, subpopulations of which are LGR5+ and GLI1+ (Ren et al., 2014; Liu et al., 2013). To test the necessity of LGR5+ progenitors in TRC renewal, we ablated these cells by treating $Lgr5^{DTR\text{-}mGFP}$ mice with Diphtheria toxin (DT). At 24 hrs post-DT, LGR5-GFP+ cells and taste buds were almost completely gone; however, recovery began by 48 hrs when sparse LGR5-GFP+ cells and scattered KRT8+ TRCs were evident. By 72 hrs, numerous LGR5-GFP+ cells and multiple small KRT8+ cell clusters were detected that also expressed type I, II and III TRC markers. By 7 days post-DT, LGR5-GFP+ cells and taste buds had increased but were still fewer than found in controls. As LGR5-GFP+ and KRT8+ taste cells reappeared coincidentally and unexpectedly rapidly, we posited $LGR5^{neg}$ progenitors were activated by DT injury. Ki67+ cells were increased 24 hrs post-DT, indicating a rapid proliferative response to LGR5+ cell killing. All TRCs differentiate from post-mitotic SHH+ taste precursor cells and in controls 3-4 *Shh*+ cells are found basally in each bud. At 24 hrs post-DT, however, *Shh*+ cell clusters spanned the height of the epithelium, presaging appearance of KRT8+ cell clusters at 72 hrs. Finally, *Gli1*+ cells were abundant at 24 hrs post-DT, leading us to hypothesize activated GLI1+ progenitors support accelerated taste epithelium recovery after LGR5+ cell killing. We will next test if persistent GLI1+ progenitors underlie taste epithelium recovery by treating $Lgr5^{DTR\text{-}mGFP}$; $Gli1^{CreER}$; $Rosa^{tdTomato}$ mice with DT and tamoxifen to track the contribution of GLI1+ cells to regenerating taste buds following LGR5+ cell ablation.

416 **The Effects Of High Fat Diet And Body Weight On Unconditioned Licking To Artifical Sweeteners In Rats**

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Some studies have demonstrated a negative relationship between obesity and sweet taste responding, while others have seen no effect of obesity on taste. We have previously used captopril to prevent weight gain on high fat diet (HFD) in a mouse model to test the role of diet exposure and weight gain independently. Our results indicate that both diet and body weight differentially affected taste-driven responding to artificial sweeteners but not sucrose. In the current study, we used a rat model to 1) test the species specificity of the effect and 2) test the role of diet exposure in the absence of captopril. Rats were divided into four groups: Ad-lib Chow, Ad-lib HFD, Pair-fed HFD, in which animals were given HFD that was calorically equivalent to the Ad-lib Chow group, and to control for the fact that pair-fed groups usually eat all of their food early in the dark cycle we included a 3-hr Time Restricted Chow group. The Ad-lib HFD group gained significantly more body weight than all other

groups, (p's <.05). Body weights for the Pair-fed HFD and Ad-lib Chow group were not significantly different (p = .639), however, both HFD groups regardless of caloric intake, gained significantly more body fat (g) per body weight (g) than the animals consuming chow (p's <.05). Rats were tested in brief access taste tests for AceK and saccharin solutions. There were no significant differences between the diet groups for either solution (p's > .05). These data suggest there is no effect of body mass, diet, or fat deposition on taste responding to artificial sweeteners in rats. This is in contrast to our work in mice, suggesting there may be species differences in the response to artificial sweeteners following HFD and weight gain.

417 **Don Tucker Finalist: Crispr- And Dreadd-Engineered Mice Selectively Drive The Excitability Of M/Tcs To Regulate Whole-Body Metabolism In Mice**

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To investigate a hypothesized effect on whole-body metabolism, we engineered mice with either enhanced or suppressed excitability of mitral and tufted cells (M/TCs) of the olfactory bulb (OB). To increase neuronal excitability, we used Tbx21-Cre x flox-Cas9 progeny to retroorbitally deliver a sgRNA directed to cleave the Kv1.3 channel in M/TCs. *Ex vivo* patch clamping recordings confirmed that CRISPR MCs had enhanced excitability- having a less negative RMP, a lower rheobase current, and increased evoked AP firing frequency. MCs of CRISPR mice were insensitive to a selective blocker of Kv1.3, and protein expression of the channel was reduced by 70%. Confocal imaging confirmed Cas9/sgRNA co-labeling in ~70% of MCs. Similar to Like global Kv1.3-/- mice, the conditional CRISPR knockouts had increased odor discrimination in a habituation/dishabituation paradigm compared to that of control (Cas9-) littermates. When challenged with a 25-week moderately-high fat diet, CRISPR males demonstrated improved health metrics over control littermates. They were resistant to weight gain, had faster glucose clearance, had reduced serum leptin and liver triglycerides, and reduced RER with a shift toward fat metabolism. In a second cohort of Tbx21-Cre x flox-Cas9 male progeny, inhibitory DREADDs were stereotaxically delivered to allow restricted expression to M/TCs. Opposite to that of the CRISPR mice, DREADDS mice had reduced odor discrimination. When assessed for metabolic health, DREADDs mice contrastingly had a reduction in thermogenesis, oxygen consumption, and caloric and water intake in the dark cycle. CNO alone did not have any metabolic effects in the absence of the DREADD receptor. We conclude that the projection neurons of the OB represent an intersection of the neuronal circuity that regulates both olfaction and metabolism.

418 **Association Between Frequency Of Sweet Cravings And Weight Loss After Metabolic Surgery**

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Metabolic surgery is the most successful treatment for severe obesity. These surgeries curb liking and cravings for sweet foods shortly after surgery, but it is unclear if these cravings associate with weight loss in the long-term. Using validated questionnaires (Food Craving Inventory, Dutch Eating Behavior Questionnaire, Eating Disorder Examination Questionnaire (EDE-Q) and Alcohol Use Disorders Identification Test-Concise (AUDIT-C)), we evaluated 36 women (43±10 years old) who underwent metabolic surgery 3.8±1.4 years ago (range 2-7 years). We assessed relationship between variables using Pearson's coefficient, and multiple linear regression when controlling for potentially confounding variables. Participants' current percent excess weight loss (%EWL) was 67±25%, and their mean weight regain from the lowest body weight achieved, 12±12 kg. Current frequency of cravings for sweets and age independently associated with %EWL and accounted for 39% of the individual differences in %EWL (adjusted R^2=0.39, P<0.001). Further, frequent cravings for sweets were associated with increased influence of emotions (r=0.65) and external food cues (r=0.69) on eating behavior, EDE-Q global score (r=0.59, all P<0.001) and weight regain (r=0.33, P=0.05). Remarkably, from all alcohol-drinking participants (86%), 52% had an AUDIT-C score indicative of hazardous alcohol use. These findings suggest that increased sweet cravings associate with reduced %EWL. Consistent with previous reports, there is high prevalence of hazardous drinking in this population, stressing the need for monitoring alcohol in addition to nutrient intake after surgery.

419 **Olfaction And Obesity - Fibroblast Growth Factor 21 Is A Predictive Biomarker In Mice**

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Obesity is one of the most challenging diseases of the 21st century and is accompanied not only by non-alcoholic fatty liver disease, but also by behavioural disorders such as smell disorders. Exercise, dietary adjustments, or time-restricted feeding are the only successful long-term treatments in humans and mice to date. Fibroblast growth factor 21 (FGF21) plays a key role in dietary regulation, but FGF21 resistance is prevalent in obesity. The aim of this study was, first, to investigate in obese mice whether weight reduction leads to improved olfaction detection ability and, second, whether these behavioural changes are associated with decreased FGF21 plasma levels. After establishing a model for diet-induced obesity, mice were subjected to three different interventions for weight reduction, namely dietary change, treadmill exercise, or time-restricted feeding. In this study, we demonstrated that only the combination of dietary change and treadmill exercise affected all parameters leading to a reduction in weight, fat, and FGF21, as well as less anxious behaviour, higher overall activity, and improved olfactory detection abilities. To investigate the interrelationship between FGF21 and behavioural parameters, feature selection algorithms were applied designating FGF21, body weight, physical activity, anxiety, and olfaction detection ability as highly weighted features. In future, the analysis of parameters such as FGF21 and odour recognition ability may be new biomarkers in the field of obesity research, particularly concerning non-alcoholic fatty liver disease, which up to now can only be diagnosed by biopsies.

420 **Don Tucker Finalist: Metabolic Responses To A Glucose Load In People With Obesity Who Are Habitual Or Non-Habitual Users Of Low-Calorie Sweeteners.**

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Although previously considered inert, findings from recent studies suggest that low-calorie sweeteners (LCS) have the potential to affect glucose metabolism. The objectives of this study were to test the premises that sweetness signaling plays a role in the regulation of postprandial glucose metabolism and that habitual consumption of LCS impairs sweetness signaling fidelity. We hypothesized that for non-habitual consumers of LCS, consuming a glucose load when sweetness is inhibited would trigger higher plasma insulin concentrations than when sweetness is present. In contrast, for habitual consumers of LCS, sweetness inhibition would have no effect on their postprandial glucose metabolism. Habitual (11 women, 6 men) and non-habitual (5 women, 8 men) consumers of LCS (i.e., intake of >5 or <1 diet soda or LCS equivalent product per week, respectively), all with obesity, completed two 5.5h Oral Glucose Tolerance Tests (OGTT) in a randomized crossover design. One of the OGTTs was a regular 75 g glucose test whereas, in the other, sweetness was inhibited by the addition of lactisole (a broad sweet taste receptor antagonist) to the 75 g glucose load. We found that postprandial glucose concentrations were not different between habitual and non-habitual consumers but were lower in women than in men ($P=0.03$). As hypothesized, compared to the control condition, sweetness inhibition resulted in higher insulin concentrations in non-habitual consumers (with bigger effects in women than in men) but had no effect in habitual consumers ($P<0.04$). These preliminary data suggest that sweetness signaling plays a role in metabolic responses to glucose and might be disrupted with chronic LCS exposure. However, longitudinal studies with controlled LCS exposure are needed to confirm the latter.

421 **Estimating The Relationship Between Liquid- And Vapor-Phase Odorant Concentrations Using A Photoionization Detector (Pid) Based Approach.**

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Olfactory research often relies on extrapolations from liquid-phase dilutions to determine the vapor-phase concentration tested. Unfortunately, the relationship between liquid dilution and vapor concentration is dependent upon the solvent and can significantly deviate from predictions based on ideal solutions. In other words, a 10-fold liquid dilution rarely results in a 10-fold decrease in the amount of molecules in the vapor phase, particularly at higher concentrations. Measurements of liquid dilution/vapor-phase equilibria are common across chemical literature, but solvent effects make it difficult to directly compare studies using different solvents and odorants. The precise quantification of these chemical vapors usually relies on gas chromatography (GC), which is expensive, requires specialized training, and is not particularly portable. Alternatively, a photoionization detector (PID) is a relatively common piece of equipment in olfactory research labs. Our approach normalizes PID measurements of each liquid dilution to an absolute concentration of the pure odorant. While this method is clearly not as accurate or sensitive as a purely GC approach: 1) it is accessible, 2) allows many odorants to be tested in a cost-efficient manner, and 3) results in estimates of vapor-phase concentrations that are roughly similar to data obtained using a GC. Here, we provide estimations of liquid- / vapor-phase concentrations for ~50 odorants belonging to several different chemical classes of odor (alcohols, amines, esters, terpenes, ketones, aldehydes, phenols, thiazoles and acids) in five different solvents. The resulting liquid-/vapor-phase equilibrium equations successfully corrected for behavioral sensitivity differences observed in animals tested with the same odorant in different solvents.

422 **Monorhinal And Dirhinal Odor Processing In Humans**

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Introduction: Humans can localize bimodal odorants that stimulate both the olfactory and trigeminal nerves. Pure odorants stimulate only the olfactory nerve (e.g., PEA: Phenylethyl alcohol). Yet, how the intranasal olfactory and trigeminal sensory systems interact and integrate information remains unclear. Here, we used olfactory functional Magnetic Resonance Imaging (fMRI) to test the hypothesis that nostril specific stimulation with PEA will differentially activate the primary olfactory cortex (POC). *Methods*: Twenty subjects with normal smell function completed two olfactory fMRI paradims (24 trials) at 3T. The timing structure of the two fMRI paradigms were identical except in one, subjects were instructed to "sniff" at the start of each PEA stimulation block (6 seconds duration). Monorhinal and dirhinal stimulation were used with an inter-stimulus interval of 24 secs. fMRI data were preprocessed and analyzed with SPM812. *Results*: Both the monorhinal and dirhinal stimulation activated the primary olfactory cortex (POC), the temporal gyrus, and the sensory motor cortex. Our results showed that both the left and right stimulation produced bilateral activity in the piriform cortex. Results showed differences in fMRI activation between sniff and no sniff stimulation conditions. *Conclusion*: Our olfactory fMRI paradigms provided an approach to obtain both the spatial and temporal information about nostril specific olfactory processing in the brain. Our paradigms also helped delineate brain networks that subserve the human ability to localize odorants. The sniff paradigm produced substantially higher fMRI activation (e.g., POC) supporting the idea that sniffing is part of olfactory processing. Our results highlight the utility of investigating olfactory network using fMRI.

423 **Perceptions Of Odors And Tastes In Time And Space**

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In natural situations, many chemical stimulus components are simultaneously present. But their odors and tastes rapidly appear and disappear from our sensations. Simulations of natural detection in laboratory studies have used binary stimuli with components that are each clear, distinct and of similar intensity. However, individual component identification is compromised by suppression. Adapting one component for 5 seconds will release other components from the suppression. In this way olfactory and gustatory systems are able to dynamically adjust saliences of items presented sequentially; effects already seen for many foods and flavors (Frank ME, Fletcher DB, Hettinger TP, 2017; Hage J, Frank ME, 2021; Hettinger TP, Frank ME, 2021). We propose that 'adaptation plus antagonism' is a universal chemosensory coding mechanism. Adaptation likely occurs at the taste and odor receptor level in the periphery. Antagonism, however, is probably mediated by interactions between multiple distinct odor or taste responses in the central nervous system. We propose that 'quality of sensation-constancy', maintained by adaptation and component suppression, limits tastes or odor perceptions to a few dominant percepts at one time. Odors and tastes fade and are quickly replaced by newly appearing stimuli. Therefore, odors and tastes are limited in time and space. Although multiple odor and taste receptors have been identified, greater recognition of their dynamic limits are needed to develop an understanding of the complex coding of tastes and odors.

424 **Don Tucker Finalist: Predicting The Combinatorial Code Of Olfaction Via Graph Neural Networks And Representation Learning**

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Our sense of smell relies on the use of approximatively 400 genes expressing functional odorant receptors (ORs), endowing us with the power to discriminate a vast number of chemical stimuli. ORs are activated by molecules, called ligands, in a key-lock mechanism where a ligand fits into the OR's binding cavity. One molecule can fit in different ORs' binding cavities and ORs can accept several different classes of molecules. The first step to crack the combinatorial code of olfaction relies on identification of these OR-ligand pairs. To date, common procedure for OR's ligand identification has been based on *in vitro* search with rather low success rates of ~3%. Moreover, the data linking a molecule to a set of ORs are scarce and only 131 ORs have an identified ligand. Thus, building a machine learning protocol taking both molecules and ORs' sequence explicitly into account remains challenging. To tackle this issue, we leverage recent advances in representation learning and combine them with graph neural networks (GNN) to build a receptor-ligand prediction model. Several methods inspired by success of representation learning in the natural language processing (NLP) have been proposed to represent protein sequences. Here we represent ORs using BERT which was previously trained on more than 200M protein sequences. We treat molecules as graphs and process ORs and molecules simultaneously using GNN. Our receptor-ligand prediction model has been evaluated on a set of more than 7500 OR-molecule pairs. The model is achieving Matthews correlation coefficient (MCC) of 0.46 in the case that all receptors are included in the training set (i.e. random split). The performance on a much more difficult deorphanization task (i.e. discarding all pairs of a given receptor) remains acceptable with a value of 0.30.

425 **Masking Effects On Isovaleric Acid Recognition By Peri-Threshold Concentrations Of Neohivernal, Geraniol, Florhydral And 2,2,3-Trimethylpyrazine.**

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Masking unpleasant odors with high levels of pleasant-smelling odorants is an ancient practice that has evolved into many enterprises: from perfumery to many consumer product formulations. However, effective odor

masking turns out to be idiosyncratic and impermeant. When a subject displays no malodor recognition there is still malodor-dependent activation at the neuronal level and with training the subject can learn to recognize the odorant in presence of the masking agent (Rodrigues-Raecke, 2019). Here, we used Sniff Olfactometry (SO, Rochelle, 2017) to investigate the psychophysics of masking during 70ms-stimulations with mixtures of a mal-odorant Isovaleric Acid (IVA) and 4 different odorants. IVA is a component of human sweat that can dominate its smell, is often described as in unpleasant terms, e.g., “gym locker”, “smelly feet”, “dirty clothes”, etc. Conventionally, high concentrations of positive smelling odorants are used to reduce the unpleasantness of IVA in clothing or environments contaminated with IVA. To investigate the masking effects of peri-threshold and sub-threshold of masking agents (Neohivernal, Geraniol, Florhydral and 2,3,5-trimethylpyrazine) on IVA, we used SO to measure the probability of recognizing IVA after 70ms stimulations with headspaces containing mixtures of super-threshold concentrations of IVA and peri-threshold concentrations of IVA-suppressors (6 IVA concentrations, 8 subjects, 8~16 replication). The masking-psychophysics of IVA-recognition ranged between 3 to 100 times its threshold.

426 **How Fast Can A Human Sniff?**

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How fast can a human sniff? This question remains unanswered. Researchers have determined that rodents sniff at frequencies ranging from 4-12 Hz during odor sampling (Kepecs et al. 2007; Macrides et al. 1982; Rajan et al. 2006; Uchida and Mainen 2003; Youngentob et al. 1987), which is within the theta range. These bouts of fast sniffing in rodents are associated with expediting odor delivery to the olfactory bulb (Wesson et al., 2009) and with high cognitive effort including sensorimotor learning (Kay, 2005). Human olfactory research has shown that the sniff functions as more than an odorant delivery mechanism, also affecting odor intensity and perception, and driving activity in olfactory cortex (Mainland & Sobel, 2005). In this study, we aim to determine how fast humans are capable of sniffing. We will examine individual variation in the upper limit of human sniff speed and will determine whether sniff speed is correlated with olfactory performance. Participants will be asked to sniff as fast as they can while nasal airflow is recorded and will complete standard olfactory testing (UPSIT and sniffin stix). Preliminary results suggest that humans are able to sniff as fast as 4 Hz, within the theta range.

427 **Configural Responses To Odor Mixtures In Head-Fixed Mice**

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Odor mixtures can exhibit configural (the mixture is considered to have an odor different from any of the individual component odorants) or elemental (the components in a mixture are recognizable) properties. However, it remains unclear whether the olfactory bulb, the brain structure that mediates the first stage of olfactory information processing, might contribute to the configural perception of certain odor mixtures. The aim of the present study was to compare elemental and configural perception of odor mixtures in head-fixed mice in a lick/no-lick discrimination task. In this study we used a binary odor mixture (citronellal-octanal) that elicited configural responses in freely moving rats (Kay et al, (2003). We followed a procedure for motivating mice by limiting their access to water as was initially described by Guo et al, (2014). All mice undergoing water restriction were monitored daily for hydration, weight, ruffled fur, and movement. The head-fixed mice were trained in a lick/no-lick discrimination task where animals were rewarded with water for licking in response to binary citronellal-octanal mixtures of two different ratios, 1:1 and 3:1 (citronellal–octanal). Water was not available for trials with the non-rewarded odor (hexanol) and licking on those trials was punished with a longer inter-trial interval. Licking was signaled by mice breaking a light path in a Bpod r2 water port with their tongue. All mice learned to avoid licking in no-lick trials during the first sessions and reached a learning criterion (60% correct responses) within 100 - 220 trials. To determine whether the perception of the odor mixtures is elemental or configural we compared the response to the mixture, the individual components, and a novel odor (isopentyl acetate). The response to the individual components and the novel odor were similar to each other and were different from the response to the mixture and to hexanol, showing that the mixture perception was configural. Supported by NIH grant DC005259 and the Brain Science Institute, Korea Institute of Science and Technology.

428 **Chemical Structure-Based Model Outperforms A Human Panelist On Odor Description Task**

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If you wanted to know what a novel odorant smelled like, would you ask a person to smell and describe it or ask a model to predict it? The relationship between stimulus and odor percept is complex, and so predicting odor perception from molecular structure has been an enduring challenge in the field. Here, we combine state-of-the-art machine learning with a large set of high-quality psychophysical data to raise the ceiling of structure-odor predictive modeling performance. We trained a neural network (NN) on over 5000 molecules characterized in perfumery databases. The model reads in molecular structure and outputs odor label (e.g. grassy, fruity, sulfurous) probabilities. To prospectively validate this model, we purchased 400 structurally diverse molecules that do not appear in or resemble molecules in fragrance databases. Next, we recruited a cohort of human subjects, trained them using odor references to describe odors with a 55-label odor lexicon, and screened to retain only those subjects with high inter- and intra-rater agreement. The 15-subject panel evaluated each of the

400 molecules in duplicate using the rate-all-that-apply method, generating stable mean ratings (panel mean test-retest R=0.80). We find that predictions from molecular structure alone are sufficient to achieve super-human performance. Across all molecule by label combinations, the median panelist predicts the panel mean with an R of 0.47; the NN just surpasses this mark (R=0.49). On a per-molecule basis, NN-predicted labels more closely match the panel mean than the ratings of a single subject for 60% of molecules. Given the choice between the median subject and the NN, you should ask the NN.

429 **Quantity Has A Quality All Its Own: Mapping Odor Character Changes Across Intensities**

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Although most odor atlases describe the odor character of a given molecule using a single description, odor character can change across intensities. Other sensory modalities have similar phenomena, for example the Bezold-Brücke effect in color vision where hue varies with luminescence and the Zürmühl-Stevens effect in audition, where perceived pitch varies with sound pressure. Without a quantitative “odor space,” it is difficult to develop general rules describing how perception shifts with changes in intensity. To develop such rules, we asked 15 trained participants to rate the applicability of 51 odor labels to 100 odorants at two concentrations corresponding to low and high intensities. Several molecules exhibited concentration-dependent changes in character that were larger than typical character differences between different molecules. Odorants with a low detection threshold were more likely to undergo large perceptual shifts and these perceptual shifts tended to occur in consistent directions in odor space. Understanding how intensity and quality interact will provide an important constraint on models of olfactory perception.

430 **Imminence Of Predator Threat Detected By The Accessory Olfactory System**

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Defensive behaviors in the presence of predator cues are typical innate behaviors in animals. Although predator signals are best detected with the summation of various sensory modalities in nature, olfactory-specific exposure to a predator specimen is sufficient to yield defensive responses in nocturnal prey animals such as mice. Animals display fixed patterns of defensive behaviors such as freezing, flight, and risk assessment in response to olfactory predator cues. Interestingly, olfactory cues from a single predator species can evoke a range of defensive behaviors with different intensities, and conversely, different predator cues can elicit one specific behavioral response. The underlying molecular and neural mechanisms for such behavioral decisions are still not well understood. In this study, we investigated a shift of defensive behavioral responses in mice toward olfactory predator cues collected from the same cat individuals. In our behavioral tests, we observed robust freezing responses to cat odors that were freshly collected, while the freezing response was reduced toward older samples. These behavioral outputs were observed only when mice had direct contact with the cat odor and abolished in mice lacking functional Trpc2. Taken together, these results suggest that fresh cat odors contain temporal cues that signal imminence of predator threat to mice, and the signal is detected through the accessory olfactory system. Our study may shed light on the molecular and neural mechanisms underlying the defensive behavioral decision making in prey animals.

431 **The Order Code In The Olfactory Bulb: Can Odorants Be Represented By The Order Of Glomerular Activation?**

Khristina Samoilova[1,2], Hirofumi Nakayama[3], Farhad Pashakhanloo[2], Dmitry Rinberg[3], Alexei Koulakov[2]

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The representation of odor identity (ID) in the responses of olfactory neurons is not understood. Here, we test the hypothesis that odor ID is carried by the order of activation of olfactory receptor (ORs) types. The order of OR activation is approximately preserved for different concentrations suggesting that it can carry information about the concentration-invariant odor ID. To test this hypothesis, we obtained glomerular calcium responses from the dorsal surface of the olfactory bulb to a large array of odorants, including mixtures, in multiple animals. Using this data, we computed the timing of activation of individual glomeruli and their relative ranking. We proposed a neuronal distance metric in the odorant space based on rank correlation (Kendall-tau). We compared the neuronal distance metric with perceptual distance measured in mice for the same odorants. We made the following observations. First, we find that odorant ID representations are similar across different concentrations of the same odorant and different animals. Second, we find that odor IDs can be embedded into a space of low dimension (D~6). Third, the representations of mixtures satisfy triangle inequality suggesting that the Kendall-tau based distances define a metric space. Using Canonical Correlation Analysis (CCA), we observed a strong correlation between the space on neural responses and the perceptual spaces of low dimension. Finally, we developed a method of embedding individual ORs into the odor space using the order-based measures which can allow us to identify ORs across animals. Overall, we proposed and validated the metric for odorant representations based on the order of OR activations. This metric yields robust representations of odor identity which generalize well across stimulus conditions and individual animals.

432 **Identification Of Entry Pathway And Binding Site Of Insect Odorant Co-Receptor.**

Jérémie Topin[1], Jody Pacalon[1], Guillaume Audic[2], Jérôme Golebiowski[3], Christophe Moreau[2]
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Insects are of major importance for our society, either benefit for agriculture or detrimental for human health as pathogen vectors. Olfaction is an essential sense for insects notably for food and host seeking. The insect odorant receptors are cationic channels, contrary to their mammal counterpart that are metabotropic receptors. Recent progress in the atomic resolution of their structures offers unprecedented opportunities for deciphering their molecular mechanisms and particularly the binding mode of their ligands. Odorants bind to the odorant receptor (OR) subunits, while the second mandatory subunit so called odorant receptor co-receptor (ORco) binds chemicals like VUAA1. Despite groundbreaking studies in insect olfaction, many questions are still not answered and most elementary functional characteristics of insect ORco remain to be defined. In particular, little is known about the binding site and entry pathway of VUAA1 and derivatives into ORco. Their identification is essential for both a better understanding of the specificity of action of VUAA1-based molecules and structural insights for the rational design of new ORco ligands. In this study, we focus our work on the molecular features of the receptor protein that determine the ligand specificity and the molecular processes that contribute to the striking sensitivity of the insect olfactory system. We therefore combined results from molecular modeling with site-directed mutagenesis and two electrodes voltage clamp (TEVC) measurement to identify both the binding pocket and the translocation pathway of VUAA1 from the extracellular space to the binding site.

433 **Sparse But Structured Representation Of Odor Space By Primary Sensory Inputs To Glomeruli Of The Mammalian Olfactory Bulb**

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In the olfactory system, convergence of olfactory sensory neurons (OSNs) onto glomeruli of the olfactory bulb (OB) generates a map of odorant receptor identity across glomeruli. How glomerular maps relate to odor space remains unclear. We approached this problem by defining the tuning properties of glomeruli in terms of the odorants to which they are most sensitive. Using high-throughput odorant delivery combined with GCaMP6s imaging of OSN inputs, we imaged glomerular responses to 185 odorants in single preparations using a concentration that evoked activation of one-to-a-few glomeruli. The resulting dataset yielded a comprehensive atlas of glomerular sensitivities and revealed key aspects of how odorant receptors and their glomerular maps relate to odor space. First, nearly all odorants activated one to several glomeruli at or below nanomolar concentrations (range, 4e-14 to 4e-9 M). Second, in this concentration range, glomeruli were extremely narrowly tuned, with ~25% of glomeruli responding to only one of the 185 odorants. Despite this sparseness, the response spectra of glomeruli responding to more than one odorant revealed common principles of OSN tuning and an underlying structure to glomerular odor representations. This structure was poorly captured by sets of physicochemical odorant descriptors, but well-represented by simpler descriptor sets derived from odorant structural features. Odorant sensitivity maps also revealed spatial clustering of glomeruli tuned to odorants sharing the same structural features. These results indicate that in low-concentration regimes, individual glomeruli and their cognate ORs are sensitively tuned to a narrow portion of odor space, and that the structure of odor representations across OB glomeruli largely reflects basic chemical features of odorant stimuli.

434 **The Behavioral Sensitivity Of Mice To Acetate Esters.**

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Measures of behavioral sensitivity provide an important guide for choosing the stimulus concentrations used in functional experiments. Despite their frequent use, a systematic survey of the relative sensitivity of mice to acetate esters is not available. To address this issue, we assayed the ability of C57BL/6J mice to detect seven different acetates (propyl acetate, butyl acetate, pentyl acetate, hexyl acetate, octyl acetate, isobutyl acetate and isoamyl acetate) using a head-fixed Go / No-Go operant conditioning assay combined with highly reproducible stimulus delivery. To aid in the accessibility and applicability of our data, we have estimated the vapor-phase concentrations of these odorants in five different solvents using a photoionization detector-based approach. The resulting liquid-/vapor-phase equilibrium equations successfully corrected for behavioral sensitivity differences observed in animals tested with the same odorant in different solvents. We found that mice are most sensitive to isobutyl acetate and least sensitive to propyl acetate. Interestingly, these estimates of murine sensitivity were similar to those previously reported in primates. These updated measures of sensitivity will hopefully guide experimenters in choosing appropriate stimulus concentrations for experiments using these odorants.

435 **Expression Of Slc27A2 In The Olfactory Epithelium In Mice**

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Lipids are involved in many physiological processes such as cell division and differentiation. Very few lipid-related proteins have been studied in the olfactory epithelium (OE). Consequently, how lipid uptake and metabolism influence the development and function of the OE is poorly understood. Slc27a2 is a very long-chain fatty acid (VLCFA) transporter and it also activates VLCFAs required for subsequent metabolic processes. Slc27a2 is observed in most of the proteomic and transcriptional screens of the OE. Here we report that Slc27a2 is widely expressed in the developing and adult OE by genetic reporter expression analysis and RNA in situ

hybridization. We found that Slc27a2's expression in the adult OE is restricted to immature olfactory sensory neurons (OSNs) and only a subset of mature OSNs. Per the expression of Slc27a2 in the immature neurons, we hypothesized a role of Slc27a2 in the growth and regeneration of the OE. Our preliminary data show that Slc27a2 knockout mice exhibit slower regeneration dynamics of the OE after methimazole-induced injury. Studying how lipid metabolism is involved in olfactory signaling and development will help us to further understand olfaction.

436 **An Optimized Method For Axonal Arbor Tracing And Quantification In The Adult Olfactory System.**

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Ciliopathies are a class of genetic disorders that impair cilia and lead to a constellation of symptoms, including olfactory dysfunction. Ciliopathy induced olfactory dysfunction is thought to be caused by defects in cilia that emanate from OSNs. Importantly, various ciliopathy models have also displayed defects in size and targeting of glomeruli, where OSN axons arborize and synapse. However, no study has detailed a mechanism for the axonal defects in ciliopathy mouse models. Immunohistochemistry provides only a gross analysis of arbor and synaptic change, but more refined information can be obtained by analysis on the level of a single axon. Papers assessing olfactory axonal arborization were limited to neonatal mice and utilized expensive analysis software. We created an optimized procedure to quantify arborization and synaptic vesicles in adult mice with open-access axonal tracing software. Mice were intranasally delivered adenovirus, transducing OSNs with a lipid anchored fluorescent protein (MP-GFP). Ten days after infection, mice were cardiac perfused, the tissue was decalcified, and 100 μm OB coronal slices were collected, cleared, and imaged. We optimized the viral load to allow for confocal images of glomeruli with a singly infected OSN to be captured. The semi-automatic software, Simple Neurite Tracer from FIJI was used to reconstruct and measure axonal arbors. Our results correspond well to previously published data. To assess presynaptic specializations on the axons, OSNs were infected with MP-GFP-2A-Synaptophysin-mCherry to label axons and synaptic vesicles, respectively. This optimized workflow allows for the investigation of the role of ciliopathy proteins in the regulation of axon synapse, providing a more detailed mechanism for ciliopathy induced olfactory dysfunction.

437 **Towards The Structural Elucidation Of Mammalian Odorant Receptors**

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Odor perception is based on odorant receptors (ORs), which belong to the large family of G protein-coupled receptors and more particularly to the rhodopsin-like family, also called class A The vast majority of odorant receptors show poor cell surface expression in non-olfactory cells due to retention of the endoplasmic reticulum (ER), hindering their structural elucidation and functional study. Here, we study at the molecular level the expression mechanisms of this sub-family of G protein-coupled receptors. In this project, we use the diversity of the odorant receptor repertoire to create new optimized synthetic receptors based on their consensus sequences. Using these consensus ORs cases, we study the role of amino acids in their expression through molecular modeling, site-directed mutagenesis and flow cytometry. Their functionality is also assessed by *in vitro* assays. We then developed a protocol to produce and purify the most promising ORs which would allow us to attempt the first structural elucidation of a mammalian OR. This research is crucial, not only to understand the strategy of our brain to perceive its olfactory environment but also to identify general mechanisms governing the function of ORs.

438 **Olfactory Training In Specific Anosmia: Association With Genetic Variations Of The Olfactory Receptor Hor7D4**

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Background and aim: Short-term, repeated exposure to odors, "olfactory training" (OT), improves olfactory function. This study explored whether OT-related changes in the perception of androstenone (AND) are associated with genetic variations of the olfactory receptor hOR7D4. Methods: 335 healthy volunteers participated. Participants performed OT with four odors known to be characterized by high levels of specific anosmia within the general population: bacdanol (BAC), benzylsalicylate (BENZ), 3-hydroxy-2-methyl-4-pyrone (3H2M4P), and AND. Detection thresholds were measured for all odors before and after OT. Buccal swabs were taken to examine the hOR7D4 genotype (GT). Results: 103 participants showed specific anosmia for AND, 46 for BENZ, 40 for BAC, and 11 for 3H2M4P. The rate of specific anosmia correlated with the molecular weight of the odorants (r=0.91). Out of 68 subjects initially anosmic for AND, 49 perceived AND after OT based on the detection threshold. The improvement in the olfactory threshold was statistically significant ($p<0.001$). The hOR7D4 GT was analyzed for all 68 subjects; 45 showed the RT/RT GT, 19 RT/WM, and 4 WM/WM. Out of the subjects able to perceive AND after OT, 30 had the RT/RT GT (61%), 16 RT/WM (33%), and 3 WM/WM (6%). Out of the subjects unable to smell AND after OT, 15 had RT/RT (79%), 3 RT/WM (16%), and 1 WM/WM (5%). Conclusion: Results from the present study confirm that exposure to odors is associated with an improvement of odor thresholds in healthy individuals. They also confirm previous results indicating a positive

relation between the rate of specific anosmia and the molecular weight of the odorant. However, the present results do not support the idea of a significant impact of hOR7D4 GT on the ability to improve AND sensitivity (p=0.35).

439 **Loss Of The Primary Ciliary Protein, Arl13B, In Immature Osns Impairs Neuronal Maturation**

Julien C. Habif[1,2], Kirill Ukhanov[1,2], Carlos de Celis[1,2], Chao Xie[1,2], Lian Zhang[1,2], Warren W. Green[1,2], Jeffrey R. Martens[1,2]

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Ciliopathies are a class of inherited disorders induced by mutations of ciliary genes and manifest in dysfunction in various organs, including the olfactory system. It is believed that ciliopathy induced olfactory dysfunction is caused by defects in the multi-cilia of mature olfactory sensory neurons (mOSNs) which possess the machinery necessary for odorant detection. We show for the first time that immature OSNs (iOSNs) possess primary cilia that express ARL13B, a canonical ciliary marker. *Arl13b* encodes a small GTPase and is a causative gene for the ciliopathy, Joubert syndrome (JS). There is a profound gap in knowledge whereby the role of ARL13B in OSNs is unknown. It is also unclear if JS patients suffer from smell impairment. To explore the role of ARL13B in iOSNs, we derived a mouse model where *Arl13b* is excised from iOSNs (*123-Cre;Arl13b$^{fl/fl}$;123-Arl13b*). The OE of *123-Arl13b* mice showed a shift in neuronal population, with more iOSNs and less mOSNs compared to WT mice at 1 month of age. Also, at that age there was an increase in the basal stem cells and more overall proliferation. BrdU lineage trace experiments revealed that OSNs in *123-Arl13b* mice had a delay in maturation compared to the WT OSNs. Also, *123-Arl13b* mice had a decrease in the number and length of cilia of mOSNs and a reduced ability to detect odorants, as tested by electro-olfactogram recordings. Finally, *123-Arl13b* mice displayed severely deformed glomeruli, speaking to a role of ARL13B in glomerular innervation. Together, our findings demonstrate that ARL13B plays an important role in the maturation of OSNs and suggests that JS has penetrance in the olfactory system.

440 **Olfactory Differences Among Closely Related Cactophilic Drosophila Species**

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Divergence of behavioral traits with adaptation to changes in the environment has been well documented in many animals, and is often accompanied by divergence in sensory systems. For instance, shifts in insect host plant preference behavior, with changes in host plant availability, are often accompanied by changes to an insect's olfactory system. Some progress has been made in understanding the neural basis of such sensory modifications within and between Drosophila species: shifts in host plants have been shown to correspond with changes in the sensitivity and selectivity of olfactory sensory neurons to odor cues. However, the extent to which these changes are lineage specific remains to be determined. This research examines six closely related pairs, 12 total Drosophila species, from the *Drosophila repleta*group, a group that feed and breed on cactus. Several species have shifted host plant preference from the cactus favored by the ancestral group – Opuntia – to either columnar cactus, or to using both the ancestral and new types of cacti, i.e., they have become polymorphic. Olfactory sensory neuron odor-evoked responses were recorded using single sensillum recording (SSR) and compared among species. These recordings reveal differences in both sensitivity to different odors and selectivity of odors. Results such as these will advance a firmer understanding of the evolution of nervous systems in response to environmental pressures such as shifts in host plant use.

441 **Singular Expression Of Trace Amine-Associated Receptors Via Cooperative *Cis*-Acting Enhancers**

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Each olfactory sensory neuron in the mouse nasal cavity chooses to express one allele of one olfactory receptor gene out of >2,000 possible alleles. The mechanism underlying this "singular"
gene choice is not well understood but likely involves *trans*-chromosomal interactions among an olfactory enhancer network. Olfactory receptor genes belong to two phylogenetically distinct families—a large family of >1,000 odorant receptor (OR) genes, and a smaller family of 14 trace amine-associated receptor (TAAR) genes. The TAARs are preferentially expressed by a distinct population of OSNs, and some aspects of TAAR gene regulation appear to differ from those of the canonical ORs. Here we identify and characterize two enhancers in the TAAR gene cluster that are necessary and sufficient for TAAR gene choice. Deleting each enhancer alone partly silences TAAR gene choice, while deletion of both enhancers completely silences TAAR gene choice in *cis*. Unlike typical OR enhancers, the TAAR enhancers are share common, evolutionarily conserved motifs including homeodomain binding sites that are known to promote OR expression. We are currently studying the effects of motif mutations in mice, and identifying additional transcription factors that interact with the enhancers to mediate TAAR gene choice. Our data show that the mechanism of singular TAAR expression shares some similarities and some unique features compared with the canonical ORs, and provide a unique model for studying mechanisms of olfactory monoallelic expression.

442 **Investigating The Role Of The Toggle Switch Motif In Odorant Receptor Proteins**

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Odorant receptor (OR) proteins are a substantial part of the human proteome (4%) and constitute the largest subgroup of the class A G-Protein Coupled Receptor (GPCR) family. Despite this abundance and the crucial role that these receptors play in chemosensation, there is little known about the mechanisms that facilitate receptor activation and signal transduction. Motifs that play significant roles in activation have been elucidated via sequence alignments with GPCRs followed by rigorous in vitro experimentation. One motif of high import in GPCRs is the 'toggle switch' (canonically found to be CWxP in non-olfactory GPCRs) which is indicated to play a key role in agonist sensing and triggering receptor activation. Previous studies have identified the corresponding toggle switch motif in mammalian ORs to be FYG. It is known that non-olfactory GPCRs are significantly more sensitive and specific relative to ORs and we hypothesize that this substitution of the CWxP motif for FYG helps afford ORs this generalizability. From a consensus human OR, we performed site directed mutagenesis to generate mutant ORs with toggle switch motifs of species that have intermediate motifs between FYG and CWxP. We then performed luciferase assays to test the influence of the motif on receptor activation upon odorant stimulation. The results indicate that a subset of the mutant receptors with intermediate toggle switch motifs are activated at lower odorant concentrations relative to the consensus OR. In comparison, a subset of the mutant receptors derived from zebrafish sequences that contain motifs similar to FYG demonstrate similar activation patterns to the consensus. Together, these results allow the identification of the role of these different amino acid positions in agonist sensing and receptor activation for ORs.

443 **Foxj1, The Master Regulator Of Motile Ciliogenesis, Is Essential For Building A Functional Olfactory Sensory Organ In Mammals**

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In vertebrate animals, olfactory sensory neurons (OSNs) are multiciliated cells (MCCs). Cilia harboring all necessary molecular machinery for odor detection are indispensable for olfaction. However, the regulatory pathway(s) controlling their genesis and molecular composition are understudied. Cilia on OSNs share the basic ‘’9+2’’ axonemal morphology with motile cilia on MCCs from other tissues (ependyma, respiratory and reproductive tracts), yet they lack motility. Foxj1 is the master transcriptional regulator of motile ciliation, and it was reported in some mammalian OSNs. In our study, using an established antibody, we report for the first time, the presence of the Foxj1 protein in nuclei of mature OSNs of the adult mouse, and intriguingly, at a ca.10-fold lower level than in respiratory MCCs that bear motile cilia. A knock-out (KO) mouse with disrupted *Foxj1* revealed a ca.4-fold decrease of immature and mature OSNs which were de-ciliated. Loss of Foxj1, however, did not affect the vomeronasal organ, which is populated with non-ciliary microvillous OSNs. In *Foxj1* KO animals, nearly half of OSNs, compared to less than 5% in the WT, were positive both for OMP and GAP43 suggesting alteration in the neuronal maturation program. Another hallmark of the Foxj1 phenotype was the severely distorted distribution of proliferating Ki67-positive cells and more than 5-fold increase of cleaved Cas3 apoptotic cells. Importantly, the entire nasal cavity of the KO mouse was congested with Ki67-positive cells. Finally, the severe loss of OSN cilia and the reduction of OSN number in *Foxj1* KO impacted axonal targeting and arborization in the glomerular layer of the olfactory bulb. Our results underscore the importance of understanding ciliary biology in OSN MCCs, and thus the etiology of olfactory sensory deficits in patients with ciliopathies.

444 **Gene Therapeutic Reversal Of Ciliary Lipid Remodeling Rescues Olfactory Dysfunction In Bardet-Biedl-Syndrome**

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Ciliopathies are a class of genetic diseases resulting in loss of cilia in multiple organ systems, including the olfactory system, and for which there are currently no curative treatments. A critical barrier to treatment is the lack of understanding of the underlying mechanisms of cilia loss. Olfactory sensory neuron (OSN) cilia are microtubule-based, actin-exclusive organelles that are critical in transducing odorant signals. Loss or shortening of olfactory cilia, as seen in multiple mouse models of the ciliopathy Bardet-Biedl-Syndrome (BBS), exhibit impairment or loss of odor detection. However, the underlying mechanism of the reduction of olfactory cilia is unknown. Here, we demonstrated that PI(4,5)P2 (phosphatidylinositol 4,5-bisphosphate), a phosphoinositide typically excluded from olfactory cilia, aberrantly redistributed into the residual cilia of BBS mouse models, which caused F-actin ciliary infiltration. Importantly, ciliary PI(4,5)P2 remodeling and F-actin infiltration were necessary for olfactory cilia shortening in BBS. Furthermore, utilizing a gene therapeutic approach, the hydrolyzation of PI(4,5)P2 by overexpression of INPP5E restored cilia length, rescued odor detection and odor perception in BBS, suggesting that INPP5E is a potential treatment for olfactory dysfunction in BBS. Together, our data indicate that PI(4,5)P2 and F-actin-dependent cilia disassembly is a common mechanism contributing to

the loss of olfactory cilia in BBS and suggest pan therapeutic targets for treating olfactory dysfunction and ciliary dysfunction in multiple organ systems.

445 **Microstructural Analysis Of Water And Sucrose Consumption In A Large Panel Of Mouse Strains Derived From C57Bl/6J And Dba/2J Parental Strains.**

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The analysis of fluid licking in mice allows investigation of the environmental, genetic, and neural controls of ingestive behavior. Licking is a rhythmic behavior controlled by a central pattern generator, in which the interlick intervals, or ILI, are normally distributed. Licks are grouped into bursts (i.e., 3+ licks separated by ILI of <1 s) separated by longer pauses. We examined the burst-pause structure of 20-min sessions of fluid licking of water and 0.1 M sucrose in 516 mice representative of 71 isogenic mouse strains, including C57BL/6J (B6), DBA/2J, 63 BXD RI strains, 3 congenic strains, and 2 F1 hybrids. Across all strains, the MPI (mean ILI <160 ms) averaged 111.0 ms for water and 111.6 ms for sucrose. The MPI for the B6 parental strain was near the all-strain average, whereas D2 was the fastest-licking strain. Strains varied in the number of licks generated (water: 262-1280; sucrose: 536-2141). Strains also varied in number of lick bursts (range: 5.8-54.3) and in average burst size (range: 23.0-104.8). These parameters were strongly correlated across water and sucrose sessions. Within bursts, some strains licked highly reliably, whereas others did not. This was quantified this as lick efficiency (i.e., % of ILI <160 ms). Strains varied in this parameter (range: 0.67-0.95). We also developed an ingestive-style index (ISI) that is independent of total meal size which quantifies the degree to which mice favor short, frequent bursts of licking (negative ISI) or long, infrequent bursts (positive ISI). The ISI was strongly correlated between water and sucrose sessions (r = 0.65) with considerable variation across strains (range: -0.47-+0.61). Strain data from these phenotypes will be compared with existing BXD sequence, transcriptome and phenotype data sets at GeneNetwork.com.

446 **Individual Differences In The Taste Of Ibuprofen-Containing Pediatric Medicine**

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Objective: To objectively and systematically determine the degree of individual variation in sensory experiences upon tasting and following swallowing a liquid pediatric formulation of ibuprofen (Motrin™). Methods. Trained adult panelists (n = 154) used validated psychophysical tools (general Labelled Magnitude Scale) to rate the taste and irritation intensities and to indicate the types of sensations (i.e., burning, tingling, stinging, numbing, cooling, scratching, urge to cough, urge to sneeze) experienced under three conditions: after tasting but not swallowing Motrin™ , immediately after swallowing it, and then after a 5-minute delay. Results. As a group, panelists gave higher irritation ratings and were more likely to experience tingling and scratching sensations and have the urge to cough after swallowing Motrin™ than after the sip-and-spit condition (p <0.001). At the individual level, the higher the irritating ratings, the more bitter (r=0.38; p <0.001) and less palatable (r=-0.41; p <0.001). Ratings of irritation after tasting Motrin™ predicted ratings immediately after (r= 0.54; p <0.001) and 5 minutes (r= 0.46; p <0.001) post swallowing. Conclusions. The highly personal nature of the chemical senses underlies why a given medicine is not accepted by every patient. Whether there is a genetic basis for personal variation in sensory experiences of ibuprofen and whether the results from taste-screening of medicines by adult panelists are transferable to pediatric patients are the goals of our research program.

Saturday, April 23, 2022

7:30 - 9:00 AM	Estero Foyer
Continental Breakfast	

8:00 - 10:00 AM	Estero Ballroom
Poster Session V	

500 **Functional Connectivity Reveals The Importance Of The Interaction Between Gender And Apoe Status For Odor Memory In Alzheimer&Rsquo;S Disease**

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Males perform more poorly than females in odor identification tasks over the lifespan; however, females are more vulnerable to Alzheimer's disease (AD). The apolipoprotein E (ApoE) ε4 allele increases risk for AD and females with the allele are show more olfactory dysfunction than those without. Recent research using functional MRI indicated that ε4+ males performing an odor memory task showed hyperactivation, brain activity which is neurotoxic (Kapoulea & Murphy, 2020). Here we used archival data to investigate whether cognitively normal males and females with and without the allele showed differential functional connectivity both within and outside the olfactory network when performing an odor memory task. Participants were non-demented adults, 65 and older, who had been genotyped for ApoE. Subjects with an ε2 allele (protective against AD) were excluded. There were approximately equal numbers of males and females and ε4+ and ε4- subjects. They were scanned at 3T while performing an odor memory task, indicating whether an odor had been previously presented. Functional connectivity was examined for a key variable: when subjects correctly indicated memory for an odor previously presented. Functional connectivity was examined with the seed voxel method, seeding regions in the hippocampus and perirhinal cortex. Results indicated that the interaction between gender and ApoE status significantly influenced connectivity between these regions and processing areas critical for the task. Males with and without the allele and females without the allele displayed differential connectivity from that of females with the ε4 allele. The implications of this for the vulnerability to Alzheimer's disease will be discussed.

501 **Dominance Communication Via Scent: Does Testosterone Play A Role In Odor-Based Perceptions Of Personality Traits?**

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The current research provides a first ever look at whether scent cues associated with testosterone influence perceptions of dominance and big five personality traits. Males (N=73) provided salivary testosterone, self-ratings of personality traits, and body odor samples. Females (N=290) smelled body odors and provided ratings of dominance, big five personality traits, and odor quality. As anticipated, testosterone predicted higher odor-based perceptions of dominance (r = .29, p = .012) and this relationship persisted even when controlling for odor quality ratings (i.e., pleasantness, sexiness, intensity; β = .17, p = .009). Higher testosterone levels also predicted odor-based perceptions of one of the five big five personality traits: neuroticism (r = .29, p = .013), but this result became marginal when controlling for odor quality variables (β = -.13, p = .09). Contrary to previous work, no significant correlations emerged between odor-based ratings and target's self-ratings of dominance or any of the big five personality traits (p's > .10). Together, these results suggest that odor cues associated with testosterone communicate important personality information, and odors of men with higher testosterone may signal a rather unfavorable personality profile (i.e., high dominance and perhaps higher neuroticism). These odor-based perceptions may ultimately translate to altered behaviors towards men with high testosterone (e.g., interpersonal avoidance or, when contact is required, favorable treatment to avoid conflicts). Thus, scent cues associated with testosterone could be an overlooked modality through which information about status, hierarchy, and personality are communicated.

502 **Olfaction, Cognitive States, Mortality, And Life Expectancies: A Multistate Survival Analysis**

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This project aimed to investigate the extent to which olfactory ability, measured by a 12-item smell identification

test, predicts transitions between clinically diagnosed cognitive states and death, as well as the degree to which olfaction is associated with cognitively unimpaired and total life expectancies in midlife to older adulthood (*N*=1501; 74% female). METHODS: Multi-state survival models (MSM) estimated the association of baseline olfaction on transition patterns through cognitive states (no cognitive impairment [NCI], mild cognitive impairment [MCI], dementia) and death. To estimate cognitively unimpaired and total life expectancies, multinomial regression models were fit using the hazard ratios (HRs) from the MSM's. RESULTS: Higher olfactory test scores were associated with a lower risk of transitioning from NCI to MCI (HR=0.86, 95% confidence interval 0.82-0.88) and from MCI to dementia (HR=0.89, 0.86-0.93). Additionally, better olfactory ability was associated with a greater likelihood of transitioning backwards from MCI to NCI (HR=1.07, 1.02-1.12). The MSMs suggest that the direct association between olfaction and mortality was not statistically significant after accounting for transitions through cognitive states. Higher olfactory test scores were associated with up to 6 additional years free of cognitive impairment, as well as 5 additional years of lifespan, compared to individuals with low olfaction. CONCLUSION: These findings suggest that higher olfactory ability is associated with a decreased risk of progressing forward through cognitive impairment, and that the association between olfaction and mortality likely occurs primarily through the pathway of neurodegeneration. These analyses highlight the differential role of olfaction as a risk factor for changes across cognitive states

503 **The Relationship Between Olfactory Dysfunction And Cognition After A Mild Traumatic Brain Injury**

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Introduction: Olfactory dysfunction (OD) is a frequent consequence of traumatic brain injury (TBI), even after a mild TBI. We recently found that OD could predict affective symptoms such as anxiety and depression in a population with mild TBI. This study aims to investigate the relationship between OD and cognition after a mild TBI. Methods: A total of 53 patients between 18 to 56 years of age were evaluated in the acute phase of a single mild TBI. Among them, 12 (22.6%) exhibited signs of OD based on the Sniffin' Sticks results. We compared them with those without OD. Then, we assessed them with the EXACT (EXAmen Cognitif abrégé en Traumatologie), a brief cognitive test to evaluate global cognitive functioning following a TBI. Results: We did not find a significant difference in EXACT scores between patients with and without OD. Moreover, OD and global cognitive functioning did not statistically correlate. Conclusion: While olfactory evaluation can be a screening tool for affective symptoms, our study does not suggest that OD can be used to predict cognitive symptoms following a mild TBI.

504 **Don Tucker Finalist: Preference For Fat As A Function Of Obesity In Lfabp -/- And Wt Mice**

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Introduction: The liver fatty acid binding protein knockout (LFABP -/-) mouse model is hyperphagic on a high fat diet, which may be due to increased gut levels of both major endocannabinoids, 2-AG and AEA, described in previous work. Similarly, obesity has been correlated with taste insensitivity in rodents and humans, leading to greater consumption of calorically dense foods. Aim: Our objective was to understand if LFABP -/- mice display a higher preference for a high fat, high palatable food over Wild-type (WT) mice and if this preference is independent from body fat percentage (BF%). In other words, is obesity driving preference or is it genotype specific? Methodology: 6 mice (3 WT and 3 LFABP -/-) were given access to a high-fat cake frosting (Crisco/sugar mixture) for 5 minutes and frosting intake was recorded. This was done at the same time each day and repeated 3 times. BF% was measured by the average of two EchoMRI readings for each animal. Results: Our preliminary results showed that LFABP -/- mice consumed more high-fat frosting during a brief access test and a strong positive correlation ($R^2 = 0.736$) between BF% and average high-fat frosting intake, regardless of genotype. Conclusion: These results suggest that the underpinnings of obesity are influencing fat liking and intake, rather than the LFABP -/- genotype. This has important implications for the overconsumption of highly palatable food in our society. Future: The preliminary results from this aim have been used to inform human subject testing. Future work will build on these preference tests using different ratios of fat:sugar frostings to determine whether preference for fatty foods changes as a function of body fat percentage. Understanding how adiposity affects taste function will help guide us to better therapies for obesity.

505 **Early Life Adversity And Altered Hedonic Interest In Odorants: Neurobiological Substrates**

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Early life adversity (ELA) significantly increases the later risk for depression. In humans, depression has been associated with disturbances in the hedonic perception of odorants. As olfaction serves a primary role in social interactions, food intake, and daily pleasures, altered olfactory perception and hedonic processing of chemosensory cues may provide insights into basic neurobiological disturbance in ELA-associated pathology as well as identify key pathways that can impact on quality of life, contributing to the development and severity of depressive pathology. Here, we used the limited bedding and nesting protocol in mice to model ELA in the form

of fragmented maternal care. The aim of this study was to determine whether ELA altered the hedonic processing of odorants, and to identify neural substrates underlying those effects. We found that ELA led to a specific reduction in the investigation of pleasant odorants. Using cellular imaging and fine structural analysis of neurons, we found altered morphology of the neurons responding specifically to pleasant odorants in the olfactory bulb, and an altered activity in the olfactory tubercle and ventral tegmental area, key structures implicated in the hedonic coding of odorants. These results highlight potential impacts of ELA on network development and response to exogenous signals, diminishing interest in hedonically pleasant odorants. The current results point to ELA altering the neural substrates implicated in basic olfactory sensory function and reward, with implications for understanding broader risk for anhedonia and symptom severity in individuals at risk for depression associated with prior history of ELA.

506 **A Simplified Method To Measure The Pattern Of Individual Sweet Hedonic Responses**

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The appeal of sweetness is a driver of sugar consumption and understanding individual differences in its appeal may help develop personalized strategies to lower added sugar intake. Current methods to quantify sweet hedonic patterns involve a range of stimulus concentrations and numerous judgements, so there is a need to simplify the testing procedure so that it can be used in large-scale population-based studies, e.g., *NHANEs* or NIH's *All of Us*. Therefore, we measured the hedonic response in 21 adult participants. In the first method, they rated five sucrose solutions (0.09, 0.18, 0.35, 0.70, 1.05 M) using a rating of liking (on a 100-point visual analog scale) with a paired comparison preference tracking procedure. Based on their ratings of all solutions, participants were classified into three sweet liking patterns (Disliker, Moderate Liker, and Extreme Liker) using a quadratic function fit. Using ordinal logistic regression to predict classification, we found that the individual slopes calculated using two of the solutions (0.09 and 1.05 M sucrose) could predict the patterns with a 76% accuracy (X^2 = 19.11 (4, 21), p <0.001), but using one single concentration (1.05 M) could not (43% accuracy, X^2 = 7.96 (4, 21), p = 0.093). Furthermore, the individual slopes from the ratings of the same two solutions could predict the preferred concentration from the preference tracking procedure (r^2 = 0.77, p <0.001). Overall, using data from two concentrations could provide similar hedonic information as five concentrations. Lager studies are the next step to validate this short test.

507 ***Laboratory-Supervised Versus Unsupervised Home Testing To Understand Person-To-Person Differences In Taste, Smell, And Chemesthesis Perception***

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Sensory perceptions determine in part what we eat and drink, which in turn impacts our health. To understand sensory-based nutrition as a whole, we need to understand person-to-person differences in taste, smell, and chemesthesis on an individual basis. Therefore, we developed the Monell Flavor Quiz (MFQ) to comprehensively enable objective measurement of an individual's chemosensation in their own home. The objective of the study was to test the reliability of a self-administered and unsupervised test versus an in-person assessment with a researcher present. Participants (N=16) rated the overall liking, liking, and intensity liking (e.g., how much they like the intensity) for four taste stimuli (sucralose, NaCl, citric acid, and PTC), three chemesthetic stimuli (nasal: alcohol; oral: menthol and capsaicin), and six smell stimuli (isoamyl acetate, coffee, tert-butyl mercaptan, trimethylamine, phenyl ethyl alcohol, and water), both at home and in-person at Monell (counter-balanced design). Both testing environments demonstrate similar test-retest reliability (Home: R^2 = 0.45; Monell: R^2 = 0.56; Z_{obs} = 0.36), however, significantly lower ratings ($F_{1, 359}$ = 10.836; p = 0.001) and greater variance was observed in the unsupervised home environment compare to in-person testing at Monell ($F_{746, 449}$ = 1.35; p = 0.001). More research is needed to better understand the resulting impact of differential variation due to the environment on the data collected. The results demonstrate the complexity and value of comprehensive testing for understanding individuals' sensorial experiences. The development of an inclusive tool such as the MFQ with remote capabilities is valuable for clinicians and researchers working to understand individual differences in chemosensory modalities.

508 **Food Cravings Are Mediated By Sensory Imagery In Reward Sensitive Adults**

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Reward sensitive individuals show increased cravings in the presence of external food cues, thus putting them at risk for overeating. Empirical data also suggest that craving can be induced experimentally by creating vivid mental (sensory) imagery of the appetitive target. However, if high reward sensitivity exacerbates craving frequency by triggering a shift in sensory imagery processes is unclear. Using cross-sectional self-reports, we investigated if reward sensitive adults show greater food cravings and if this relationship is mediated by the ability to create vivid mental images of odors and foods. Our participants (n=169, mean body mass index 26.48 kg/m2) recorded reward sensitivity with Sensitivity to Reward and craving frequency with the Food Craving Index Questionnaire. The vividness of mental imagery for odors and foods was determined by the Vividness of Olfactory Imagery Questionnaire (VOIQ) and the Vividness of Food Imagery Questionnaire (VFIQ), respectively. VOIQ scores positively correlated with VFIQ (P<0.001), suggesting similarities in imagery modalities. Higher reward sensitivity score predicted greater food craving frequency (P<0.001). Further, adults with higher reward sensitivity reported a greater perceived ability to form vivid mental images for both odors and foods (VOIQ P<0.001; VFIQ P=0.01). The overall total indirect effect of reward sensitivity and food craving

frequency via the mediating variables (i.e., the vividness of imagery scores of odors and foods combined) was significant (P<0.001). Our findings suggest that the greater perceived ability to form vivid mental images of odors and foods influence food cravings in reward sensitive adults. Interventions to reduce vividness of image may be beneficial to prevent cravings potential overeating in reward-sensitive adults.

509 **Chemosensory Rich Electronic Cigarette Additives Enhance Nicotine Sampling And Exposure Rather Than Reward**

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Adolescent use of nicotine-containing electronic cigarettes (e-cigs) has dramatically increased in recent years. Along with nicotine, e-cigs often possess additional additives, with fruit additives being the most popular among adolescents. These additives are a primary reason for e-cig experimentation (*i.e*. vaping) in young people and have been shown to potentiate nicotine reward and reinforcement in both humans and rodents. Less is known, however, about the chemosensory contributions of e-cig additives on nicotine use. Notably, the volatile odors of e-cigs are of particular importance to the sensory perception and enjoyment of vaping. Here, we first examined how the popular e-cig additive, strawberry, influences nicotine reward and aversion utilizing a conditioned place preference/aversion (CPP/CPA) paradigm wherein stimuli were presented as a vapor. While nicotine vapor alone dose-dependently produced both CPP and CPA, the addition of a strawberry additive did not enhance nicotine CPP. However, mice exposed to nicotine + strawberry vapor had higher plasma cotinine concentrations than nicotine only mice. We then examined how adolescent mice sample e-cig odors by analyzing their odor-evoked respiration acquired in an unrestrained whole-body plethysmograph. While mice investigated all stimuli through sniffing and could readily discern between stimuli, they spent more time in high frequency, investigatory sniffing when presented with the odor of strawberry or nicotine + strawberry compared to nicotine alone. Together, these data suggest that the odors of e-cig additives may alter the perceived sensory profile of nicotine vapors rather than the reward value, ultimately leading to increased nicotine consumption. Ongoing work aims to explore the role of tubular striatum dopamine receptors in this effect.

510 **Mothers&Rsquo; Dietary Habits Predict Their Daughters&Rsquo; Habits, But Not Their Sons**

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Childhood is a critical period in the acquisition of long-term food preferences. Mothers often are the gatekeepers and their own beliefs and practices impact what they feed their children. In this cross-sectional study, we aimed to determine whether the sex of the child (n=155; 3-10 y) affected the concordance in intakes and liking of foods rich in added sugar and protein with that of their mothers. Several dietary measures were obtained: 24-hr diet recalls to determine % daily kcal from added sugar and protein; pediatric-adapted/adult liking surveys to assess variation in liking of these foods; healthy eating index (HEI) to assess how dietary patterns align with recommendations; and level of sucrose most preferred as determined from forced-choice, paired-comparison tracking procedure. More than half of mothers' (62%) and children's (57%) intake of added sugar exceeded recommendations (≤10% daily kcal). The more mothers reported liking sweet-tasting manufactured foods, the more added sugar in their diet (P=0.002) and the higher sucrose concentration most preferred (P=0.008), validating that the individual differences in diet were sensory based. The % daily kcal from added sugar was negatively correlated with the % kcal from protein (P=0.001) and overall quality of mothers' diet, as indicated by HEI (P=0.001). While similar relationships were seen among children's diets, the relationship between mothers' HEI scores and the % daily energy from added sugar and protein positively correlated with that of their daughters only (P's<0.03), while maternal liking of savory foods correlated with that of their sons (P<0.001). These findings suggest that sex is a powerful determinant in what mothers feed their children which in turn provides the foundation for later sex-differences in health and disease.

511 **Early-Life Exposure To A Non-Nutritive Sweetener Increases Unconditioned Licking For Fructose And Quinine And Decreases *Tas1R3* Expression In Adult Food-Restricted Male And Female Rats.**

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Non-nutritive sweeteners (NNS) are touted as healthy sugar alternatives, but their long-term impacts remain to be explored. Here we assessed if early-life exposure to the NNS acesulfame potassium (AceK) affects "sweet" receptor expression and brief-access licking of a representative sugar, fructose, and bitterant, quinine, in adulthood. In 2 cohorts, Sprague-Dawley rats (n=24, equal sexes) were evenly distributed into 2 groups that from postnatal day (PND) 26-77 either received daily home-cage 0.1% AceK (15ml/kg) access (group NNS) or not (group CNTL). At >PND220, the rats were trained and tested to lick water and a concentration array of first fructose (0.6-0.03M) and then quinine (1.0-0.01mM) presented one at a time in 10-s trials across 30-min sessions. When 23-h food restricted, NNS rats licked high concentrations of fructose more avidly than CNTL

rats; this difference did not persist when the rats were tested while ad libitum fed. When 23-h water restricted, NNS rats licked more quinine overall than did CNTL rats. The lingual epithelium containing the circumvallate papillae was extracted from the rats of the 2nd cohort (n=6/group) and was run through qPCR to quantify "sweet" receptor expression. *Tas1r3* mRNA was significantly reduced in food-restricted NNS rats, compared to CNTL rats. Collectively, the findings suggest that early-life AceK exposure increases hedonic-based adult fructose responsiveness under food-restricted conditions and decreases quinine avoidance under water-restricted conditions. Changes in taste-based behaviors to fructose, may be due, at least in part, to altered sweet receptor expression at the taste bud level. This suggests that NNS intake during a critical developmental phase can have lasting effects on the peripheral and behavioral aspects of taste function.

512 **The Impact Of Incidental Taste Exposure On Learning-Related Cortical Responses**

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Experience has been widely shown to impact learning and sensory perception. In conditioned taste aversion (CTA), an animal learns to avoid a taste that has previously been paired with malaise. Experience, or familiarity with taste stimuli that later become the conditioned stimulus (CS) has long been known to reduce the strength of aversion learning (Lubow and Moore 1959; Lubow 1973; De La Casa and Lubow 1995). Recently, we demonstrated that even taste exposure (TE) with "incidental" (i.e., non-CS) stimuli (such as salty and sour tastes) can influence later learning towards novel stimuli, albeit in the opposite direction of CS pre-exposure. Specifically, exposure to salty and sour tastes strengthens a later CTA learning to novel sucrose (Flores 2016; Flores 2018). One potential interpretation of these results is that TE changes the neural dynamics underlying the processing of new tastes in GC. Recent work using *in-vivo* electrophysiology in female Long Evans rats (Flores 2021) has confirmed that TE increases the discriminability of GC ensemble and single-unit responses to different tastes. Here, we evaluate how this increased discriminability after TE changes GC firing rate dynamics in response to a novel taste before and after CTA learning and how these changes may track behavior in Long Evans Rats. We hypothesize that increased discriminability will correlate with the strength of CTA performance. These findings support the notion that unlike what current models of learning portray, even a simple yet omnipresent incidental exposure can have a lasting impact on learning and neural processing.

513 **Achems Undergrad Award Finalist: Mice Condition Cephalic-Phase Insulin Release To The Flavor Of Maltodextrin, But Not Saccharin Or Sucrose**

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Oral stimulation with glucose elicits cephalic-phase insulin release (CPIR) in mice. Glucose-containing saccharides also elicit CPIR once the glucose molecule has been liberated by amylases and alpha-glucosidases in the mouth. CPIR is beneficial because it attenuates postprandial hyperglycemia. Here, we asked whether C57BL/6 mice can condition CPIR to flavors that do not normally elicit CPIR—i.e., saccharin or glucose-containing saccharides mixed with acarbose (an inhibitor of amylases and alpha-glucosidases). During conditioning, we offered mice 1-hr or 23-hr access to flavored glucose solutions across five days. During CPIR testing, we measured immediate changes in plasma insulin after mice completed 200 licks for a flavorant. In Experiment 1, mice ingested 30 mM saccharin while 1 M glucose was co-infused via an implanted intragastric catheter. This procedure failed to condition CPIR to the flavor of saccharin. In Experiment 2, mice ingested a mixture of 30 mM saccharin + 1 M glucose during conditioning, but failed show a CPIR to the flavor of saccharin. In Experiment 3, mice ingested 1 M sucrose during conditioning, but did not display a CPIR to the flavor of 1 M sucrose + 5 mM acarbose. In Experiment 4, mice ingested 32% maltodextrin during conditioning, and displayed a CPIR to the flavor of 32% maltodextrin + 5 mM acarbose. In Experiment 5, we added a Kool-Aid flavor to the 32% maltodextrin to determine whether increasing flavor intensity would enhance the conditioning process. The Kool-Aid did not cause any enhancement. Our results show that mice can condition CPIR to the flavor of maltodextrin but not sucrose or saccharin. Based on the selectivity of the conditioning process, we propose that it reflects a specific adaptation for tolerating diets rich in complex carbohydrates.

514 **Somatostatin Expressing Cells Of Central Amygdala Projecting To The Nucleus Of Solitary Tract, Parabrachial Nucleus, And The Lateral Hypothalamus Are Distinct Cell Populations**

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The nucleus of solitary tract (NST) and parabrachial nucleus (PBN) represent the first and second central synapses of ascending gustatory information. Neural processing in these nuclei is influenced by descending input from forebrain regions such as the central nucleus of the amygdala (CeA) and the lateral hypothalamus (LH). In mice, we have shown that somatostatin (Sst) expressing neurons of CeA that project to NST and PBN are largely distinct cell populations and optogenetic inhibition of the CeA/Sst-to-NST subpopulation increases the intake of high concentrations of quinine with no apparent effect on sucrose intake. Given the interconnectivity between LH and CeA, one possibility is that increased quinine intake was not solely due to silencing the CeA/Sst-to-NST neurons but also involved collaterals to LH neurons, which in turn project to NST. To answer this question, we injected retrograde viral tracers into the NST (HSV-DIO-EYFP) and LH (HSV-DIO-mCherry) of Sst-cre mice. For comparison, we performed additional experiments with injections into LH (HSV-DIO-mCherry) and PBN (HSV-DIO-EYFP). Quantitative analysis of retrograde-labelled cells in CeA showed that CeA/Sst cells projecting to NST or PBN are largely distinct from those projecting to LH. These results indicate that the

increased intake of quinine we observed during optogenetic studies did not involve nonspecific manipulation of LH-to-NST neurons but rather specific inhibition of the CeA/Sst-to-NST pathway.

515 **Anatomical And Functional Dissection Of The Anterior Olfactory Nucleus To Nucleus Of Lateral Olfactory Tract Pathway In Mice**

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The olfactory bulb projects to multiple olfactory cortical areas including the piriform cortex and anterior olfactory nucleus (AON), which play distinct roles in odor-guided behaviors. The function(s) of the AON and its connected brain regions are not well established. In order to gain genetic access to the AON neurons, we wished to identify a molecular marker for these neurons through a differential gene expression search in the Allen Brain Atlas. This search led to the identification of the neuromedin B receptor (NMBR) gene as the top candidate that is highly expressed in the AON compared to the rest of the brain. Using the CRISPR-Cas9 gene-editing approach, we generated an NMBR-Cre knock in mouse line. Anatomical tracing from the AON neurons revealed specific projection to the nucleus of lateral olfactory tract (NLOT), part of the cortical pallial amygdala. In addition, whole-cell patch clamp recordings combined with optogenetic activation showed that the AON/TT neurons make monosynaptic and polysynaptic connections onto NLOT neurons. Furthermore, in vivo fiber photometry revealed odor and/or sniff induced calcium signal elevation in the AON neuron axonal terminals in the NLOT of freely behaving mice. Finally, ablation of excitatory neurons in the NLOT not only impaired olfactory guided food search and social discrimination but also disrupted aversive behavior to a synthetic predator odor. Taken together, these results indicate that the AON/TTàNLOT pathway plays a critical role in olfactory-guided behaviors.

516 **Preservation Of Sniffing-Elicited Olfactory Networks In Patients With Congenital Anosmia**

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Sniffing, as a natural mean to efficiently sample odorants, has been shown to activate the olfactory cortex, even when sniffing odorless air. Some patients with hyposmia/anosmia regain their olfaction by sniffing odors regularly as an olfactory training, in which the role of sniffing is still unsettled. To clarify this, we recruited congenitally anosmic patients (CA) to highlight the difference in sniffing-induced brain networks as compared to normal subjects (NS). We recruited 9 patients in CA group and age- and sex-matched 15 volunteers in NS group (29.9 ±6.2 years old). Functional MRI with a block-designed sniffing paradigm was undertaken. Upon group-level analysis, CA group shared a spatially similar pattern but less intensive activation than NS, although the between-group contrast displayed no significant voxel surviving. In independent component (IC) analysis, it yielded 34 ICs. By sorting with the temporal relevance to sniffing task, we can further identify primary/secondary olfactory network, olfactomotor, somatosensory and integrative networks. After a between-group analysis of the ICs, we did not find any evidence of compensatory network activation in CA. On the contrary, the NS presented a more intensive activation in the integrative network and in another temporally irrelevant somatosensory network. This finding reflected that NS may take advantage of an integrative network activated by sniffing, during which the central processing of olfaction was enhanced. It highlighted the pertinent role of signal integration and network crosstalk in sniffing. It may also imply that through the stimulation of the odors and the repeating acts of sniffing, olfactory training can improve or rebuild the olfactory integrative network and ultimately achieve restoration of olfactory functionality.

517 **Neural Networks Involved In Olfaction - Food Intake Interactions : Role Of The Hypothalamus In Odor Processing**

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Body weight of most animals is remarkably stable over time, suggesting that a complex physiological system balances food intake and energy expenditure over the long term. This homeostatic control system is the Hypothalamus - and more precisely the arcuate nucleus (ARC) of the hypothalamus. By integrating internal (hormones) as well as external (sensory cues) feeding signals, the ARC regulates homeostasis and controls eating behaviour. It contains 2 populations of neurons, Agouti-related peptide (AgRP) and pro-opiomelanocortin (POMC) neurons, whose activity is respectively modulated by hunger and satiety hormones such as ghrelin and leptin. In addition, a recent study showed that the activity of AgRP neurons was also modulated by food odors, since the presentation of palatable but hidden food leads to a clear decrease in the activity of these neurons (Betley et al., 2015, Chen et al., 2015). The sense of smell is, indeed, a central driver of food-seeking, appetite, and food preference in vertebrates. However, the functional connection between AgRP neurons and the Olfactory Bulb (OB) - first cortical relay of the olfactory system- remains unexplored. The aim of this study is to determine whether hypothalamic AgRP neurons modulate odor processing in the OB. In order to investigate this question, AgRP DTR mice were used. In this transgenic mouse model, perinatal ablation of AgRP neurons is achieved by toxin-mediated ablation of AgRP neurons in the first week after birth (Luquet et al., 2005). Control mice and mice lacking AgRP neurons were then compared and subjected to multiple behavioural Tests. Our data show that mice with AgRP neuron’s ablation tend to have different Olfactory behaviour, suggesting that beside regulating food intake, hypothalamic AgRP neurons impact OB-mediated food-odor processing.

518 **Neural Associations Between Well-Being And Odor Perception**

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Objective- We investigated (1) neural processing underlying olfactory perception in healthy people with distinct 'levels' of well-being (WB) (2) central-nervous processing of odors associated with various degrees of WB. Methods- The experiment included pre-testing and fMRI scans. During pre-testing 100 subjects rated intensity, valence and WB for 14 pleasant odors. This resulted in selection of two odors (flower+ orange) strongly and two odors (grass+ coffee) weakly associated to WB which were then delivered to subjects with different WB state (high and low) in MRI. Results- In presence of odors strongly associated to WB, low WB group had increased bilateral angular gyrus (AG), left inferior frontal gyrus (IFG) activity for ON>OFF. Posterior orbitofrontal cortex (OFC) and bilateral IFG activated for ON (weakly associated odors) > OFF in the low WB. Left lateral OFC activated in the high WB group only in presence of strongly WB odors. For high vs low WB groups, low WB showed stronger activity in the right AG in presence of strongly WB odors whereas no voxel survived for weakly odors. Conclusion- This was possibly because low WB group was more sensitive to odors that added an emotional value and meaning to them. Overall, odors may play an important role in lifting mood or altering emotional state especially in people with low WB.

519 **Extracting Positional Information In An Odor-Guided Droplet Reach Task**

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Olfaction acts as a remotely-guiding sense used by an organism to navigate towards or away from a food source, mate, or survival cue. A growing body of literature has uncovered how spatial information of an odor could be mapped across various regions in the brain. However, it is not well known how odor source localization is represented in the region responsible for maintaining precise and predictive motor control, the cerebellar cortex. Here we have built and implemented a droplet reach task to track mouse limb and nostril kinematics as well as sniff rate over the course of a reach. Trained, head-fixed, water-deprived mice reached consistently for a scented droplet (ethyl butyrate) presented 1-2 mm from the tip of the nose and varied across one of 3 randomized positions (0, 30, 60 degrees from nose midline). We observed the angle of their nostrils and relative sniff frequency dynamically changing throughout a reach towards a scented target. Preliminary data suggest that nostril displacement coincides with the initiation of the reach onset, then is held to the matched direction of the droplet across the reach. These results imply shared motor dynamics where the nose is continuously updating olfactory information in concert with reaching. Future utilization of this task can answer questions of how various active sensing inputs (i.e., whisking vs. sniffing) can assist in fine motor discrimination and proper limb targeting. Upcoming experiments will focus on decoding neural transformations of sensorimotor information across the cerebellar, granule cell layer during odor-guided reaching as well as airflow modeling of nasal kinematics in parallel.

520 **Long Term *In Vivo* One-Photon Ca^{2+} Imaging Of Taste-Responsive Cells In The Parabrachial Pons In The Awake Freely Licking Rat.**

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The observation that cells at all levels of the taste pathway respond primarily to a single taste quality, constant over time, is the basis of labeled line theory. However, studies have shown that only a subset of cells in each neural structure are narrowly tuned. Further, tuning changes over time have been observed. Here, we used *in vivo* one-photon Ca^{2+} imaging of taste-responsive cells in the parabrachial nucleus of the pons (PbN) of awake, freely licking rats to verify and expand those findings. Rats were prepared for Ca^{2+} imaging by infusion of GCamp7s and implantation of a GRIN lens above the PbN. When recovered, rats were water-deprived and a miniscope (Inscopix, Inc.) was mounted above the GRIN lens. Rats were then placed in a chamber with a lick spout for delivery of taste stimuli (sucrose, NaCl, citric acid, quinine, MSG). Each trial consisted of 5 consecutive taste stimulus licks preceded and followed by 6 artificial saliva licks delivered on a VR5 schedule. Between 7-16 cells per rat were present across multiple sessions over 2-12 wks. Results showed that taste-responsive, lick bout and anti-lick cells could be identified in the PbN. For all taste cells, the best stimulus varied across sessions. While taste cells often responded to one stimulus consistently across sessions, that stimulus was not always their best stimulus. In some cells, the breadth of tuning was relatively stable across sessions but in others it waxed and waned systematically across sessions. Thus, on a given day, there was a subset of cells that were narrowly tuned to a single taste quality, but the identity of these cells was different across days. We suggest that these data reflect long term changes in input originating in the periphery. Further, these data point to a combinatorial view of taste coding in the PbN.

521 **Tensor Based Independent Component Analysis (Ica) Approach Towards Olfaction**

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Introduction: Brain activations for olfactory stimulations are less prominent as compared to other senses like visual and auditory. This has partly to do with how olfactory stimuli are presented to the participants. However, the way data are analysed also plays an important role when it comes to visualising these activations. General linear model (GLM) based approach is very common and has been extensively used and studied. Its dependence on the canonical haemodynamic response factor (HRF) requires careful interpretations. Model free tensor-ICA (independent component analysis) could help to better understand the data related to olfaction. Methods: Tensor-ICA, was used to analyse the functional data from 38 healthy participants. Four odors (peppermint, cherry, strawberry and spearmint) were presented to the participants via an olfactometer using a block design where 10 blocks comprised of 12s air and 8s odor were presented intranasally to both nostrils. Tensor-ICA was analysed using multi-variate linear decomposition (MELODIC) toolbox provided by FMRIB software library (FSLv6.02). 20 ICAs were used for decomposition of the data in spatial and temporal components. Results: We found that 6 out of 20 components were related to olfactory activations comprising areas like piriform cortex, orbitofrontal cortex, and entorhinal cortex. A matching time course with the olfactory stimulations could also be seen. Conclusions: Tensor-ICA allows a model-free decomposition of the variance in the olfactory signal, into different activation and artefactual components, including their spatial maps and time courses. The model-free Tensor-ICA validated that the olfactory paradigm was spatially and temporally associated with a functional network.

522 **Structural Plasticity Of Peripheral Taste Axons**

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Cell turnover in the taste bud requires taste neurons to form new connections with new sensory cells over time. Do taste neurons alter their structure to accommodate taste bud cell renewal? To address this question, we developed an *in vivo* two-photon microscopy approach that allows for the observation of a single taste nerve arbor (portion of the axon innervating taste buds) at multiple time points for up to 100 days. We found that the terminal branches of taste arbors continuously and rapidly remodel – adding or subtracting terminal branches every 5.5±0.8 hours. The speed of this structural plasticity is faster than predicted by rate of taste bud cell renewal. The inhibition of new taste bud cell entry into the taste bud using Hh-inhibitors did not impact the rate of terminal branch change (U=312, p=0.61), and even complete taste bud loss did not prevent terminal branches from remodeling. These findings indicate that taste nerve arbor remodeling is not regulated by taste bud cell renewal. We also observed that adult taste branch retraction was typically predicted by a retraction bulb and/or fiber blebbing (86%), indicating that retraction uses conserved mechanisms. Furthermore, the rapid remodeling of individual arbors was coupled with limited loss and no addition in the total number of arbors per taste bud. Thus, arbors per neuron is stable and possibly dictated by the taste neuron type. Taste neurons appear to cope with the unstable cellular environment associated with taste bud cell renewal by maintaining a stable number of nerve arbors that are each capable of high-speed remodeling. The concurrent stability and plasticity of taste neuron arbors may explain how taste neurons remain functionally stable despite the challenge associated with continuous taste bud cell renewal.

523 **Regional Peak Mucosal Cooling Predicts Treatment Outcomes Of Nasal Valve Obstruction**

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Remodeling narrow nasal valve may effectively treat nasal obstruction symptoms, yet its precise mechanism is not fully understood. In this study, 20 patients with internal nasal valve obstruction underwent in-office radiofrequency (RF) treatment (Aerin Medical, Inc), based on the concept that RF energy creates a submucosa thermal lesion, induces fibrosis during wound healing that may result in the nasal valve expansion. Under local anesthesia, a small probe topically delivers 18s of less than 60°C RF energy at up to 5 positions along the upper lateral nasal valve region. Patients' Nasal Obstruction Symptom Evaluation score improved significantly at 90 days post-treatment (NOSE: pre 78.89±11.57; post 31.39±18.30, P=5e-7). Computational fluid dynamics (CFD) models were constructed based on the pre- and post-procedure CT scans to identify variables that may predict treatment outcome. There were no statistically significant changes in CFD computed nasal resistance (pre-0.096±0.065; post:0.075±0.026Pa/(ml/s); P=0.063) nor the measured peak nasal inspiratory flowrate (PNIF, pre 60.16±34.49; post 72.38±43.66ml/s; P=0.13). As validation, PNIF correlated significantly with nasal resistance (r=0.47, P=0.004). Among all the variables, only the peak mucosal cooling posterior to the nasal vestibule significantly correlated with the NOSE at baseline (r=-0.531, P=0.023) and with post-treatment improvement (r=0.659, P=0.003). Nasal airway volume in the nasal valve area increased only ~7% post-treatment, yet this minimal remodeling has a profound effect on perceived nasal obstruction, corroborating our previous hypothesis that subjective relief of nasal obstruction correlates with perception of regional mucosal cooling rather than nasal resistance or peak flow rate, a potential target for future effective, personalized therapeutic approaches.

524 **Modulation Pathways Of Mchr1 In Olfaction**

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Melanin concentrating hormone (MCH) is an orexigenic neuropeptide synthesized by neurons in the lateral hypothalamus that project throughout the brain. These areas include multiple areas involving olfaction including the olfactory bulb, piriform cortex, and olfactory tubercle. MCH signaling has been shown to contribute to the modulation of several olfactory driven behaviors, such as feeding and maternal behaviors. In rodents, a single receptor for MCH exists. Melanin concentrating hormone receptor 1 (MCHR1) is a g-protein coupled receptor enriched in neuronal primary cilia. The primary cilium is a signaling center and highly conserved organelle that projects from nearly every cell type including neurons. In the olfactory bulb, MCHR1 localizes to the cilia of neurons surrounding the glomeruli and in the granule cell layer. Previously, we have shown that in anesthetized animals there was an enhancement in glomerular response post MCHR1 antagonist application and a depression in glomerular response post MCH application. To more specifically test the role of MCHR1 in olfactory signaling, we have generated a MCHR1 knockout mouse using CRISPR/CAS9. MCHR1 knockout mice were crossed with Thy1-GCaMP6f mice to assay glomerular responses to odor stimulation. Using epifluorescence imaging of the dorsal surface of the OB, MCHR1 knockout and wildtype animals respond similarly to changes in odor concentration. However, we find that glomerular responses are enhanced following administration of an MCHR1 antagonist ($t(51) = 3.382$, $p<0.01$) in awake wildtype animals. We then tested MCHR1 antagonist in MCHR1 knockout animals and observed no change in glomerular response. Together these data suggest that MCH/MCHR1 signaling contributes to modulation of olfactory function.

525 **An Exogenous Cannabinoid Decreases Olfactory Sensitivity In Non-Fasted Mice**

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The endocannabinoid system is a widespread neuromodulatory network that influences numerous aspects of sensory perception including olfactory processes. Previous research in rodents has suggested that endocannabinoids may regulate food intake through an olfactory-dependent mechanism (Soria-Gómez & Bellocchio et al. 2014). Specifically, cannabinoid type-1 (CB1) receptors within the granule cell layer of the main olfactory bulb (MOB) were proposed to stimulate ingestion in fasted mice by enhancing their olfactory sensitivity. To further explore this phenomenon, we used an operant conditioning go/no-go assay with highly reproducible odor stimulus delivery to measure olfactory thresholds in mice. Infusions of the CB1 agonist, WIN 55,212-2, (WIN) directly into the granule cell layer (GCL) of the MOB in these animals, yielded a significant decrease in behavioral sensitivity as compared to vehicle or no manipulation ($p = 0.001$). Intrabulb infusions of the CB1 antagonist, AM251, into the GCL did not have a significant effect on olfactory sensitivity compared to vehicle ($p = 0.35$). Further, peripheral injections of WIN also did not influence odor detection ($p = 0.76$), contrary to previous findings utilizing this manipulation. These results indicate that exogenous cannabinoids acting on granule cells, blunt rather than enhance olfactory sensitivity, at least in non-fasted mice. Additional research is needed to uncover how metabolic state (e.g., fasting) influences cannabinoid signaling within the olfactory bulb and ultimately odor perception.

526 **Lateral Hypothalamic Projections To The Olfactory Bulb Constitute An Anatomically Distinct Subpopulation Of Orexin Neurons**

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The obesity epidemic has driven a growing body of research on the relationship between metabolism and the brain. Indeed, recent work has shown that inducing an imbalanced metabolism in mice via the consumption of a high-fat diet can drive functional and structural changes in the olfactory bulb (OB). The underlying mechanisms behind this relationship, however, remain poorly understood. We hypothesize that brain areas known to be involved in homeostatic regulation could be directly projecting to the OB. To test this hypothesis, we injected a monosynaptic retrograde tracer conjugated to Alexa Fluor 555 (CTB555) into a single hemibulb of six adult C57BL6/J mice. We discovered a number of tracer-labeled neurons located in the lateral hypothalamus ipsilateral to the OB injection site in all of our preparations. Orexin is a neuropeptide known to be involved in feeding behavior that is released from a subpopulation of neurons located in the lateral hypothalamus. We therefore tested whether the hypothalamic OB-projecting neurons included orexin neurons by combining tract tracing with immunohistochemistry for orexin-A, and then quantified the numbers of single- and double-labeled neurons. In our examined mice, 22 % ± 4 (s.e.m.) of the OB-projecting neurons in the hypothalamus expressed orexin-A, indicating that orexin-A neurons constitute a small fraction of the hypothalamic input to the OB. Remarkably, only 7.3 ± 1.2 % of all the orexin-A neurons projected to the OB, suggesting that the orexin-A neurons that innervate the OB constitute an anatomically distinct subpopulation. Overall, these results support a model in which the hypothalamus could modulate the OB by releasing orexin-A, which may serve as a mechanism behind metabolic modulation of the olfactory system.

527 **Adult Neurogenesis In The Mouse Accessory Olfactory System**

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In rodents, both sensory neurons in the vomeronasal organ and inhibitory interneurons (granule as well as periglomerular cells) in the accessory olfactory bulb are continuously replaced by adult neurogenesis, thereby (re)shaping the output of the accessory olfactory system (AOS). Notably, the precise physiological function of adult neurogenesis in the AOS remains unclear. Here, we begin to describe characteristics of neurogenesis in

both peripheral and central AOS tissues. Using a novel genetic approach, we label newly generated vomeronasal sensory neurons as well as accessory olfactory bulb interneurons. After tamoxifen injection, neuronal stem cells in *Id2CreERT2*$^{+}$ *:: Rosa26R-tdTomato* mice express tdTomato upon coincident Id2 promoter activity. Descendants of these stem cells are thus labelled with tdTomato. Introducing the *Id2* stem cell marker as an AOS lineage tracer, we show (i) horizontal and tangential migration of sensory neuron precursors. We demonstrate that (ii) differentiated vomeronasal neurons appear two days after tamoxifen treatment. Finally, we provide insight into (iii) the migration of accessory olfactory bulb interneuron precursors into the accessory olfactory bulb.

528 **Repeated Odor Presentations Evokes A Relatively Short-Term Form Of Adaptation In Mitral/Tufted Glomeruli In The Mouse Olfactory Bulb.**

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Neural circuits that can adjust their responsiveness to changes in an organism's sensory experience would facilitate odor recognition and localization in natural environments. Imaging experiments from the olfactory receptor neuron axon terminals and mitral/tufted apical dendrites innervating the glomerular layer in the mouse olfactory bulb revealed that repeated odor stimulation with brief interstimulus intervals evokes a form of adaptation that is computed within the bulb. Here we used 2-photon calcium imaging in awake mice to record odor-evoked signals from the apical dendrites of mitral/tufted cells innervating the glomerular layer. Odors were varied across a ~100-fold change in concentration, and we tested several different interstimulus intervals from seconds to days. Adaptation was modest in most glomeruli at lower concentrations at all interstimulus intervals. In contrast, higher concentrations evoked diverse responses across the glomerular population where many glomeruli exhibited substantial adaptation, while others adapted minimally. Most adapting glomeruli showed a substantial, but incomplete recovery with a 30 second interstimulus interval between odor presentations. Most glomeruli responded a with similar response amplitude across recording trials, which were separated by a minimum of 3 minutes, and in some cases were measured on different recording days. The results indicate that mitral/tufted glomerular adaptation represents a relatively short-term process that we hypothesize is important for making rapid adjustments to the current sensory environment in natural odor scenes.

529 **Electrophysiological Characterization Of Periglomerular Cells In The Mouse Accessory Olfactory Bulb**

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The mouse accessory olfactory system plays a central role in detecting chemical cues during conspecific social interactions. The vomeronasal organ is the system's peripheral sensory structure and information is sent along the vomeronasal nerve to the accessory olfactory bulb (AOB). Here, AOB mitral cells receive excitatory synaptic input via multiple glomeruli. These glomeruli are surrounded by local interneurons, collectively designated as periglomerular cells (PGCs). Their physiological function(s) as well as whether PGCs form a homo- or heterogeneous neural population remains unknown. Using whole-cell patch-clamp recordings from visually identified PGCs in acute slices of the mouse AOB, we investigate PGC biophysical properties. To detail cell type-specific features, both passive and active membrane properties are analyzed. We demonstrate that, given their large input resistance, PGCs are highly sensitive to electrical stimulation. With fast action potential kinetics, PGCs discharge at high frequencies. In addition, we describe voltage-dependent currents with distinct activation and inactivation properties, including potassium, sodium and calcium currents. Our results reveal the biophysical properties of an elusive AOB neuron population and, thus, provide first insight into physiological PGC characteristics.

530 **Effect Of Olfactory Bulb Pathology On Olfactory Function In Normal Aging**

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Decline of olfactory function is frequently observed in aging and is an early symptom of neurodegenerative diseases. As the olfactory bulb (OB) is one of the first regions involved by pathology and may represent an early disease stage, we specifically aimed to evaluate the contribution of OB pathology to olfactory decline in cognitively normal aged individuals without parkinsonism or dementia. This clinicopathological study correlates OB tau, amyloid β (Aβ) and α-synuclein (αSyn) pathology densities and whole brain pathology load to olfactory identification function as measured with the University of Pennsylvania Smell Identification Test (UPSIT) and clinical data measured proximate to death in a large autopsy study including 138 cases considered non demented controls during life. Tau pathology was frequently observed in the OB (95% of cases), while both Aβ (27% of cases) and αSyn (20% of cases) OB pathologies were less commonly observed. A weak correlation was only observed between OB tau and olfactory performance, but when controlled for age, neither OB tau, Aβ or αSyn significantly predict olfactory performance. Moreover, whole brain tau and αSyn pathology loads predicted olfactory performance, however only αSyn pathology loads survived age correction. In conclusion, OB tau pathology is frequently observed in normally aging individuals and increases with age but does not appear to independently contribute to age-related olfactory impairment. Results suggest that further involvement of the brain seems necessary to contribute to age-related olfactory decline.

531 **Don Tucker Finalist: Neural Circuit Basis For Generating Spindle Oscillations In The Developing Olfactory System**

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We recently demonstrated that the olfactory system in awake, neonatal rat pups exhibit highly stable activity patterns: starting at birth (P0) until around postnatal day 15 (P15), odor stimuli evoke spindle oscillations that are coherent between the olfactory bulb (OB) and the piriform cortex (PC). Here, we investigated the circuit-level mechanisms underlying the generation of these spindle oscillations. To determine the source of spindle oscillations, we recorded local field potential (LFP) activity simultaneously from the OB and PC in unanesthetized rat pups (P4-P9, n=30). Granger causality revealed a significant information flow from OB to PC, and PC to OB in the spindle frequency range, suggesting that the generation of spindle oscillations relies on feedback from PC to OB. To determine the circuit-level organization of potential cortical feedback projections to the OB, we applied current source density analysis (n=9) that revealed a sink in the glomerular layer, followed by a sink in the granule cell layer (presumably the target of cortical feedback projections). To investigate the causal effect of feedback projections on oscillatory activity, we recorded odor-evoked activity from OB before and after pharmacological inactivation of the lateral olfactory tract (n=9) or olfactory peduncle (OP, n=5) through lidocaine injection. Inactivation of either tract caused a significant increase in oscillation frequency in the OB. Thus, feedback modulates spectral characteristics of oscillations generated in the OB and may control different functional states of the neonatal olfactory system. Together, our results suggest that the neonatal olfactory system already exhibits adult-like circuit motifs, including net inhibitory feedback projections, capable of generating complex and flexible activity patterns.

532 **Self-Reported Chemosensory Changes In Alcohol Consumption Groups And Associated Impact On Quality Of Life**

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Chemosensory alteration in excessive alcohol drinkers substantially impacts their quality of life (QoL). Early assessment of chemosensory decline can help in early prediction of disease severity and associated comorbidities in heavy drinkers (HDs). Here we examined smell and taste self-reports of individuals with different alcohol drinking behaviors and association with change in their overall QoL. Participants (n=466; 224 Females, 242 Male) were recruited between June 2020 and September 2021 into the COVID-19 Pandemic Impact on Alcohol study. Alcohol Use Disorders Identification Test (AUDIT) consumption scores across four time points (at enrollment and after 4, 8, and 12 weeks) were analyzed by group-based trajectory modeling to stratify participants into three groups (non-drinkers, NDs; moderate drinkers, MDs; and HDs). Linear mixed effects analysis of self-reported taste and smell data revealed a significant reduction in smell ability of HDs compared to NDs ($F_{1,224}$=4.40, p=0.03) after adjusting for age and smoking status, but group differences in taste ability did not reach significance ($F_{1,241}$=3.55, p=0.06). Smell/taste reports did not significantly differ between the MDs and NDs. Further, the reduced smell and taste ability of HDs significantly associated with deterioration in several QoL domains, including physical health (smell: β=0.13, p=0.003; taste: β=0.13, p=0.01) psychological health (smell: β=0.15, p=0.001; taste: β=0.13, p=0.01), social relationships (smell: β=0.20, p<0.001; taste: β=0.30, p<0.001), and environmental health (smell: β=0.18, p<0.001; taste: β=0.21, p<0.001). The reduced smell and taste function of HDs and association with poorer QoL indicates that early assessment of chemosensory changes may be crucial in identifying the risk for poorer outcomes in heavy drinkers.

533 **Evidence Of The Involvement Of The White Matter Integrity In Olfactory Dysfunctions In Parkinson'S Disease.**

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Olfactory dysfunction (OD) is a frequent symptom of Parkinson’s disease (PD) that appears in early stages. Studies suggest that PD-related OD is different from other forms of non-parkinsonian OD (NPOD; related to sinunasal disease, viral infection, trauma, etc.), as PD patients maintain trigeminal sensitivity while patients NPOD typically exhibit reduced trigeminal sensitivity. The difference in trigeminal sensitivity between the two types of patients leads to distinct imaging features. Indeed, in previous work, we identified a specific pattern of functional connectivity between olfactory and trigeminal chemosensory brain processing area in PD patients and in NPOD patients. Here, we aim to further understand these results by investigating white matter fiber integrity

in PD patients and NPOD subjects. We hypothesized the presence of a specific alteration of white matter fibers between the chemosensory regions in PD compared to NPOD patients. Specifically, we aimed to assess potential differences in white matter fiber integrity between the chemosensory regions using diffusion MRI in 15 patients with PD and compare them to 15 patients with NPOD and to 15 controls. Group differences and similarities will be discussed in light of the existing literature. In summary, this study will provide a better understanding of PD-related OD with the aim of differentiating it from NPOD, an important step towards the use of olfaction as an early marker and potentially as a screening tool for PD.

534 **Prevalence Of Smell And Taste Loss In Youth With Covid-19**

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Chemosensory dysfunction is a common and early symptom of COVID-19, even in otherwise asymptomatic patients. In COVID-19-positive adults, the prevalence of smell loss is ~67% and of taste loss is ~42%. Despite the promise of tracking smell and taste as a discriminatory symptom of COVID-19, there has been little effort to quantify the prevalence of these symptoms in youth. Here we aim to examine the extent to which smell and taste have been assessed among youth with active COVID-19. To date, only 4.7% (N = 39/826) of studies including COVID-19 positive youth assessed smell and/or taste loss. We use random-effects meta-analysis to pool 39 studies including individuals younger than 18 years old, with confirmed or suspected COVID-19 diagnosis in which a measure of smell and/or taste was reported (24 secondary reports from medical records or parental reports, 13 self reports, 2 direct testing) and estimate the effect of chemosensory dysfunction due to COVID-19 in youth (age 0-17 years, 11 months, 29 days). Based on self-reports alone, the prevalence of smell loss is 14% (vs. 8% secondary reports) and the prevalence of taste loss is 18% (vs. 7% secondary reports). The only paper using a standardized direct test for smell loss (an adult version of the Sniffin' Sticks odor identification test) indicates a much higher prevalence of smell loss of 86% (N = 79). Prevalence increases from age 10, but no sex differences are revealed. We highlight the need for guidelines to assess chemosensory loss in children with suspected COVID-19. At a minimum, we recommend the use of self-reports to document the prevalence of chemosensory loss due to iCOVID-19 in youth, and possibly mitigate the burden of the COVID-19 pandemic in this age category.

535 **Transient Changes In Oral Chemesthesis, Taste And Smell In Covid-19 &Ndash; Longitudinally Intensive Data From A Small Case-Control Series**

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Anosmia is common with influenza or rhinovirus infections, but loss of taste or chemesthesis is rare. Reports of true taste loss with COVID19 were viewed skeptically until confirmed by multiple studies. Nasal menthol thresholds may be elevated in some with prior COVID19, but data on oral chemesthesis are lacking. Many patients recover quickly, but precise timing and synchrony of recovery are unclear. We collected broad sensory measures over 28 days, recruiting adults (18-45 yrs) who were COVID19 positive or recently exposed (close contacts per CDC criteria). Participants received nose clips, red jellybeans (Sour Cherry, Cinnamon) and scratch-n-sniff cards (ScentCheckPro). To assess changes during disease onset, we identified 4 cases enrolled before Day 1 of illness; 4 controls (close contacts who never developed COVID) were matched via age, sex and race. Variables included sourness and sweetness (Sour Cherry jellybeans), oral burn (Cinnamon jellybeans), mean orthonasal intensity of 4 odors (ScentCheckPro), and perceived nasal blockage. Data were plotted over 28 days, creating panel plots for each case and control. Controls exhibited stable ratings over time, in contrast to COVID-19 cases, who showed sharp deviations over time. No single pattern of taste loss or recovery was apparent, implying different taste qualities may recover at different rates. Oral burn was transiently reduced for some before recovering quickly, implying acute loss may be missed in data collected post-infection. Major deviations in odor intensity were unexplained by nasal blockage. Collectively, daily testing shows orthonasal smell, oral chemesthesis and taste are all altered by COVID19, and such disruption is dyssynchronous for different chemical senses, with variable loss and recovery rates across modalities and individuals.

536 **Changes In Cell Dynamics In The Olfactory Bulb In Mouse Models Of Multiple Sclerosis**

Sema Kaya[1], Carsten Holzmann[2], Markus Kipp[1], Martin Witt[1]

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Olfactory deficits are among the most common initial symptoms of neurodegenerative diseases such as Alzheimer´s disease, Parkinson´s disease and multiple sclerosis (MS). They can appear years before their clinical manifestation. MS is characterized by focal demyelination, axonal loss, gliosis and inflammation. Animal models used here comprise experimental autoimmune encephalitis (EAE), cuprizone (Cup) and a combined cuprizone/EAE (Cup/EAE) MS animal model. The EAE model is based on autoimmune reactions, while the cuprizone is a toxic model. Both lead to demyelination of the CNS in mice. Our previous report revealed signs of

inflammation in the olfactory bulb (OB) such as significantly increased density of T cells and microglia. We here studied cell dynamics of OB interneurons, oligodendrocyte activity and demyelination using immunohistochemistry (IHC). Results revealed a reduced number of tyrosin hydroxylase (TH) positive interneurons, as well as increased glial cell activity (Olig-2), and demyelination (Myelin Basic Protein (MBP)) within EAE, Cuprizone and Cup/EAE- affected mice. The IHC investigations also showed a highly proliferative activity (Ki-67) in the OB in all three animal models. The results of our study let us assume that changes within the cell dynamics of olfactory neurons and the glial cells, but also the demyelination in the olfactory bulb could be reasons for olfactory impairment in MS. The combination of these animal models could also offer a new experimental approximation for investigations and for understanding chemosensory dysfunctions in human MS.

537 **Chemosensory Losses In Active Probable Delta And Omicron Variants Breakthrough Covid-19 Cases**

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Chemosensory loss is a COVID-19 hallmark but it is unclear if the Delta (DL) and Omicron (OM) variants similarly impact smell and taste function, and whether vaccination results in less severe symptoms. 80 subjects with prior confirmed/clinical probable diagnosis of COVID-19 and 125 controls performed sensory tests via Zoom using the NIH toolbox 9-item scratch and sniff odor id test and bitter intensity ratings of 1mM quinine. 39 subjects had active COVID-19 (symptom onset <14d) at the time of testing, and most (36/39) were vaccinated. 25 of these active cases were likely infected by the DL variant with the rest as probable OM cases based on diagnosis dates. The other 41 positive cases occurred prior to the DL surge in the US with diagnosis >14d prior to sensory testing (x=6.5m). 9 of the 41 subjects reported smell loss (8 long-haulers); objective testing confirmed smell loss comparable to the active cohort in 7 (78%). 16 of the remaining 32 (50%) without reported smell loss still had objective losses. All probable DL-variant cases (100%) had objective smell loss based on age and gender adjusted normative cutoffs, although only 16/25 reported smell/taste loss. None of the likely OM cases reported smell/taste loss, yet 5/11 (45%) subjects had objective smell loss, higher than in controls (34%). For taste function, while COVID+ subjects with self-reported chemosensory loss rated quinine as less bitter, the difference was not significant ($p>0.05$). The results demonstrate (1) the DL variant may cause similar if not more severe impact on olfactory function while the impact of the OM variant is less profound and (2) vaccination does not fully prevent chemosensory loss. Results also add to evidence that self-reported chemosensory loss is useful but may not capture the full spectrum of losses from COVID-19.

538 **Ambient Air Pollution And Olfactory Sensitivity In 11 Locations Across The Globe**

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Over 90% of the global population live with air pollution above the WHO limit, and these numbers continue to increase. Exposure to outdoor air pollution has been linked to cardiovascular disease, stroke, chronic obstructive pulmonary disease and lung cancer, oxidative stress as well as an increase in the risk for acute respiratory infections. Although pollution is largely harmful to the respiratory system, we only have started to collect information about its potential effects on the sense of smell. Lifetimes of particles present in the atmospheric air has been confirmed to be strongly linked with olfactory sensitivity in humans (Williams, Ringsdorf, 2020), further supporting the notion that atmospheric conditions alter olfactory perception. A few reports including adults representing indigenous tribes suggest lower olfactory sensitivity to be related with lesser exposure to air pollution (Sorokowska, Sorokowski, Hummel, Huanca, 2013). In line with this, residents of the rural Tlaxcala characterized by low air pollution have been found more sensitive to odors than the inhabitants of highly polluted Mexico City (Guarneros, Hummel, Martínez-Góme, Hudson, 2009). In the present study, we compare the olfactory sensitivity of individual subjects (n=811) inhabiting 11 locations across the globe with the composition of atmosphere pollutants. The aim of this analysis is to estimate the overall relationship between air pollution and olfactory sensitivity, and further to point to the most harmful atmospheric pollutants for our sense of smell.

539 **Behavioral Chemosensory Interventions: Multiple Flavors Of Olfactory Training To Improve Diet Quality**

Valentina Parma[1], John E. Hayes[2], Valerie B. Duffy[3]

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Smell dysfunction is associated with several negative health outcomes, including unhealthy diet. The prevalence of persistent smell dysfunction has dramatically increased because of the COVID-19 pandemic. Critically, current recommendations for smell dysfunction are limited to orthonasal olfactory training (OT), which is effective in ~30% of patients with post-viral smell loss. The effects of OT on diet are lacking. A strong need exists to (a) improve OT efficacy, and (b) assess impact of olfactory improvement on diet quality. Here we describe a protocol for a multi-arm 3 (sensory method) by 2 (engagement) Randomized Clinical Trial (RCT) with OT using intent-to-treat analysis to test efficacy of six personalized, modular, multicomponent 12-week online interventions and 24-week follow-up on improving smell function and diet quality. Along with the standard OT exposure method (OrthoOT–), we propose two novel methods: RetroOT– and DietOT–. In two RetroOT arms, a polymeric matrix is chewed to deliver odorants retronasally. In two DietOT arms, ecologically relevant exposure occurs by enhancing dietary diversity, including fruits, vegetables, and whole grains. Each sensory-only training method (OrthoOT–, RetroOT–, DietOT–) will be paired with a parallel mindfulness arm

(OrthoOT+, RetroOT+, DietOT+) to determine whether active cognitive engagement during exposure boosts efficacy. To monitor and improve adherence, all arms will include goal setting sessions, health coach guidance, online step-by-step instructions, and social media support. Findings from intent-to-treat analysis align with rigorous requirements to minimize bias in reporting OT efficacy and support evidence-based recommendations for health providers to use with patients with OD and address Healthy People 2030 goals for chemosensory disorders.

540 **The Essence Of Male Scent Promotes Female Puberty And Estrus**

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Pheromones are chemical signals that trigger a response in another member of the same species. In mice, it has been shown that exposure of females to male pheromones leads to puberty advance and estrus induction, phenomena known as the Vandenbergh and Whitten Effects, respectively. These effects can be triggered by conspecific male urine, suggesting that they are related to the chemical composition of this stimulus. Although these phenomena were among the earliest known examples of pheromonal actions, the identities of these chemical signals remain mysterious. In agreement with previous work, we found that these behavioral effects could be triggered by low molecular weight nonvolatile constituents of male mouse urine. By combining high-performance liquid chromatography, calcium imaging from the mouse vomeronasal organ, and mass spectrometry (MS), we identified physiologically active fractions of male urine. We then isolated two small molecules in male urine, termed Calin319 and Calin381, that accounted for much of the vomeronasal neuronal response to male urine. By MS-MS and MS^n and direct synthesis, we identified the structure of Calin319 as 2,6-dimethyl-2-heptyl glucuronide. We found that Calin319 and Calin381 were sufficient and necessary to advance juvenile female puberty and induce female estrus. Besides acting as a primer pheromone, a blend of these two male compounds also acts as a releaser pheromone that resulted in increased investigatory behavior by female mice. These findings demonstrate that Calin319 and Calin381 are crucial male pheromones that regulate female reproductive behavior in mice. This study resolves the long-standing mystery of the molecular code of male urinary chemicals that control female gonadal function.

541 **How Does Covid-Related Masking And Social Distancing Affect Social Olfactory Communication? **

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Odor is an important component of human social interactions. Everyone has a unique body odor, and this odor can provide a wide variety of social information. Due to the current COVID-19 pandemic, there has been a shift in human interaction due to masking and social distancing, limiting our access to these important olfactory cues. Additionally, many people have struggled with COVID-related olfactory loss. In this study, we aimed to examine the influence of COVID safety protocols on perceptions of social olfactory information. Data collection is ongoing and results are preliminary (n=39). We developed a questionnaire to examine how people's perceptions of others' body odors have changed in the face of COVID-related masking and social distancing, including whether these measures interfere with the conscious perception of social odor cues, and whether others' body odor has become more intrusive since the start of the pandemic (*impact*, Cronbach's α =.64). We also developed a brief questionnaire to assess participants' level of COVID risk in their social behaviors (Cronbach's α=.80) and collected information on personality type and self-reported olfactory function. Participation in risky behaviors was significantly higher for unvaccinated than vaccinated individuals ($t(36)=-3.883$, $p<.001$) We found that covid impact scores were positively correlated with extraversion ($r(39)=.35$, $p=.03$) and body odor disgust sensitivity (BODS) scores ($r(39)=.37$, $p=.02$), but negatively correlated with odor awareness sensitivity (OAS) (r=.52, p<.001). Preliminary evidence suggests that the impact of social restrictions during the COVID pandemic influences individuals differently depending on personality factors and sensitivity to social odors.

542 **Self-Grooming Promotes Social Attraction In Mice Via Chemosensory Communication**

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Self-grooming is a stereotyped behavior displayed by nearly all animals. Though often conceptualized as a solitary or asocial behavior, self-grooming is also implicated in social communication. However, how self-grooming influences behaviors of nearby individuals has not been directly tested, partly due to the technical challenge of inducing self-grooming in a reliable and temporally controllable manner. We recently found that optogenetic activation of dopamine D3 receptor expressing neurons in the ventral striatal islands of Calleja robustly induces orofacial grooming in mice. Using this optogenetic manipulation, we examined social preference of observer mice toward a mouse displaying more grooming versus a counterpart displaying less grooming in a three-chamber apparatus. We found that observer mice spent more time investigating mice that groomed more regardless of biological sex. Grooming-induced social attraction unlikely depended on visual or auditory cues as social preference persisted when the observer mice could not visualize the other mice and grooming mice did not produce robust vocalizations during orofacial grooming. Interestingly, ablation of the main olfactory epithelium in observer mice eliminated social preference toward mice that groomed more. Moreover, observer mice exhibited strong preference to orofacial secretions collected from mice that groomed

more, indicating that grooming-induced social attraction depends on volatile chemosensory cues broadcasted from grooming mice. Collectively, our study establishes self-grooming as a means of promoting social attraction among mice via volatile cues, suggesting an additional benefit for animals to allocate a significant amount of time to this behavior.

543 ***Arc*-Expressing Accessory Olfactory Bulb Interneurons Play A Role In Chemosensory Social Behavior**

Kelsey E. Zuk[1,2], Julian P. Meeks[3]

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The accessory olfactory system (AOS) is critical for the development and expression of sex-typical social behavior in terrestrial mammals. The first dedicated circuit in the AOS, the accessory olfactory bulb (AOB), exhibits cellular and network plasticity in both male and female mice after social experience. An AOB interneuron subtype, internal granule cells (IGCs), has been shown to selectively express the plasticity-associated immediate-early gene *Arc* following social experience. In this work, I sought to better understand how *Arc*-expressing IGCs shape AOB information processing and the display of social behavior. I used Arc-CreERT2 mice to selectively and permanently label *Arc*-expressing IGCs following male-male resident-intruder interactions. Using whole-cell patch clamp electrophysiology, I found that *Arc*-expressing IGCs displayed increased intrinsic excitability for several days after a single resident-intruder interaction. Further, Arc-expressing IGCs displayed a similar increase in excitability across a week of repeated resident-intruder interactions. During these repeated interactions, I found that resident mice increase their aggression. I then tested the hypothesis that *Arc*-expressing IGCs participate in increasing resident aggressive behavior. Using a combination of Arc-CreERT2 mice and chemogenetics, I found that disruption of *Arc*-expressing IGC activity during repeated resident-intruder interactions completely abolishes the increase in resident aggression. Taken together, this work demonstrates that *Arc*-expressing AOB IGCs participate in the establishment and expression of sex-typical social behavior. These findings increase our understanding of central chemosensory processing, experience-dependent plasticity, and the role of specific AOB cell types in mammalian social behavior.

544 **Using Affect Misattribution Procedures (Amp) To Study Emotional Responses To Food Odorants.**

Hannah Kelson, Justin Ong, Zoe Alcott, Arbenita Hasani, Yumi Higashiyama, Arya Kumar, Gomez Andrea, Solla Leto, Acree Terry
Cornell University, Ithaca, NY, United States

Whether or when affective stimuli have primacy over cognitive stimuli are important issues in brain science (Murphy 1993, Mueller, 2017). Experiments designed to study these issues require precise control over the timing, duration and precision of the stimulations in the psychophysical protocol used to collect data. This paper examines the use of sniff olfactometry (Rochelle, 2017) and an Affect Misattribution Procedure (AMP) (Murphy, 1993) to determine if brief odor puffs (70ms) can reduce the pleasantness ratings of neutral images (Chinese characters) before and after the odorants were conditioned with “Sad” or “Happy” images. All images were randomly chosen from a stock online database. Two common food odorants (hexanal, HEX – with a “green vegetable odor” or 2,3,5-trimethylpyrazine, TMP - with a “toast or fresh bread like odor”) were used in a baseline-study to show that these had on effect on the visual images of randomly selected Chinese characters displayed for 16ms, 700ms after the odorants were puffed. Finally, the subjects rated the pleasantness of the Chinese characters paired with the food odorants. The higher ratings of pleasantness of the Chinese characters paired with odorants, HEX or TMP, previously paired with “Happy” images indicated a positive affective image was transferred to a neutral Chinese character by an odorant. In this experiment the “Sad” conditioned odorants were more effective in reducing pleasantness in the neutral characters than the “Happy” conditioned odorants increased pleasantness. In future experiments we plan to reduce the concentration of the odorants below their recognition threshold but above their detection threshold to study what role cognition plays in these experiments.

545 **The Organization Of Odorant Binding Proteins (Obps) In The *Drosophila* Sensory System**

Keehyun Park[1], Hyungjun Choi[1], Rahel Asefa Wayessa [1], I Joon Han[2], Chaiyoung Jeong[1], Jung Yoon Jang[1], Min Sung Choi[1], Jae Young Kwon[1]

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The fruit fly *Drosophila melanogaster* has a complex chemoperception system. The odorant binding proteins (OBPs) are known to be involved in chemoperception together with chemosensory receptors. They are generally considered to help transfer hydrophobic odorants in the aqueous lymph solution to olfactory receptors. Many publications implicate that OBPs have other diverse roles in many physiological pathways. Transcriptomics experiments have shown that *Drosophila* OBPs are abundantly expressed in various organs. However, their overall expression patterns and exact functions have not been fully deciphered. Therefore, we established a *Drosophila OBP-Gal4* driver library which can be used with various UAS reporters. With these drivers, we completed a catalog of *Drosophila OBP-Gal4* expression patterns, which shows *Drosophila* OBP expression in the labellum, antennae, pharynx, maxillary palp, leg, wing, brain, ventral nerve cord (VNC), and intestine. Analyzing the patterns, we found that some OBPs show interesting patterns that seem to be related to proprioception and glial cell function. Furthermore, we could specifically identify which cells in the labellum express OBPs using reporters of Gustatory receptors and accessory cells. We constructed CRISPR-Cas9 mutant lines that showed expression in the labellum to study the roles of OBPs in gustatory sensation. With these OBP mutant flies, we conducted electrophysiology and behavioral experiments. Overall, our study provides an integrative resource of the *Drosophila* OBP gene family.

8:00 - 10:00 AM	Great Egret
Olfactory Test Training	

This practical session is meant to provide a very practical overview about techniques that are used in a clinical context to assess chemosensory functions, including olfactory, gustatory, and trigeminal functions. In addition, techniques to address psychological/cognitive issues related to olfactory function and dysfunction will be shown. The various techniques will be presented by researchers experienced in clinical chemosensory research, including Bob Pellegrino from Philadelphia, Caroline Huart from Brussels, and Akshita Joshi and Thomas Hummel from Dresden. There will be 4 stations, and the participants would rotate clockwise through stations 1 to 4. They will stay at each station for 15 min. The 4 stations will be: Station 1: Smell testing (e.g., Sniffin Sticks, UPSIT, CCCRC test, SSParoT, retronasal testing): Thomas Hummel, Dresden, Germany; Station 2: Taste testing (e.g., taste sprays, taste strips, electrogustometry, PROP/PTC test): Robert Pellegrino, Philadelphia, PA, USA; Station 3: Trigeminal testing (e.g., lateralization, AMMOLA-test, oral capsaicin test, CO2 threshold): Akshita Joshi, Dresden, Germany; Station 4: Psychological testing/questionnaires (e.g., SNOT, QOD, WHO wellbeing, MOCA): Caroline Huart, Brussels, Belgium

10:00 - 12:00 PM	Calusa ABCD
ACCESSIBLE AND HIGHLY-COLLABORATIVE CHEMOSENSORY SCIENCE	

Chair(s): Valentina Parma & Renee Hartig

10:00 **Accessible And Highly-Collaborative Chemosensory Science**

Renee Hartig[1,2], Ha Nguyen[3], Oghogho Braimah[4,5], André M. Chagas[6], Lucia Prieto-Godino[7], Valentina Parma[3]

[1]Department of Psychiatry and Psychotherapy, University Medical Center, Johannes Gutenberg University, Mainz, *, Germany, [2]Focus Program Translational Neuroscience, Mainz, *, Germany, [3]Monell Chemical Senses Center, Philadelphia, PA, United States, [4]Edo Specialist Hospital, Benin City, *, Nigeria, [5]University of Benin, Benin City, *, Nigeria, [6]University of Sussex, Brighton, *, United Kingdom, [7]Francis Crick Institute, London, *, United Kingdom

Diversity, equity and inclusion (DEI) drive excellence and innovation in several fields, including chemosensory science. However, engaging in DEI science is perceived as costly - both in terms of resources needed and changes to solidified research habits. In this symposium, we showcase success stories that demystify chemosensory science with a strong DEI component as costly and that provide concrete strategies to be used by students, early career and senior scientists and clinicians across the world. The present symposium intertwines a strategy for applying research findings to diversity across geographical regions and the influence of such diversity on the adaptation of the olfactory system in invertebrates. We also present a means for equitable production of equipment in visual neuroscience and how this can be bridged to chemosensation. Further, an example of how diversity in gustatory genetics affects the production of drugs is presented along with a clinical approach to address the challenges of using chemosensory tests cross-culturally with the use of SCENTinel within a local population in Nigeria. Taken together, this symposium crosses chemosensory domains and the underlying diversity serves as a primary example of promoting scientific innovation through creative and accessible means.

10:10 **Evolution Of Neural Circuits**

Lucia Prieto-Godino
The Francis Crick Institute, , *, United Arab Emirates

Sensory systems encode the world around us to guide context-dependent appropriate behaviours that are often species-specific. This must involve evolutionary changes in the way that sensory systems extract environmental features and/or in the downstream sensory-motor transformations implemented. However, we still know little about how evolution shapes neural circuits. We address these fundamental questions using as models the olfactory systems of different fly species. We employ a multidisciplinary approach, including comparative connectomics, the development of genetic tools across species, fast volumetric calcium imaging, single cell transcriptomics and behaviour. I will present a project where the combination of these methods in a collaborative fashion has uncovered the role of a local inhibitory interneuron population in the evolution of odour-guided behaviours. I will end by showcasing collaborative and open science approaches we employ in the lab and in our outreach efforts.

10:40 **What It Really Takes To Make Chemosensory Tests Cross-Cultural? Experience With Rapid Smell Testing In Nigeria.**

Oghogho E. Braimah[1,2], Stephanie Hunter[3], Pam Dalton[3], Valentina Parma[3]

[1]Edo Specialist Hospital, Benin City, *, Nigeria, [2]Department of Surgery, University of Benin Teaching Hospital, Benin City, *, Nigeria, [3]Monell Chemical Senses Centre, Philadelphia, PA, United States

Olfactory tests are critical tools in the diagnosis and management of chemosensory disorders. Yet, they are not routinely used. Obstacles to routine clinical implementation are cost, duration, and expert administration. The

smell tests most commonly used are odor identification tests, which are prone to cultural biases due to the presentation of unfamiliar target odors, descriptors and/or distractors. Here we set out to validate the US-developed multi-function rapid smell test SCENTinel in Benin City, Nigeria. A group of 346 adults (200 F, age: mean±sd, range: 32±9, 18-70 years old, 100% Black) was recruited at the Ear Nose and Throat clinic of the Edo Specialist Hospital to complete one of nine versions of the SCENTinel test, comprising odor detection, intensity rating and identification. The group included 13 participants with various forms of smell loss (N=6 anosmia; N=1 hyposmia; N=6 fluctuations, 8F, 38±13, 21-64 years old). Results indicate that 93% of individuals with normosmia meet the accuracy criteria for SCENTinel ivs. 69% of individuals with smell disorders. Each subtest confirms greater smell ability in the normosmic group (detection: 92% vs. 69%; intensity: 99% vs. 31%; identification: 71% vs. 54%). The performance of normosmics at the odor identification subtest is variable across versions. Fail rates were as follows: coconut (77%), lemon (46%), wood (32%), orange (31%), bubblegum (17%), flower (15%), coffee (14%) and strawberry (13%). All in all, we conclude that SCENTinel passes the feasibility test in Nigeria, yet the odor identification component needs to be adjusted.

11:10 **A Worldwide Taste Project - Our Experience**

Ha Nguyen[1], Katherine Bell[1], Amy Huang[1], Mackenzie Hannum[1], Cailu Lin[1], Vicente Ramirez[1,2], Carol Christensen[1], May Cheung[1], Danielle Reed[1]

[1]Monell Chemical Senses Center, Philadelphia, PA, United States, [2]University of California Merced, Merced, CA, United States

The majority of human chemosensory research is conducted on people of European ancestry, but we know that studying only a small portion of the population of the world paints an incomplete picture of the breadth of human sensory experience. We have recently explored a low-cost method of recruiting a worldwide sample of people of diverse ancestry by capitalizing on new technologies which have accelerated this research program, e.g., remote testing capacities and mailout taste kits. We have tested 186 people who are of recent African, Asian, and European ancestries. Our experience of these methods is that mailout and zoom testing is feasible and may scale to large numbers of participants (e.g., group zoom testing). In a separate study, this method was compared with traditional lab-based sensory testing, revealing no differences in outcomes. Engaging diverse participants in sensory research studies is a new path forward in ensuring research rigor and generalizability.

11:30 **Open Hardware In Neuroscience, Better, Faster, Funner**

Benjamin H. Paffhausen
CNRS Toulouse

When approaching a new problem the experimental design should be directed by the scientific question not by the present machines or funding availability. The open hardware movement is closing such a gap by making nearly every device, existent or not, accessible to researchers. The state of the art will be framed and approaches for newcomers to electronics and prototyping will be presented. It will be shown using self-made examples that every machine consists of elements that are individually documented and understandable. One's specific desire shall combine those into a specialized device. Recent developments and increasing mass production have made every sensor actor and chip highly available, cheap and well documented. The presented data and live demos will show that such an open approach does not have to hide behind commercial solutions.

10:00 - 12:00 PM	Calusa FGH
Parosmia Clinical Symposium	

Chair(s): Thomas Hummel

10:00 **Introduction To Parosmia Clinical Symposium**
Thomas Hummel
University of Dresden Medical School

10:05 **Philadelphia: Clinical Appearance Of Parosmia**
Robert Pellegrino
Monell Chemical Senses Center

10:25 **Edinburg: Molecular Triggers Of Parosmia**
Jane Parker
University of Reading

10:45 **Boston: Olfactory Epithelium In Parosmia**
Eric Holbrook
Harvard University

11:05 **Brussels: Central Nervous Involvement In Parosmia**
Caroline Huart
Cliniques universitaires Saint-Luc

11:25 **Aarhus: Coping With Parosmia**
Alexander Fjældstad
Aarhus University

11:45 **Joint Discussion**

12:00 - 2:30 PM	Lunch On Own
Lunch On Own	

2:30 - 4:30 PM	Calusa ABCD
Industry Symposium: Understanding bitterness, and its implications for food formulations	

Chair(s): Robin Dando

2:30 **Predicting And Modulating Bitterness**
Masha Niv
The Hebrew University of Jerusalem

3:00 **Can Bitter Get Better? Saliva And Bitter Acceptance**
Ann-Marie Torregrossa
University at Buffalo

3:30 **Bitter Or Bitters? Qualitative Differences Between Bitter Stimuli**
John Hayes
Pennsylvania State University

4:00 **Bitter Taste Modulation: A Molecular Approach**
Guy Servant
Firmenich, Inc.

2:30 - 4:30 PM	Calusa FGH
The Barry Davis Funding Workshop	

This workshop will include an overview of research, training, and funding opportunities for graduate students, postdoctoral fellows, and early stage investigators. The discussion will provide practical information on how grant applications are processed within NIH/NIDCD, including Institute and study section assignments, the peer review process, Advisory Council activities, pay lines, and the roles of program and review staff.

Chair(s): Susan Sullivan

2:30 - 3:30 PM	Great Egret
Meet the Editors	

Chemical Senses is the premier journal focused on the science of smell, taste and chemesthesis in humans and other animals. It is also the official journal of five scientific societies devoted to chemosensory science, including the Association for Chemoreception Sciences. This session will discuss the many advantages of publishing in your society journal, the journal's review and publication processes, and journal policies and new initiatives. After a short presentation by Editor-in-Chief Steven Munger, the session will include a Q&A session with a panel of the journal's executive editors to address questions from the audience and add their own perspectives.

7:00 - 9:00 PM	Calusa ABCD
Presidential Symposium: How COVID-19 Affects Taste, Smell, The Brain and The Mind	

Chair(s): Nirupa Chaudhari

7:00 **How Covid-19 Affects Taste, Smell, The Brain And The Mind**
Nirupa Chaudhari
Department of Physiology & Biophysics and Department of Otolaryngology, University of Miami Miller School of Medicine, Miami, FL, United States

There are many, many puzzling aspects of the COVID-19 pandemic. What inherited, acquired and environmental conditions allow some infected people to remain asymptomatic, while others suffer serious illness? The link with other respiratory illnesses was evident from the onset of this new viral disease. Yet, mysteries were presented early on – why are taste and smell affected? Is there really taste loss, or simply a common general confusion between taste and smell? Once the mechanisms of anosmias started being addressed, deeper questions of mechanisms presented themselves. If SARS-CoV-2 virus interacts with select cell types, what are the pathways for the virus to gain access to the brain and is this really the source of neurological symptoms. How does the immune system in its diversity interact with the brain and the nervous system at large? These are some of the intriguing topics that Drs. Reed, Lomvarda and Bartley, our invited speakers, will address. We hope to understand mechanisms that we study as chemosensory scientists, and mechanisms that affect us as individuals, families and communities.

7:05 **Genetics Of Covid-19 Susceptibility: Phenotypes And Ancestry**
Danielle Reed
Monell Chemical Senses Center, Philadelphia, PA, United States

COVID-19 has presented us with a biological puzzle: why do some people become much more ill than others or have such a range of symptoms? Genetic researchers worldwide have pooled their information about COVID-19 susceptibility (how likely someone will become infected if exposed) and severity (if infected, how likely someone will be hospitalized or die). This global effort has identified some high-risk alleles for both susceptibility and severity, particularly a high-severity-risk allele on chromosome 3. The allele frequency differs worldwide, which may partly explain why death from COVID-19 is more common in some places than others. Another biological puzzle is why some people lose their sense of taste and smell with COVID-19 infection, whereas others do not. Here too, genetics may account in part for these person-to-person differences; in particular, there is an influential allele in a little-studied olfactory gene. Such information can be useful in the fight against COVID: the need for a one-size-fits-all emergency public health message is often at odds with personalized medicine, but as we grow more familiar with this coronavirus and learn to live with its effects, we may incorporate genetic information when assessing risks of becoming infected or becoming very ill if infected. While COVID-19 and its effects on the chemosensory system are a scourge, we are learning more about infectious disease and human variation, as well as chemesthesis, taste, and smell.

7:35 **Mechanisms Of Covid-19 Induced Anosmia**
Stavros Lomvardas
Zuckerman Neuroscience Institute, Columbia University

8:05 **Anti-Sars-Cov-2 And Self-Reactive Antibodies In Neuropsychiatric Covid-19**

Christopher Bartley[1,2], Thomas Ngo[1,2], Ravi Dandekar[2,4], Eric Song[3], Bonny Alvarenga[2,4], Claire Johns[5], Sharon Wietstock[2,4], Colin Zamecnik[2,4], Jayant Rajan[2,4], Joseph Derisi[6,7,8], Serena Spudich[3], Shelli Farhadian[3], Samuel Pleasure[2,4], Michael Wilson[2,4]

[1]Department of Psychiatry and Behavioral Sciences UCSF, San Francisco, CA, United States, [2]Weill Institute for Neurosceinces, San Francisco, CA, United States, [3]Yale University School of Medicine, New Haven, CT, United States, [4]Department of Neurology, UCSF, San Francisco, CA, United States, [5]Department of Pediatrics, UCSF, San Francisco, CA, United States, [6]Biomedical Sciences Graduate Program, San Francisco, CA, United States, [7]Department of Biochemistry and Biophysics, University of California, San Francisco, CA, United States, [8]Chan Zuckerberg Biohub, San Francisco, California, San Francisco, CA, United States

Background: COVID-19 patients are at increased risk for new para- and post-infectious neuropsychiatric symptoms. Objective: To identify anti-neural antibodies in neuropsychiatric COVID-19. Methods: We profiled anti-SARS-CoV-2 and self-reactive antibodies in the cerebrospinal fluid (CSF) and sera of COVID-19 patients (4 – 84 years, n = 31) with para- or post-infectious neuropsychiatric symptoms and uninfected, neuropsychiatrically intact controls (n = 12). Neuropsychiatric COVID-19 syndromes included encephalopathy, encephalitis, seizure, cognitive impairment, and psychosis. Biofluids and five monoclonal antibodies (mAbs) from one patient were screened for SARS-CoV-2 antibodies by Luminex-based immunoassay and SARS-CoV-1/2 phage display immunoprecipitation sequencing (PhIP-Seq). Self-reactive antibodies were screened for by mouse brain immunostaining, human PhIP-Seq, and mouse brain immunoprecipitation mass spectrometry (IP-MS). mAbs were additionally characterized by whole human protein array. Antigens were validated by cell-based assay or western blot. Results: 25/31 CSF IgG and 2/5 mAbs bound SARS-CoV-2 antigens. 20/31 CSF and 4/5 mAbs immunostained brain tissue—including both anti-SARS-CoV-2 mAbs. By IP-MS and PhIP-Seq, COVID-19 CSF enriched more neural autoantigens than control CSF. Validated autoantibodies included ciliary proteins NINL and IFT88 and nuclear proteins THAP3 and TCF4. By peptide mapping, one anti-SARS-CoV-2 mAb bound the spike fusion peptide and cross-reacted with human FIP1L1, with which it shares some sequence similarity. Conclusions: Most COVID-19 CSF harbored anti-SARS-CoV-2 and anti-neural antibodies. Some anti-SARS-CoV-2 antibodies cross-react with host proteins, suggesting molecular mimicry. The pathogenic relevance of anti-neural antibodies in COVID-19 is unknown.

8:35 **Panel Discussion**

9:00 - 12:00 AM	Mangroves & Belvedere
Closing Dance Party	

Friday, April 29, 2022

11:00 - 1:00 PM	Gather.town
Virtual Poster Session	

1 **Birth And Death Of Taste Cells In Circumvallate Papillae Of Mice**
Thomas E. Finger, Rubaio Yang, Yannick Dzowo, Robert S. Lasher, John C. Kinnamon, Courtney E. Wilson
Univ. Colorado Sch. Medicine, Aurora, CO, United States

Cells in taste buds have a limited lifespan and undergo continual replacement by proliferative basal cells situated along the basal lamina (BL). Using serial blockface EM sections though circumvallate taste buds in mice, we have identified the progression of taste cells from their origins along the BL to ultimate senescence and death within the taste bud. Dividing cells, recognized by lack of nuclear envelope and rearrangement of chromatin, have an irregular, ragged appearance and always lie outside of the nominal boundaries of the taste bud. Apparent immature cells lie in the basal region of the taste bud with shapes ranging from irregular and roundish to vertically elongate, often with apical extensions but reaching only part way up in the taste bud. At this stage, many immature cells begin to exhibit features consistent with a Type II cell morphology, including rounded nucleus and appearance of organelles. Elongate, not fully mature Type II cells have an apical process not reaching the taste pore but may form specialized contacts with nerve fibers including atypical mitochondria that typify a channel synapse. Mature Type II cells have a single apical microvillous process extending into the taste pore and prominent synapses with nerve fibers. Senescent Type II cells exhibit numerous cytoplasmic vacuoles while retaining synaptic contacts and an apex still reaching the taste pore. Still older Type II cells withdraw from the taste pore although maintaining synaptic contacts, and begin fragmentation while apparently being engulfed by Type I cells. At terminal stages, dying cells with fragmented nuclei have a dense cytoplasmic matrix and are fully engulfed by Type I cells. We see no obvious examples of dying Type III cells, which may relate to their lower overall numbers and relative longevity.

2 **Response Profiles Of Vagal Gustatory Neurons**
Bryan Fowler, Saima Humayun, Shannon Landon, LIndsey Macpherson
University of Texas, San Antonio, TX, United States

There are two major pathways conveying gustatory information from the oral cavity to the brainstem: the anterior pathway in which geniculate ganglion neurons relay taste information from the fungiform and palate taste buds, and the posterior pathway in which petrosal neurons of the vagal complex relay taste signals from foliate and circumvallate taste buds. In addition to this anatomical segregation, there are also differences in the composition and proportions of types of taste receptor cells in these taste papillae, and they produce different downstream behavioral and reflex responses. While a wealth of data is now available to examine the tuning profiles of individual geniculate ganglion neurons, we currently lack equivalent information for the vagal ganglion neurons of the posterior taste pathway, making it difficult to evaluate how the activity of these neurons may contribute to downstream taste signaling pathways. We use in vivo calcium imaging to show that vagal neurons react to taste stimuli with similar response qualities to geniculate neurons, i.e. repeatable responses with narrow tuning profiles at moderate stimulus concentrations. However, the proportions of neurons responding to taste qualities are significantly different between the two ganglia, with relatively more vagal neurons responding to bitter or umami stimuli and fewer to salty or sweet stimuli than geniculate neurons. The surprising over-representation of umami-responding vagal neurons led us to explore the potential physiological relevance of activating this population. We find that MPG+IMP elicits more salivation at the posterior tongue than at the anterior tongue. Together, these data provide a better understanding of the response profiles and physiological roles of gustatory signaling through the posterior taste pathway.

3 **Taste Nerve Arbor Morphology Is Not Determined By Taste Bud Size Or Cellular Composition**
Lama Hanbali, Lisa Ohman, Robin Krimm
University of Louisville School of Medicine, Louisville, KY, United States

On the tongue, taste buds are localized to three classes of papillae, which differ in size and cellular composition. How do these differences in taste bud size and cellular make up influence the portion of the taste nerve fibers which innervate the taste bud (arbors)? To address this question, we compared the morphology of arbors in circumvallate (CV) and fungiform (FF) taste buds and their proximity to receptor cells. Taste-transducing cells were immunohistochemically stained and nerve fibers were labeled with sparse cell genetics and images were captured using confocal microscopy. Locations where nerve fibers and receptor cells were overlapping (contacts) were identified using the Imaris software and branching structures of individual nerve arbors were traced using Neurolucida. We confirmed that CV taste buds are larger than FF taste buds ($p<0.0001$). However, the size of arbors in CV taste buds compared to FF taste buds is not different as measured by convex hull. The arbors from CV taste bud were less complex in that they had fewer terminal branches than arbors from FF taste buds ($p<0.05$). This indicates tastes bud size does not regulate arbor size or branching. Although CV arbors were less complex than FF arbors, they contacted the same number of taste-transducing cells (CV=1.51, FF=1. 87). We found that CV taste buds have a larger number of Car4 cells per taste bud ($p<0.0001$), but have the same number

of PLCB2 cells, as compared to FF taste buds. However, the percent of arbors contacting only PLCB2, only Car4, or both cell types was similar between arbors in CV and FF taste buds. This suggests that contacts between different cell types is similar between arbors entering FF and CV tastebuds. Taken together, neither taste bud size, nor cellular make-up influences taste nerve arbor structure.

4 **Mapping Of Gustatory Pharyngeal Neural Responses In Sea Lampreys**

Hasan Polat[1], Gianfranco Grande[1], Zeenat Aurangzeb[1], Réjean Dubuc[2], Barbara Zielinski[1]
[1]University of Windsor, Windsor, ON, Canada, [2] Université de Montréal, Montreal, QC, Canada

In sea lampreys, jawless fish of ancient extant lineage, taste buds are positioned to monitor the chemical content water entering the pharynx from the exterior through seven paired gill pores. The pharyngeal taste buds adjacent to the first and second gill pores are innervated by the glossopharyngeal (IX) nerve and the taste buds associated with gill pores three to seven are innervated by the vagal (X) nerve. We investigated neural taste responses at these different pharyngeal locations, by testing neural responses to the tastants sucrose, amino acids, bile acids and bitter compounds. We did not see differences in the tastant repertoire between the pharyngeal regions, indicating that the differential innervation and distribution of taste buds is not likely linked to the chemoreception of different tastants. However, the response magnitude varied at the different locations, and may code the rostral-caudal location of the tastants, as this spatial difference may signal the location of the food source to the lamprey.

5 **Pre- And Post-Covid-19 Assessments Of Taste And Smell In A Prospective Cohort Study In New Jersey**

Vaishnavi Coneti[1], Valerie B. Duffy[2], Daniel B. Horton[3], John E. Hayes[4], Patricia Greenberg[5], Tracy Andrews[5], Emily S. Barrett[5], Jeffrey L. Carson[6], Martin J. Blaser[7], Reynold A Panittieri Jr.[6], Shristi Rawal[1]
[1]Department of Clinical and Preventive Nutrition Sciences, Rutgers School of Health Professions, Newark, NJ, United States, [2]Department of Allied Health Sciences, University of Connecticut, Storrs, CT, United States, [3]Department of Pediatrics, Rutgers Robert Wood Johnson Medical School, New Brunswick, NJ, United States, [4]Department of Food Science, College of Agricultural Sciences, The Pennsylvania State University, University Park, PA, United States, [5]Department of Biostatistics and Epidemiology, Rutgers School of Public Health, Piscataway, NJ, United States, [6]Department of Medicine, Rutgers Robert Wood Johnson Medical School, New Brunswick, NJ, United States, [7]Center for Advanced Biotechnology and Medicine, Rutgers University, Piscataway, NJ, United States

Evidence for adverse impacts of COVID-19 on chemosensation comes exclusively from retrospective studies that lack data on chemosensory function prior to SARS-CoV-2 infection. We prospectively investigated the effects of COVID-19 on chemosensation in a university and hospital-based cohort in NJ. A total of 329 participants, without prior SARS-CoV-2 infection, completed baseline chemosensory assessments, including self-rated function, 8-item odor identification task, and whole-mouth taste intensities of bitter (1mM quinine) and salt (1M NaCl). Of these, 53 were subsequently diagnosed with COVID-19, and 14 completed follow-up chemosensory assessments. At baseline, all 14 participants reported both their taste and smell as excellent or good. At follow-up (38±35 days after COVID-19 diagnosis), 36% (5 of 14) reported problems with their smell, and 29% (4 of 14) reported problems with taste, including dysgeusia. From baseline to follow-up, the mean odor identification score decreased from 7.8±0.3 to 6.9±1.4 (p=0.03). Of note, the ability to accurately identify smoke declined, from 92.9% (13 of 14) correct assessments at baseline to 71.4% (10 of 14) correct assessments after COVID-19 diagnosis. From baseline to follow-up, the average smell intensity of the 8 odors declined, reaching statistical significance only for smoke (pre-COVID: 65.3±26.2; post-COVID: 37.6±36.6, p=0.01). The average whole-mouth taste intensity of bitter and salt solutions declined from 77.3±16.3 to 67.9 ± 24.7 (p=0.07). This is the first prospective evaluation of the impact of COVID-19 on taste and smell using a pre- and post-test design. Our results confirm previous retrospective findings, and suggest a potential increase in susceptibility to fire hazards after a COVID-19 diagnosis.

6 **Selectively Fluorinated Citronellol Analogues Indicate A Hydrogen Bonding Donor Interaction With The Human Or1A1 Olfactory Receptor**

Mengfan He[1], Weihong Liu[2], Chen Zhang[2], Yingjian Liu[2], Hanyi Zhuang[2], David O'Hagan[1]
[1]University of St Andrews, St. Andrews, *, United Kingdom, [2]Hanwang Technology Co., Ltd. Hanwang Tower, Beijing, *, China

The plant monoterpene alcohol, citronellol, is a well-known linear musk and it has been shown to be an agonist of the olfactory receptor OR1A1.[1, 2] In order to probe electronic and steric aspects of the interaction of citronellol with OR1A1 in more detail we have prepared individual isomers of selectively fluorinated and selectively methylated analogues, modified at the C-2 position of citronellol. These compounds were prepared as single stereoisomers by syntheses routes starting from pulegone. The C-2 methylated isomers gave very poor responses with the receptor indicating a sensitivity towards sterics, however, the C-2 mono fluoro isomers were significantly more active and C-2 difluorocitronellol showed the best activity. The increased response with two electronegative fluorines is indicative of an increased acidity of the hydroxyl hydrogen and suggests a hydrogen bonding donor ability of citronellol in its interaction with OR1A1. This hypothesis was explored further with site specific mutants of the OR1A1 receptor. 1. L. Ahmed, Y. Zhang, E. Block, M. Buehl, M. J. Corr, R. A. Cormanich, S. Gundala, H. Matsunami, D. O'Hagan and M. Ozbil, *Proc. Nat. Acad. Sci.*, 2018, 115, E3950-

E3958. 2. A. Stary, C. Suwattanasophon, P. Wolschann and G. Buchbauer, *Biochem. Biophys. Res. Commun.*, 2007, 361, 941-945.

7 **Human Nasal Beta-Amyloid 42 Reflects Cognition Decline In Alzheimer&Rsquo;S Disease**

Da Hae Jung[1,2], Gowoon Son[1,3], Oh-Hoon Kwon[2], Kyung Hee Kook[1,2], Sheng-Min Wang[4], Hyun Kook Lim[4], Cheil Moon[1,2]

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Introduction: The key in Alzheimer's disease (AD) therapy is a timely and accurate diagnosis for prompt drug intervention. However, due to the high cost and invasiveness of conventional biomarker analyses, including brain positron emission tomography (PET) imaging and cerebrospinal fluid (CSF)-based assays, easy accessibility to these screening tests is often hindered. There is, therefore, a great need to develop a more accessible biomarker screening test using less invasive and cost-effective peripheral body fluid biomarkers. Previous studies examined the non-quantitative expression of beta-amyloid (Aβ) in normal and AD patients' nasal discharge fluid. They identified higher expression of oligomeric Aβ in AD patients, showing a correlation with cognitive decline. However, the quantitative measurements of nasal $A\beta_{42}$ levels, including the full AD continuum, remain unknown. Here, we assessed whether quantified human nasal $A\beta_{42}$ levels could identify patients with AD and differentiate them from non-AD patients. *Methods*: 161 subjects (cognitively normal (CN), n=32; preclinical, n=29; mild cognitive impairment (MCI), n=73; AD, n=27) underwent neuropsychological battery tests. Their nasal discharge samples were collected, and nasal $A\beta_{42}$ levels were measured via enzyme-linked immunosorbent assay (ELISA). *Results*: We found that the second-highest quartile (Q3) group of nasal $A\beta_{42}$ constituted the majority of patients with AD diagnosis (p=0.036). The Q3 group also outnumbered the other groups in the most cognitively impaired subjects in all three neuropsychological battery tests (p=0.023; p=0.008; p=0.037). *Conclusions*: Quantified nasal $A\beta_{42}$ is strongly associated with cognition measurements. Nasal $A\beta_{42}$ suggests the possibility for discriminating AD from non-AD.

8 **Selection Of Odors In Multimedia Based On Odor Categories Watched With Objects In Scenes**

Kwangsu Kim[1,2], Jisub Bae[3], JeeWon Lee[1], Sun Ae Moon[1], Oh-Hoon Kwon[2], Cheil Moon[1,2]

[1]Department of Brain and Cognitive Sciences, Graduate School, Daegu Gyeongbuk Institute of Science and Technology , Daegu, *, South Korea, [2]Convergence Research Advanced Centre for Olfaction, Daegu Gyeongbuk Institute of Science and Technology, Daegu, *, South Korea, [3]Brain Engineering Convergence Research Center, Daegu, *, South Korea

Multimedia has mainly focused on visual and auditory senses, although humans detect numerous stimuli using five senses. As one of the attempts to extend senses used in multimedia, olfactory stimulation has been used in multimedia content for enhancing the sense of multimedia's reality. Matching odors with objects in scenes is mainly conducted when selecting odors for multimedia. However, it is impractical to select all odors matched with all objects in scenes and offer them to viewers. As an alternative, offering an odor in a category was suggested to represent odors belonging to the category. However, it is still unclear whether viewers' responses to videos with multiple odors (e.g., rose, lavender, lily) from a category (e.g., flower) can be comparable. Therefore, we studied whether odors belonging to the same categories could be similar by monitoring congruency and five frequency bands (delta, theta, alpha, beta, and gamma) of the EEG data in videos. We conducted questionnaires and EEG experiments to validate the effects of odors belonging to similar categories. Our result showed that odors in the similar odor categories were higher congruency to video clips than odors in the different odor categories. In our EEG data, mainly delta and theta bands were clustered in both video clips when odors were offered in similar categories. However, alpha, beta, gamma bands were not clustered depending on the categories. Our studies suggested that choosing the odors based on odor categories in multimedia can be partially feasible.

9 **Designing A &Ldquo;Smell-Aid&Rdquo; Through Enhancing Intranasal Air And Odorant Delivery Patterns**

kai Zhao, Zhenxing Wu, Gabriela Zappitelli, Bhakthi Deshpande
Department of Otolaryngology - Head & Neck Surgery, The Ohio State University, Columbus, OH, United States

Innovations to enhance sensory functions have importantly advanced human civilization, e.g. for vision: the microscope, telescope, and eye glasses; for hearing: stethoscope, hearing aids, etc.- they all serve to enhance the external stimuli to enable us to see or hear things that we wouldn't otherwise be able to. But we have no equivalent technology for sense of smell. We attempt to design prototypes of"Smell-Aid" that may enhance the odorant delivery to the olfactory epithelium, using: (a) a nasal foam plug with a diagonal channel embedded, confirmed by computer modeling that would direct air/odor flow upwards to the olfactory region; (b) a clip (similar to synchronized swimmers use) pinching a critical nasal valve region that may intensify the nasal airflow vortex to the olfactory region. Detection threshold to phenylethyl alcohol (PEA) were measured in 58 healthy controls, in counterbalanced order, without interventions (baseline) vs with a "pinch" and with the nasal plug

inserted up or down. A significant correlation was found between degree of olfactory improvement and baseline olfactory sensitivity (r=-0.41, p<0.05), with most improvement in subjects with less sensitive smell function to begin with. This makes sense - as an analogy, corrective lenses may have limited effect on a perfect 20/20 vision but can significantly improve suboptimal vision. Thus, we divided the sample based on the median of PEA thresholds (16.5) into “average” (8-16.5, n=30) and “super smeller” (>16.5, n=28), and found that PEA thresholds were significantly improved in the average group (baseline 12.5+-2.8, pinch 14.75+-5.4, plug up 14.41+-4.9, p<0.05), but not among the “super smeller” nor in plug down condition. Novel approaches to enhance nasal airflow and olfactory odor delivery may one day lead to an OTC smell aid.

10 **Thalamocortical Input To Rat Primary Gustatory Cortex Is Modulated By Inhibition**

Melissa S Haley, Dylan Gordon, Alfredo Fontanini, Arianna Maffei
Stony Brook University, Stony Brook, NY, United States

Information about the physicochemical properties of taste stimuli are transmitted to gustatory cortex (GC) via projections from the gustatory thalamus (VPMpc), and inactivation of VPMpc dramatically decreases taste-related activity in GC. The underlying synaptic architecture by which VPMpc recruits GC circuits is unknown. Here we used optogenetic circuit-breaking, ex vivo whole cell patch clamp recordings, and pharmacology to examine the laminar distribution of VPMpc afferents in GC and investigate the role of inhibition in shaping thalamocortical transmission. We found that VPMpc axons are broadly distributed across GC and make functional synapses in all layers, with the strongest input onto L4 neurons. Furthermore, VPMpc inputs show frequency-dependent short-term dynamics. In agreement with our previously published data, we could distinguish two subpopulations of thalamorecipient pyramidal cells based on the presence of VPMpc-evoked inhibition. Neurons in these two populations differed in the amplitude of the VPMpc-evoked responses as well as in baseline excitability. To identify the source of this feedforward inhibition we recorded from GABAergic neurons in GC and found that VPMpc axons synapse directly onto fast spiking GABAergic cells. To further investigate the role of VPMpc-evoked inhibition, we used pharmacological tools to activate specific GABA receptors and found that inhibition can modulate VPMpc-evoked responses via pre- and postsynaptic activation of GABA receptors. Our results identify two mechanisms by which VPMpc-evoked inhibition can modulate thalamocortical transmission in GC – a feedforward, postsynaptic mechanism driving polysynaptic inhibition onto GC neurons, as well as a feedback, presynaptic mechanism where VPMpc-evoked GABA release can act directly on VPMpc axon terminals.

11 **Tnf-A Orchestrates Experience-Dependent Plasticity Of Excitatory And Inhibitory Synapses In The Anterior Piriform Cortex**

Chunyue Geoffrey Lau, Anni Guo
City University of Hong Kong, Hong Kong, *, Hong Kong

Homeostatic synaptic plasticity, which induces compensatory modulation of synapses, plays a critical role in maintaining neuronal circuit function in response to changing activity patterns. Activity in the anterior piriform cortex (APC) is largely driven by ipsilateral neural activity from the olfactory bulb, and hence is a suitable system for testing the effects of ongoing activity on cortical circuits. Pro-inflammatory cytokine tumor necrosis factor-α (TNF-α) can modulate excitatory and inhibitory synapses, but its role in APC plasticity is unexplored. Here we examined the role of TNF-α in adjusting synapses in the mouse APC after experience deprivation via unilateral naris occlusion. Immunofluorescent staining revealed that activity deprivation increased excitatory synaptic density and decreased inhibitory synaptic density in wild-type mice, consistent with homeostatic regulation. Quantitative RT-PCR showed that naris occlusion increased the expression of *Tnf* mRNA in APC. Critically, occlusion-induced plasticity of excitatory and inhibitory synapses was completely blocked in the *Tnf* knockout mouse. Together, these results show that TNF-α is an important orchestrator of experience-dependent plasticity in the APC.

12 **Central Processing Of Olfactory And Trigeminal Stimuli In Migraine Patients With And Without Aura**

Coralie Mignot[1], Vanda Faria[1,2,3], Marie Frost[4], Thomas Hummel[1], Gudrun Gossrau[4], Antje Hähner[1]
[1]Smell and Taste Center, TU Dresden, Dresden, *, Germany, [2] Department of Psychology, Uppsala University, Uppsala, *, Sweden, [3]Pain and Affective Neuroscience Center, Department of Anesthesiology, Critical Care & Pain Medicine, Boston Children's Hospital, Harvard Medical School, Boston, MA, United States, [4]Pain Center, TU Dresden, Dresden, *, Germany

Accumulating data emphasizes the importance of olfaction in migraine pathophysiology. However, there are only a few studies evaluating how the migraine brain processes olfactory stimulation, and no studies comparing patients with and without aura in this context. In female migraine patients with aura (n=14) and without aura (n=20), event-related potentials were recorded during a pure olfactory or a pure trigeminal stimulus, in the left or in the right nostril. Data were analyzed both, in the time domain and in the time-frequency domain. Segmentation into microstates and source reconstruction analyzes were also performed. For left-sided stimulation, patients with aura had higher amplitudes of event-related potentials in response to the trigeminal stimulus and a disturbed olfactory processing. Their neural activity was enhanced for both stimuli in several regions (trigeminal: bilateral gyrus rectus, right inferior frontal gyrus, right cerebellum, right insula, right somatosensory cortex; olfactory: gyrus rectus, bilateral frontopolar cortices, right middle frontal gyrus, right inferior frontal gyrus, right entorhinal cortex, right insula, inferior occipital gyrus). They also experienced a lower number of microstates in the brain responses to the stimulations. Altogether, it seems that trigeminal and olfactory stimulations are processed differently in migraine patients with or without aura when delivered in the left nostril. Patients with aura in particular show hyperactivity in pain related structures and seem to have a disorganized olfactory processing. The overlap between nociception and olfaction provides a clue to understand

why patients with aura appear to be more impaired, as some of the structures shared by both sensory systems show differential activity patterns between patients groups.

13 **Respiratory-Linked Sleep Spindles In Human Olfactory Cortex**

Justin Morgenthaler, Andrew Sheriff, Chris Cyr, Navid Shadlou, Julia Jamka, Josh Rosenow, Stephan Schuele, Greg Lane, Christina Zelano
Northwestern University, Chicago, IL, United States

Nasal breathing drives neural oscillations in the olfactory epithelium, olfactory bulb, and piriform cortex, and other limbic and neocortical areas. Recent findings in rodents suggest respiration coordinates UP/DOWN state transitions during sleep, which are linked to hippocampal sharp-wave ripples important for memory consolidation. Depolarizing UP states are grouped by slow oscillations and drive thalamocortical spindles and sharp-wave ripples in the hippocampus. Human sleep spindles (12-15 Hz) occur during stage 2 sleep. Could breathing rhythms function to group neuronal activity in piriform cortex in depolarizing UP and hyperpolarizing DOWN states, given the lack of thalamic relay in between the periphery and piriform cortex? If spindle activity is correlated to nasal breathing, then it should have a skewed distribution across respiratory cycles during stage 2 sleep, such that more spindles would occur during inhalations. Here we used intracranial EEG to test this hypothesis by analyzing human piriform cortical oscillations during sleep. Preliminary results from one subject show significantly increased spindle activity during inhales in human piriform cortex during stage 2 sleep ($p < 0.05$, FDR corrected for multiple comparisons).

14 **Chemosensory Coding Strategy In The Gustatory Cortexof Active Licking Mice.**

Camden Neese[1], Cecilia G Bouaichi[2], Martin Bauer[3], Tom Needham[3], Richard Bertram[4], Roberto Vincis[2]
[1]Department of Statistics at FSU, Tallahassee, FL, United States, [2]Department of Biological Science and Program in Neuroscience at FSU, Tallahassee, FL, United States, [3]Department of Mathematics at FSU, Tallahassee, FL, United States, [4]Department of Mathematics, Program in Neuroscience and Program in Biophysics at FSU, Tallahassee, FL, United States

A large body of experimental work in behaving rodents has shown that individual neurons in the gustatory cortex (GC) represent taste through time-varying changes in their spiking activity. The predominant view is that the neural firing rate, over an often arbitrary and seconds-long time interval, represent the sole “unit” of taste information. However, it is not known whether the phase of spikes relative to lick timing is used by GC neurons to encode taste information. This is particularly relevant when liquid gustatory stimuli are sensed by rodents through licking - a stereotyped behavior by which fluids are actively introduced in the mouth. To address this question, we recorded spiking activity from >500 single GC neurons in mice permitted to freely lick to receive four liquid gustatory stimuli and water. We then developed a set of data analysis tools, including Support Vector Machine and elastic shape analysis, to first determine the ability of GC neurons to discriminate gustatory information and then to quantify the degree to which spike rate and spike phase, relative to consecutive licks, are used by each coding neuron in distinguishing tastes. Our results show that while GC neurons primarily encode taste information using a rate code, the phase of spiking relative to licks is also used by >50% of GC coding neurons. In addition, comparison of the relative contribution of the two coding modalities revealed that the phase of spike time within a lick cycle is used to complement rate information when a GC neuron is discriminating multiple (> 2) tastes. Overall, our analysis demonstrates a powerful approach for quantifying rate vs. phase coding, and demonstrate the extent to which each coding scheme is used by GC neurons in extracting chemosensory information.

15 **Fast Sniffing As A Potential Mechanism For Entrainment Of Human Neural Oscillations**

Andrew Sheriff, Guangyu Zhou, Julia Jamka, Torben Noto, Gregory Lane, Christina Zelano
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Human nasal breathing entrains neural oscillations across a wide range of brain areas. Thus breathing rhythms directly impact brain rhythms, suggesting that modulations of respiratory behavior could modulate neural oscillations. The impact of respiratory modulations on brain oscillations is relatively unexplored; in particular, the impact of fast sniffing behaviors on neural oscillations in humans is unknown. Neural oscillations in olfactory, hippocampal, and neocortical areas in rodents are modulated by breathing across the range of respiratory frequencies depending on behavioral state. Although humans breathe an order of magnitude slower than rodents at rest, human inspiration is accompanied by increased oscillatory power across a broad range of frequency bands that dissipate during exhalation. We hypothesized that ultra-fast sniffing in humans (if capable, see other poster) would 1. impact the frequency of respiratory-aligned oscillations in the human brain, 2. entrain oscillations that persist through exhalation, and 3. induce oscillations across a wider range of brain areas compared to natural breathing. To test these hypotheses, we collected intracranial EEG data from surgical patients with medically intractable epilepsy. Participants were presented with plain or odorized air and instructed to sniff fast or slow. Multiple olfactory, limbic, and neocortical areas significantly coupled to fast sniffing and in some cases persisted after the fast sniffing bout. The frequency of respiratory coupling was increased in some areas with fast sniffing. These data help consolidate findings between rodent and human studies of respiratory entrained neural oscillations and suggest a potential translational mechanism of fast sniffing to engage limbic and cortical circuits.

16 **Role Of Cortico-Cortical Connectivity In Processing Chemosensory Signals By The Gustatory Cortex.**

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Flavor perception is a multisensory experience requiring smell and taste. Sampling an odor-taste mixture associates the odor with the quality and hedonic value of the taste, resulting in behavioral preferences for odors paired with palatable tastes and avoidance of odors paired with unpalatable tastes. The gustatory cortex (GC) is considered a principle site for processing multimodal chemosensory signals. Recent electrophysiological and behavioral studies show that cortico-cortical interactions between the gustatory cortex and posterior piriform cortex (pPC) influence functional and behavioral responses to odors and tastes. However, it remains unclear how odor-taste mixtures are represented in the gustatory cortex when multiple odors are paired with the same taste. Moreover, it is unclear whether connections from posterior piriform cortex target functionally distinct populations of chemoresponsive neurons in the gustatory cortex. To this end, we injected the posterior piriform cortex in rats with a virus to drive the expression of channelrhodopsin-2 (ChR2) and implanted a drivable multielectrode optrode into the gustatory cortex. Following surgery, rats were given repeated experience with four odor-taste mixtures: two palatable (isoamyl acetate-sucrose and methyl valerate-sucrose) and two unpalatable (benzaldehyde-citric acid and ethyl butyrate-citric acid). Next, we recorded single-unit activity in the gustatory cortex of alert rats during the intraoral delivery of the individual odors, the individual tastes, and the odor-taste mixtures. Immediately following each chemosensory delivery session, we photo-activated ChR2-expressing posterior piriform fibers in the gustatory cortex to identify the population of neurons likely modulated by input from the posterior piriform cortex. We found that ~40% of chemoresponsive neurons in the gustatory cortex are modulated by photostimulation of fibers from the posterior piriform cortex. Both the laser-responsive and non-laser responsive populations are broadly tuned and respond to multiple different odors, tastes, and odor-taste mixtures. Interestingly, a population decoding analysis revealed that both groups accurately represented tastes and odor-taste mixtures, but only the laser-responsive population was able to accurately represent odors. Our preliminary findings suggest a functional role between the chemosensory cortices and support the hypothesis that the gustatory cortex is a key component of the network processing multimodal chemosensory signals.

17 **Assessing Retronasal Odor Perception And Its Relation To Eating Behavior Among Young Children**

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Flavor perception is a critical determinant of food choices, which directly relate to risk for disease. An understanding of flavor perception in young children would inform interventions for eating behavior in early life and reduce health risks later in life. Often referred to simply as “taste”, flavor is in fact a multisensory experience that combines gustation and retronasal olfaction. Although taste preferences are stable from birth, the development of retronasal smell perception remains unknown, partly due to the difficulty in assessing sensory function in young children. The primary goal of this study is to implement a novel protocol for assessing flavor preference in toddlers to investigate development of retronasal odor perception. Subjects were recruited and tested in the local community. Young children ages 3 to 6 years old (n=50) and one of their parents (n=50) were asked to drink solutions containing either a taste or odor compound. Participants rated the solutions on a pictorial liking scale. Video recordings of facial and vocal responses to the solutions were also obtained. Ratings for sweet and bitter taste were stable with age, demonstrating validity of the rating scale. In order to examine changes in flavor perception with age, all solutions were analyzed to determine perceived intensity and valence. Intensities for positive and neutral odors decreased with age. Valences of positive and negative odors changed with age, indicating higher variability in odor valences in children. This suggests that intensity and valence of retronasal odor perception differ between children and adults and can potentially be modified by experience. Ongoing work focuses on the relationships between retronasal odor perception and individual differences in eating behavior within children.

18 **Mesenchymal Bmp-Alk3 Signaling Promotes Taste Papilla Cell Differentiation Through Suppressing The Production Of Inhibitory Secretory Proteins**

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BMP signaling is one of the major pathways involved in epithelial-mesenchymal interactions that govern taste papilla formation. In our previous studies, we have demonstrated that conditional knockout of type I BMP receptor *Alk3* (*Alk3cKO*) in mouse tongue mesenchyme (*Wnt1-Cre* driven) resulted in an absolute absence of sonic hedgehog (Shh)+ taste papilla placodes in E12 tongue epithelium. To understand how mesenchymal ALK3-mediated BMP signaling (BMP-ALK3) regulates taste papilla formation, a bulk RNA-Seq analysis was performed. We identified 287 differentially expressed genes in tongue epithelium (DEGs, *Alk3cKO* versus controls) as compared to the 63 DEGs in the tongue mesenchyme. The development of Shh+ taste papillae was inhibited when E12 wild type tongues were co-cultured with *Wnt1-Cre*/*Alk3 cKO* tongue mesenchyme or cultured with *Alk3 cKO* mesenchyme-conditioned medium demonstrating an enhanced secretion of inhibitory factors from the *Wnt1-Cre*/*Alk3 cKO* tongue mesenchyme. To identify the secretory factor(s), protein fractions (<10, 10-30, 30-50, 50-100, and >100 kDa) were obtained from *Alk3 cKO* and control tongue mesenchyme-conditioned medium for functional analyses in E12 wild type tongue cultures. Shh+ taste papilla development was suppressed in cultures fed with proteins at 10-100 kDa molecular weight. Further, liquid chromatography-mass spectrometry was performed and identified 72 over-produced proteins from the *Wnt1-Cre*/*Alk3 cKO* tongue mesenchyme compared to controls. Functional analyses are ongoing to identify individual inhibitory secretory protein(s). Together, our data indicated that mesenchymal BMP-ALK3 signaling suppresses the production of inhibitory secretory proteins and promotes taste papilla development.

19 **&Shy;Dll4-Notch1 Signaling Determines Apical Vs Basal Neuronal Cell-Fate Determination In The**

Vomeronasal Organ Of Rodents

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The Vomeronasal organ (VNO) is a part of accessory olfactory system (AOS) that plays a primary role in the detection of pheromones that trigger a spectrum of sexual and social behaviors. Most of the mammals have uniform AOS system with only one type of vomeronasal neurons (VSNs). However, rodents have two main classes of VSNs – 1) VSNs in the apical zone of the vomeronasal epithelium express V1R receptors, Gai2 G-protein subunit, Meis2 transcription factor (TF) and project their axons to the anterior portion of the accessory olfactory bulb (AOB) and 2) VSNs in the basal zone contain V2R receptors, Gao subunit, Tfap2e TF and project to the posterior portion of the AOB. These two VSN cell types form from a common pool of stem cells. Notably, the mechanisms underlying their cell fate determination are not fully understood. To address this question, we performed single cell RNA sequencing of whole VNOs from adult C57B6 wildtype males. Single cell clustering analysis identified non-symmetric expression of the Notch1 receptor and Dll4 ligand among Neurog1+ precursors along the Meis2- and Meis2+ differentiation trajectories respectively. In-vivo conditional Notch1 receptor loss of function experiments, at Ascl1+ progenitor stage, shifted VSN differentiation towards the apical cell fate. Interestingly, inducing Notch Intracellular domain (NICD) gain of function at Ascl1+ progenitor stage redirected the cells towards non-neuronal Sustentacular cell fate, whereas NICD induction at later Neurog1+ precursor stage shifted neurogenesis towards the basal VSN fate. Overall, our research demonstrated that Dll4-Notch1 signaling controls the apical Vs basal VSN cell fate determination in rodents.

20 **Don Tucker Finalist: Microglia-Neuroblast Interactions In The Developing Rostral Migratory Stream**

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OBJECTIVE: This work investigates if microglia ablation affects neuroblast migration down the rostral migratory stream (RMS). METHODS: Mice were electroporated with a tdTomato plasmid in the cerebral lateral ventricles at postnatal day 0 (P0) to label proliferating neuroblasts. To selectively deplete microglia, CX3Cr1-cre-ERT2/ROSA26-eGFP-DTA were injected with tamoxifen at P0 and sacrificed at P3 or injected every 3 days until sacrifice at P14. Immunohistochemistry was performed with primary antibodies against Iba1 and cleaved caspase-3 (CC3). Stained cells were segmented and quantified using Fiji (ImageJ) software. Differences in cell density were evaluated with a Student t-test. To examine microglia phagocytosis of neuroblasts, CX3CR1-GFP/DCX-DsRed mice were stained with primary antibodies against CD68, CLEC7A, and MERTK. RESULTS: Microglia line the rostral migratory stream (RMS) and contact migrating neuroblasts during early postnatal development. Microglia ablation did not impact the distance neuroblasts migrated down the RMS. However, microglia depletion for 14 days was associated with an accumulation of apoptotic cells in the RMS. Microglia wrapping neuroblasts in the RMS express phagocytic markers, suggesting active phagocytosis of neuroblasts in the developing RMS. CONCLUSIONS: Microglia closely interact with migrating neuroblasts in the developing RMS. Microglia ablation did not impact the migratory capacity of neuroblasts in the RMS, suggesting microglia are not necessary for neuroblast migration. However, phagocytic microglia were observed to wrap neuroblasts, while microglia ablation was associated with an accumulation of apoptotic cells. Microglia may thus regulate neuroblast migration by restricting neuroblast number or selectively eliminating those showing aberrant migration.

21 ***Gli2* And *Gli3* Regulate Horizontal Basal Cell Mediated Regeneration Of The Olfactory Epithelium**

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The olfactory epithelium (OE) is a specialized neuroepithelium that is replenished by two presumed stem cell populations: rapidly dividing globose basal cells (GBCs), and relatively quiescent horizontal basal cells (HBCs). While HBCs and GBCs both contribute to OE regeneration, the signaling pathways that control this process are not well understood. Recent work indicates that HBCs contain primary cilia, cellular organelles that coordinate signals from multiple pathways. Notably, primary cilia are essential for proper mammalian Hedgehog (HH) signaling, making the HH pathway an attractive candidate in the control of HBC function. GLI proteins are the transcriptional effectors of the HH pathway – GLI1 functions exclusively as a transcriptional activator and is also a target of HH signaling; GLI2 is the major transcriptional activator of the HH pathway; conversely, GLI3 acts largely as a transcriptional repressor. My data suggest that both *Gli2* and *Gli3* are expressed in all HBCs. Notably, following methimazole-induced OE injury, *Gli2*, but not *Gli3*, is expressed broadly in both apical and basal cells of the reconstituting OE. To assess possible GLI function in HBCs, I utilized a constitutively active form of GLI2 to stimulate the HH pathway specifically in HBCs. Our data indicate that HBC-specific activation of GLI2 causes hyperproliferation of HBCs of mixed identity, which then fail to differentiate to olfactory sensory neurons following injury. To assess the requirement for endogenous *Gli2* and *Gli3* in OE regeneration, I conditionally deleted *Gli2* and *Gli3* using an HBC-specific *Cre* driver. My data indicate delayed OE regeneration and reduced p63 expression in *Gli2;Gli3* mutant mice at full recovery (8 weeks post-injury). These data suggest a novel role for GLI proteins in adult HBC function.

22 **Dose Dependent And Cell Autonmous Effect Of Gli3 On Oec Development And Gnrh-1 Neuronal**

Migration

Ed Zandro/M Taroc[1,2], Paolo/E Forni[1,2]

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Gonadotropin releasing hormone-1 (GnRH-1) is the master regulator hormone of sexual development and pubertal onset of mammals. This hormone is released by a subset of neurons known as the GnRH-1 neurons (GnRH-1ns), which controls the hormonal axis between the hypothalamus, pituitary gland, and the gonads. The GnRH-1ns reside within the hypothalamus but originate from the olfactory placode in the nose. During embryonic development the GnRH-1ns migrate with a neural crest derived glial cell type known as olfactory ensheathing cells (OECs) along the axons of the terminal nerve to get to the brain and eventually to their final positions within the hypothalamus. Perturbations in the development and/or migration of the GnRH-1ns or their ability to release GnRH-1 in humans leads to a disorder known as hypogonadotropic hypogonadism (HH), characterized with delayed or absent puberty. Previous studies have shown that OECs are crucial for GnRH-1ns migration. We recently demonstrated that loss of transcription factor Gli3 in mouse caused a loss of OECs leading to GnRH-1 migratory defects. Whether cell autonomous loss of Gli3 in the OECs is the cause of this defect is still unknown. To test the cell autonomous role Gli3 in OEC development, and GnRH-1 migration, we utilized two mouse models 1) $Gli3^{Pdn/Pdn}$ which is a hypomorphic Gli3 model and will elucidate if Gli3 has a dose dependent effect and 2) Neural crest specific Sox10Cre/$Gli3^{Flx}$ Gli3 conditional knockout. Our data suggest that Gli3 has dose dependent and cell autonomous effect on OECs development and GnRH-1 migration.

23 **Evc2 Regulates Taste Papilla Development Through Tuning Hedgehog Signaling Activities In A Stage- And Region-Specific Manner**

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Mutations of the *Evc2* gene have been identified in patients with Ellis–van Creveld syndrome which is a genetic disorder characterized by short limb dwarfism, polydactyly, abnormal development of fingernails as well as regional loss of taste papillae. To understand how taste papilla and bud development is affected in Ellis–van Creveld syndrome, *Evc2* knockout mice were used to thoroughly examine the alterations in taste organs. Our phenotypic analyses showed that in *Evc2* knockouts taste papillae developed in a significantly higher number and size. *Evc2* is an essential positve regulator of Hedgehog signaling. To investigate to which extent Hedgehog signaling activity was affected in *Evc2* knockout tongues, *Gli1* and *Ptch1* expression were examined with qRT-PCR and in situ hybridization. We found that *Evc2* knockout resulted in a decreased expression of *Gli1* in the epithelium, and of both *Gli1* and *Ptch1* in the mesenchyme. To further investigate the molecular regulation of *Evc2* in taste papilla placode initiation, RNA-seq were performed to identify downstream targets of Evc2/Hedgehog signaling. Pathway analysis revealed that the majority of up-regulated genes were related with extracellular matrix (ECM), suggesting EVC2/Hedgehog signaling inhibits taste placode initiation by down-regulating ECM-related genes. At E18.5, we observed enlarged and flatten fungiform papillae in *Evc2* knockout mice, which mimics the phenotype in human Ellis–van Creveld syndrome patients. BCL11B, an important transcription factor for papilla morphogenesis, was down-regulated in the abnormal fungiform papillae in *Evc2* knockout mice. Together with other reported data, our results indicate the Evc2 plays a stage- and region-specific roles by tuning Hedgehog signaling activities and other factors during taste organogenesis.

24 **Identifying Humans From The Smell Of Their Ear**

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Mammals can identify conspecifics, and humans, by their body-odor. This has driven an effort to develop machines that can do the same. However, these machines, also referred to as electronic noses, or eNoses, have seen only limited success at this task. A critical step for body-odor based identification is selection of the body-odor to be smelled. Most studies have concentrated on mouth and armpit. These body regions, however, pose specific complications: the mouth has high humidity that complicates real-time sampling, and the armpit is often covered in cosmetics. By contrast, the ear may pose an attractive target for this task. The inside of the ear is 1. accessible, 2. typically not washed or doused in cosmetics, 3. it is a bodily cavity without excessive humidity, and 4. It contains cerumen, a known source of body-VOCs. To test the hypothesis that humans can be identified by the smell of their ear, we used a PEN3 eNose equipped with a custom-tip made of Teflon. We initially sampled 10 individuals across 10 days. Each sample entailed 5 repetitions, each lasting 50 seconds. Using a Fine KNN classifier, we found that within a sampling day, we achieved 82% accuracy, which is significantly better than the 10% chance in this task. In other words, consistent with our hypothesis, humans can be identified in real time by the smell emanating from their ear. However, due to drift, this level of performance drops to 12% when comparing across days. Here we will present this basic result, its comparison to other body-regions in the same body-odor donors, and our efforts to deal with drift.

25 **Tasens Bands: A Novel Method For Self-Administered Taste Testing**

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Objective: To validate a “taste sensor application”, or TASENS (patent pending) for screening taste loss and reduction at home. Materials and Methods: The test consist of 24 taste bands (4x7cm), with two longitudinal agar strips on one side of the band, used to deposit the taste chemical, along with a software application (tablet/computer) for selecting the perceived taste. Six bands (two concentrations, only on the right, only on the left and both agar strips) for each taste profile – salty (sodium chloride; Conc. 1, 0.04g/ml; Conc. 2, 0.1g/ml), sweet (sucrose; Conc. 1, 0.12; Conc. 2, 0.2), sour (citric acid; Conc. 1, 0.09 ; Conc. 2, 0.13), and bitter (quinine; Conc. 1, 0.0009 ; Conc. 2, 0.0024)– were included in the test. We performed a 12-item screening test to check for taste ability (based on the standard 32 strip method) in the 55 participants and asked them to rate their taste function on a visual analogue scale (VAS; 1 to 10). Results: Only 5 of the 55 participants were below the 10th percentile, and thus categorised as hypogeusics (as defined by Landis et al. in 2009). The average TASENS scores for males (n=21) was significantly lower than females (n=34) ($p=0.016$; M=14.57, F=18.20), and so was the negative correlation (Spearman rho) with age ($p<0.001$; M=-0.275, F=-0.133), which is in line with normative taste test results as shown by Landis et al. in 2009. Their TASENS scores were positively correlated with the screening test scores (0.614; $p<0.01$). Conclusion: TASENS bands can be used as a preliminary self-administered taste test for easy follow-up of patients after surgery or viral infection. The next step in the development of this taste test would be to involve more participants with taste disorders to validate its results in the patient population.

26 **Lipopolysaccharide-Induced Inflammation Elevates Bitter Taste Responses And Induces Epigenetic Changes In Taste Bud Cells And Progenitor Cells**

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Taste disorders, including taste loss and taste distortion, are associated with various diseases, such as infection, autoimmune disease, and cancer. Taste disorders contribute to decreased appetite, food aversion, low quality of life, and poor general health. However, the mechanisms of taste disorders, particularly of taste distortions, remain poorly understood. Here we report that in an inflammation model induced by bacterial-derived lipopolysaccharides (LPS) mice showed markedly elevated neural and behavioral responses to bitter taste compounds. Consistently, the expression of many Tas2rs, the bitter receptor genes, was strongly up-regulated in the taste epithelium by LPS treatment. Using single-cell assays for transposase-accessible chromatin with sequencing (scATAC-seq) we discovered that LPS increased chromatin accessibility of multiple Tas2rs. Additionally, scATAC-seq analysis clearly identified cell clusters corresponding to types I, II, and III taste bud cells, as well as progenitor and stem cells. scATAC-seq also confirmed cell-type-specific chromatin accessibility of many previously characterized taste-cell-selectively-expressed genes, such as Tas1rs and Tas2rs. Furthermore, LPS-induced inflammation brought about broad changes in chromatin accessibility in progenitor and stem cells. Together, our results revealed an epigenetic mechanism connecting inflammation and altered bitter taste.

27 **Cyclophosphamide Reversibly Induces The Loss Of Taste Bud Innervation In Mice.**

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Many patients undergoing chemotherapy report taste alterations in the form of reduced, distorted, or complete loss of taste that can last for months after initial treatment. This represents a significant detrimental effect on a patient's quality of life and recovery. Previous studies using cyclophosphamide (CYP) showed a significant loss of Taste Receptor Cells (TRCs) one week after treatment, with the recovery of these cells after two weeks. While this pattern of TRC loss during chemotherapy likely contributes to the initial reduction of taste, this mechanism does not fully explain the prolonged taste disruption /distortion reported by patients. Thus we hypothesize that chemotherapy disrupts synaptic connectivity between TRCs and gustatory fibers, producing both reduced taste sensitivity and taste distortion that contribute to prolonged taste dysfunction. We examined the gustatory fibers throughout the initial time course of chemotherapy using a single dose of 100 mg/kg CYP. Immunofluorescence was used to quantify changes in TRCs and gustatory fibers over the course of treatment in the CV and fungiform papillae. 2-photon imaging were used to track the changes in Phox2b-Cre;Ai9 labeled gustatory fibers innervating fungiform taste buds in anesthetized mice over the course of 12 days. Along with the loss of TRCs, there is a significant decrease of gustatory fiber innervation in the taste buds at 4-8 days post CYP treatment, returning to normal levels when the TRCs return 16 days post treatment. 2-photon imaging shows that this loss of innervation may affect some fungiform taste papilla more than others. Together, this work suggests that the gustatory sensory fibers are indeed affected by CYP and future work may determine if this contributes to the prolonged loss of taste reported by patients undergoing chemotherapy treatments.

28 **Big Data Analysis Of Food Product Reviews Highlights Opportunities For Personalizing Sweetness Levels**

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The food industry strives to provide the tastiest and most attractive products to entice customers to buy their products. This results in the abundance of very sweet, very salty, very fatty food. In contrast, the world Health Organization recommends reducing the consumption of sugars, salt, and fat. This creates tension between taste and health in regards to ingredients used in modern industrialized food. We hypothesized that for some consumers the highly palatable or “extra-tasty” flavor may be too strong, resulting in a misfit un terms of both hedonics and nutritional parameters. We focused on sweet and oversweet taste, and analyzed reviews of food products sold through Amazon and iHerb using word counts, natural language processing and machine learning. Analysis of over 200000 reviews of ~30000 products on Amazon and ~350000 reviews of ~2400 products on iHerb, showed ~7000 and ~8000 respective reviews mention oversweetness (23% and 28% out of reviews mentioning sweetness), with 2200 and 1400 reviews referring directly to the purchased products. Connecting these oversweet reviews to the products' ingredients reveals that products that include particular sweeteners (e.g. glucose and corn syrup on iHerb, or sucralose in both datasets) have more oversweet reviewers than average. 19 products had at least 40 reviews for which at least 10% were oversweet. For 15 of these products the average liking by consumers reporting oversweetness is significantly lower (by 0.9 stars on average, 1-5 scale) than by the rest of the consumers. Our findings that some sweeteners are more likely to lead to oversweetness and that oversweet is less liked, suggest an opportunity for personalized products with reduced sweetness. These products will be healthier and tastier for the subgroup of users, and will benefit the manufacturer by expanding the target audience of the products.

29 **Olfactory Abilities In Alexithymic Individuals**

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Alexithymia is a psychological construct characterized by altered emotional processing. Although emotion and olfaction are closely linked, only few studies have investigated olfactory processing in alexithymia, reporting difficulties in processing olfactory emotional stimuli in individuals with alexithymia. However, these studies do not allow comprehensive conclusions on whether alexithymic individuals present lower olfactory abilities or only altered affective reactions and awareness to odors. Therefore, we conducted three pre-registered studies that aimed to clarify this relation using Bayesian statistics. In Study 1, we assessed olfactory functions using the Sniffin’ Sticks test in a sample of 144 participants (131 women, mean age 23.73) divided in groups of high (HA), medium (MA) and low (LA) levels of alexithymia. The analysis revealed a BF_{10}<0.33, indicating that olfactory abilities were not connected on alexithymia level. In Study 2, the intensity, pleasantness, and familiarity of 16 odors were assessed in a sample of 124 women (mean age 22.87). The analyses indicated that odor ratings did not depend on alexithymia levels (BF_{10}<0.33). Finally, in Study 3, we investigated the attitudes towards odors, the awareness for odors, and the ability to form olfactory images in mind in a sample of 383 participants (292 women, mean age 24.03). The analyses revealed strong evidence in support of the hypothesis that HA group presents lower odor awareness (BF_{10} > 100) and lower affective importance for odors than LA (BF_{10} > 100). Given that alexithymia is considered an important transdiagnostic risk factor for a range of psychopathologies, learning more about the affective reactions and awareness to odors in alexithymic individuals may have important implications for the understanding of those disorders.

30 **A Simplified Method To Measure The Pattern Of Individual Sweet Hedonic Responses**

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The appeal of sweetness is a driver of sugar consumption and understanding individual differences in its appeal may help develop personalized strategies to lower added sugar intake. Current methods to quantify sweet hedonic patterns involve a range of stimulus concentrations and numerous judgements, so there is a need to simplify the testing procedure so that it can be used in large-scale population-based studies, e.g., *NHANEs* or NIH’s *All of Us*. Therefore, we measured the hedonic response in 21 adult participants. In the first method, they rated five sucrose solutions (0.09, 0.18, 0.35, 0.70, 1.05 M) using a rating of liking (on a 100-point visual analog scale) with a paired comparison preference tracking procedure. Based on their ratings of all solutions, participants were classified into three sweet liking patterns (Disliker, Moderate Liker, and Extreme Liker) using a quadratic function fit. Using ordinal logistic regression to predict classification, we found that the individual slopes calculated using two of the solutions (0.09 and 1.05 M sucrose) could predict the patterns with a 76% accuracy (X^2 = 19.11 (4, 21), p <0.001), but using one single concentration (1.05 M) could not (43% accuracy, X^2 = 7.96 (4, 21), p = 0.093). Furthermore, the individual slopes from the ratings of the same two solutions could predict the preferred concentration from the preference tracking procedure (r^2 = 0.77, p <0.001). Overall, using data from two concentrations could provide similar hedonic information as five concentrations. Lager studies are the next step to validate this short test.

31 **Binge Eating Suppresses Flavor Representations In The Mouse Olfactory Cortex**

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Appropriate feeding behavior is the foundation of maintaining homeostasis. Elevated feeding speed (binge eating) is a common trait of eating disorders and is associated with obesity. It is also known that flavor perception has an active role in regulating feeding. However, the effects of feeding speed on flavor sensory feedback remain unknown. By using miniscope in mice, we show that binge eating suppresses neuronal activity in the anterior olfactory (piriform) cortex (aPC). This binge-induced suppression is due to local GABAergic interneurons in aPC, but not due to degraded odor inputs from the olfactory bulb. We further excluded the inhibitory effect from serotonergic modulation in aPC by using *in vivo* serotonin imaging. Taken together, our results provide clear circuit mechanisms of binge-induced flavor modulation, which may explain binge-induced overeating due to suppression of sensory feedback of food items.

32 **A Novel Voluntary Intake Method To Investigate Taste-Elicited Affective Reactions In Mouse**

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Human infants, primates and rodents elicit homologous facial reactions that reflect a degree of affection and aversion to taste stimuli. These spontaneous reactions were used in Taste Reactivity test (TR test) to evaluate hedonic response in animal model. Oral stimulation in conventional TR test was conducted with artificially intraoral infusion of taste solutions. In this study, we developed a novel TR test method by voluntary intake, which taste solutions are delivered similarly to normal ingestive behavior. We analyzed mice oral and facial hedonic reactions and were able to obtain positive affective reactions to sucrose and oleic acid solutions in concentration-dependent manner. Therefore, we propose this method to evaluate mice hedonic response of taste stimuli.

33 **Odor Modulation Of Drug Cue-Reactivity In Alcohol-Dependent Adults.**

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Drug cue-reactivity, the array of physiological, cognitive, and behavioral effects elicited by drug-related stimuli, is an established methodology to investigate drug craving and dependence. Previous functional neuroimaging (fMRI) studies of drug cue-reactivity (i.e. brain activation in response to drug-related versus neutral visual cues) indicate disrupted limbic-prefrontal circuits in the pathophysiology of drug dependence. Given the ability of odors to engage limbic-prefrontal circuits, we sought to explore how a neutral odor influenced drug cue-elicited activation and striatal-prefrontal connectivity. 27 normosmic, non-treatment seeking, alcohol-dependent, adults (23 M, 4 F; mean age 27.1, range 21-39) underwent a validated fMRI task during which either lavender (LAV) or odorless propylene glycol (PG) was delivered within blocks of alcohol (ALC) or neutral (NEU) visual cues. While ALC>NEU+PG demonstrated a pattern consistent with previous studies (i.e. increased activation in bilateral striatum (bS) and medial prefrontal cortex (mPFC); clusters determined by Z>2.3, corrected cluster threshold p<.05), region of interest analyses indicated a significant picture x odor interaction in mPFC (p<.05). LAV normalized drug cue-reactivity in mPFC, reducing activation during ALC to levels measured during NEU. Moreover, mPFC was functionally connected to bS during ALC+LAV, but not ALC+PG. A trend toward a reduction in subjective craving was also noted during LAV compared to PG (p=.083). These findings suggest that engagement of limbic-prefrontal brain circuits via odors may have a role in restoring dysregulated prefrontal circuits and processing of drug cues. Other odors and the relationship between them and brain activation in response to drug cues and craving requires more exploration.

34 **Salient Feature Selection In An Odor-Guided Discrimination Task**

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In a complex odor environment, how do animals identify salient stimuli? In this study, we investigate which stimulus features are relevant for solving an odor discrimination task. Briefly, the mice were trained to discriminate between overlapping multi-component odor mixtures in a digging task. Once the mice reached criterion performance, they were challenged to perform the task with single components of the mixtures on 10% of trials, hereafter referred to as “pop-outs.” We find that mice are able to use a single salient feature of the rewarded mixture to perform the task. However, only features that differed in identity between two mixtures were salient. When presented with the sole component that differed between the mixtures (acetophenone) mice successfully dug in the correct pot. However, when mice were presented with the structurally similar and vapor pressure matched component (methylbenzoate) that differed only in concentration between two mixtures, the mice either correctly aborted the trials or continued searching for the rewarded odors. Our findings suggests that when mixtures that differ by two features are presented, identity or concentration, mice prioritize using the identity of a single component over concentration differences, or the mixture as a whole. This finding begs the

questions of how or even if mixtures are encoded in discrimination tasks and how does the olfactory system filter salient versus irrelevant features of an odor mix?

35 **Interaction Of Trigeminal, Somatosensory, Olfactory, And Respiratory Signaling In The Mouse Olfactory Bulb**

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Odor-cued aversive conditioning induces odor-specific plasticity throughout the olfactory system. However, it remains unclear whether information about an aversive, non-olfactory stimulus converges with olfactory signals in the olfactory bulb. We used *in vivo* calcium imaging of population-level neural activity in mitral cells and olfactory sensory neuron (OSN) terminals in the olfactory bulb to test whether aversive trigeminal or somatosensory stimulation could impact olfactory bulb signaling and whether the evoked activity interacted with ongoing respiration- and odor-evoked activity. Stimulation of the trigeminal nerve via CO_2 presentation or direct electrical stimulation evoked robust bilateral neural activity in the olfactory bulb's mitral cells in anesthetized mice. No such response was observed in the OSN terminals. Naris occlusion eliminated both spontaneous respiration-locked and trigeminal nerve shock-evoked mitral cell activity ipsilateral to the occlusion, but contralateral responses persisted. Similar results were observed using electrical stimulation of the tail, which evoked bilateral, respiration-coupled bursts of population activity in olfactory bulb mitral cells that was eliminated when intranasal airflow was prevented. These findings illustrate that aversive non-olfactory stimuli, including those used in olfactory fear conditioning paradigms, can evoke strong, presumably centrifugal modulation of early olfactory signaling. This convergence of odor- and shock-related activity suggests that the early olfactory circuit could be a locus for associative learning. The apparent respiratory gating of bulbar signals driven by aversive sensory stimulation suggests that the olfactory bulb may be an unexpected intermediary between respiration and pain perception (Iwabe et al. 2014). Keywords: trigeminal, plasticity, coding

36 **Fear Generalization And Extinction Learning Alter Primary Sensory Input To The Brain**

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Generalization of learned fear is typically thought to occur in brain networks far removed from the sensory periphery, such as the amygdala and prefrontal cortex. However, work from our lab and others has demonstrated that odor-cued conditioning paradigms inducing generalizing fear, where mice become afraid of odors beyond those experienced during training, boost neural responding these odors in the periglomerular and mitral cells of the olfactory bulb. Do these generalization-related changes demonstrate learned changes in those neuronal populations, or do they reflect upstream plasticity in the input from the olfactory periphery? If so, how does that plasticity relate to the mouse's beliefs about the odors? To answer these questions, we used single-odor fear conditioning in mice that generalized to be afraid of multiple odors, as demonstrated on an olfactory avoidance task. *In vivo o*ptical imaging of exocytosis from populations of olfactory sensory neuron (OSN) terminals in the olfactory bulb revealed a large increase in OSN output evoked by not only the shock-predictive odor (the CS) but also by other odorants to which the mouse had generalized its behavioral fear response. Extinction training, in which the fear conditioned mouse was subsequently taught that the original CS no longer predicted a shock, reversed the learning-induced changes in odor-evoked OSN output for all odors. "Novelty-based refinement training", in which fear conditioned mice were exposed to the alternate odors to which they had generalize their fear but not exposed to the CS, narrowed the range of odors that evoked fear and exhibited facilitated OSN output. The ability to narrow fear, at the sensory and behavioral levels, to just the stimuli that are appropriately supposed to cause fear, has clinical implications.

37 **Long-Term Odor Memory Is Required For Efficient Odor Plume Navigation**

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Mice use the sense of smell to adapt to the environment in order to survive. The cognitive ability to navigate turbulent odor-plumes allows a mouse to find important survival variables such as home location, food, predators, competitors, sexual partners, among others. However, it is not clear how the brain solves this complex task. Here, we present a novel computational model of a virtual mouse navigating an odor plume to investigate the role for odor memory in adaptive behavior. The odor plume is spatially turbulent and the odor gradient breaks over time. The modeled speed of the movement of the virtual mouse is given by x and y velocities that change with the odor gradient. Because the plume changes spatiotemporally in a turbulent fashion, the virtual mouse faces difficulties to find the odor source when a long-term memory is not present. A spatiotemporal odor memory is provided by averaging over time the previous odor concentrations experienced by the mouse in each visited location. In this way, the memory develops an odor cognitive map of the arena, a process that we assume is linked with the activity of place cells in the hippocampus. In the learning phase the mouse is trained on a subset of odor plumes. Then, once the odor memory has been created, the virtual mouse performance is tested in a different subset of odor plumes. The simulation results show that the virtual mouse without a long term-memory can find the source of the odor plume in ~50% of the trials. In the condition with long-term memory the virtual mouse reaches the target ~80% of the trials. The model results suggest that long-term memory can provide a cognitive map of the odor plume gradient that improves significantly the mouse performance in a modeled odor plume navigation task.

38 **Computational Molecular Interaction Maps Of Signaling Events Within The Olfactory Epithelium**

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In the olfactory epithelium (OE), multiple mechanisms, like odor detection, cell regeneration, and differentiation are vulnerable to a variety of external and/or internal factors. However, the understanding of the cell-to-cell communications and molecular events associated with these mechanisms are still not fully characterized. To provide a global vision of the OE and cross-talks between its different cell types, we prepared maps related to signaling and molecular events in sustentacular cells, microvillous cells, Bowman's glands, trigeminal nerve fibers, horizontal basal cells, globose basal cells, and olfactory sensory neurons accessible via an interactive, searchable, web-based platform through MINERVA, a well-established tool used for the presentation of disease maps (https://www.sbi.uni-rostock.de/minerva/). The molecular single-cell and interaction maps we developed will serve to conceptually visualize and analyze complex mechanisms within single cell types as well as among different cell types. The developed maps provide various entry points to the users to access the manually curated information at the cellular, process/pathway, and molecular level. The maps are designed with the aim to serve heterogeneous communities involved in olfaction including clinicians, research scientists, systems biologists, and industrial partners. In the web platform of the maps, users can identify and prioritize diagnostic/therapeutic markers associated with various olfactory diseases. For this, we developed various user-friendly plugins that help in mapping and analyzing experimental and clinical data directly onto the map. Here we provide a quick overview of manually annotated known signaling events within OE cells and highlight knowledge gaps that need further investigation.

39 **Involvement Of The Intranasal Trigeminal System In Chronic Rhinosinusitis (Crs)**

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In CRS patients, nasal obstruction can be explained by structural deformities, polyps, or edematous nasal mucosa. However, in some cases, no major deformity or inflammation is present to explain the sensation of nasal obstruction of these patients. Here, nasal obstruction may result from an alteration of the afferent neural pathways of the trigeminal system, responsible for airflow perception. The aim of this study is to assess the involvement of the intranasal trigeminal system in reduced nasal patency in CRS patients. Methods: We carried out a prospective case-control study of 15 patients with CRS, 18 patients with a deviated nasal septum (DNS) and 16 healthy controls. We used Peak Nasal Inspiratory Flow (PNIF) and Visual Analog Scales (VAS), to assess objective and subjective nasal patency respectively. We further examined sensitivity of the intranasal trigeminal system using the Trigeminal Lateralization Task (TLT). Finally, we measured expression of trigeminal receptor TRPM8 in mucosal biopsies taken intraoperatively from CRS patients and DNS patients by quantitative real-time PCR. Results: CRS patients had significantly lower objective nasal patency than healthy controls ($p=0.046$) and subjective nasal patency than both DSN patients ($p<0.001$) and healthy controls ($p<0.001$). In line with this, CRS patients had significantly lower trigeminal sensitivity than DNS ($p=0.047$) and healthy control ($p=0.005$). We did not observe a group difference in the expression of TRPM8. Conclusion: The present data suggests that reported nasal obstruction in CRS patients may be linked to a combination of deficient perception of nasal airflow by the trigeminal system and a mechanical obstruction not visible by nasal endoscopy. The association with trigeminal receptors remains to be elucidated.

40 **An Olfactory-Specific Microbiota: Implications For Function**

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The impact of the microbiota on human and animal health has become an increasing area of interest. The role that the microbiota plays in gut and mental health support the concept of a microbiota-gut-brain axis and its bidirectional functionality. Research is limited for the impact of microbiota on olfactory function. The gut microbiota affects olfaction indirectly through the production of neurotransmitters but the nasal microbiota may have direct functional relevance to olfaction. The nasal microbiota and its impact on olfaction are understudied and there is a need to define what the microbiota composition is of the olfactory epithelium and what implications it has on olfactory function. Our pilot work using 16S rDNA sequencing has demonstrated a unique composition of microbes present in the olfactory epithelia and within distinct regions of the olfactory recess. In culture work with these separate regions we find that the total colony forming units are higher in the ethmoid when compared by weight to the respiratory epithelium and the olfactory epithelium moderately between the two. In whole-genome shotgun metagenomic data, the olfactory tissue yielded a significantly higher proportion of microbial reads and much lower host DNA, suggesting the microbial community in that tissue is an integral

and essential part of the function. This preliminary data is suggestive of a compositional and abundance difference between the respiratory epithelium and the olfactory epithelium collectively, with significantly distinct regions within the ethmoid region. We suspect that this olfactory microbiota plays an important role in olfaction and we expect that this population would be sensitive to conditional changes, such as heat stress or antimicrobial therapies, that could result in a level of dysbiosis.

41 **Aav-Php.S-Mediated Delivery Of Reporters To Chemosensory Ganglia**

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Adeno-associated viruses (AAVs) are essential tools for defining central circuits and functional neural interactions. A synthetic serotype, AAV-PHP.S, was reported to target peripheral nervous system neurons in dorsal root ganglia, and enteric neurons. We sought to expand the use of this virus to cranial ganglia, particularly for chemosensation. Fluorescent neurons were seen in the dorsal root, nodose, petrosal, trigeminal, and geniculate ganglia. A time course of expression in the geniculate ganglion showed that GFP was detected by 2 days post-injection, and reaching the maximum of labeled neurons (≈70%) by 7 days. By 7 days post-injection, GFP-labeled nerve fibers could be detected in peripheral projections such as within the circumvallate taste buds, while GFP fibers were evident in fungiform taste buds only by 14 days. Similarly, in the medulla and spinal cord, GFP-labeled sensory afferent fibers (gustatory, trigeminal) were present, allowing us to image in detail the projections to resident CNS neurons. Further, we did not detect any GFP positive neuronal somata in the CNS. To label cell-type selective neurons, we injected a Cre-dependent GCaMP6s in AAV.PHP.S into Mafb-mCherry-Cre mice. In the geniculate ganglion, ~90% of Mafb-expressing neurons (auricular and T2 types) were fluorescent while other neuron types remained unlabeled. We also tested neuronal responses by imaging GCaMP6s-expressing neurons in anesthetized mice while stimulating the pinna. This constitutes the first demonstration of mechanosensitivity in these auricular neurons (which were so named based only on neuroanatomy). Our study expands the use of AAV to many peripheral somatic and visceral sensory neurons, and studies of their sensitivities, receptive fields and circuits, that until now have been difficult to assess.

42 **Chemogenetic Inhibition Of Somatostatin Neurons In The Nucleus Of The Solitary Tract Differentially Modulates Bitter And Sweet Taste Signals**

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The rostral nucleus of the solitary tract (rNST) is the first central taste circuit and houses a network of heterogeneous neurons. A recent study (Jin et al., 2021) proposed that one genetically distinct neuron type, comprised of rNST neurons expressing somatostatin (Sst), exclusively processes bitter taste. However, in the caudal NST, Sst neurons are a mixed population of excitatory glutamatergic and inhibitory GABAergic cells, suggesting that rNST Sst neurons might be multifunctional. To explore this, we made injections of a cre-dependent AAV virus expressing an inhibitory DREADD (hM4Di) into the rNST of Sst-cre mice and then tested licking in response to concentration series of bitter (quinine) and sweet (sucrose) stimuli after I.P. injections of CNO or saline. Consistent with the previous report, inhibition of Sst neurons increased quinine licking (taste: water ratio, P=0.0004, N=6), suggesting that bitter signaling was suppressed. More surprisingly, sucrose licking also increased (taste-water licks, P=0.04, N=5), similar to our previous observations using DREADDs to suppress rNST GABA signaling. To explore the possibility that effects on bitter and sweet-driven behaviors might arise from glutamatergic Sst versus GABAergic Sst neurons, we performed fluorescent in situ hybridization for Sst, VGLUT2, and VGAT in the rNST. Preliminary data based on counting 249 Sst-positive cells (2 mice, 4 rNST sections) show that ~2X as many Sst neurons are GABAergic (N=162) than glutamatergic (N=81). Thus, rNST Sst neurons are heterogeneous and have the potential for multiple functions. Ongoing optogenetic neurophysiological studies in mice expressing ChR2 in Sst neurons are directly assessing the gustatory response profiles of these cells and their ability to modulate taste activity in other neurons.

43 **Anatomical And Functional Dissection Of The Anterior Olfactory Nucleus To Nucleus Of Lateral Olfactory Tract Pathway In Mice**

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The olfactory bulb projects to multiple olfactory cortical areas including the piriform cortex and anterior olfactory nucleus (AON), which play distinct roles in odor-guided behaviors. The function(s) of the AON and its connected brain regions are not well established. In order to gain genetic access to the AON neurons, we wished to identify a molecular marker for these neurons through a differential gene expression search in the Allen Brain Atlas. This search led to the identification of the neuromedin B receptor (NMBR) gene as the top candidate that is highly expressed in the AON compared to the rest of the brain. Using the CRISPR-Cas9 gene-editing approach, we generated an NMBR-Cre knock in mouse line. Anatomical tracing from the AON neurons revealed specific projection to the nucleus of lateral olfactory tract (NLOT), part of the cortical pallial amygdala. In addition, whole-cell patch clamp recordings combined with optogenetic activation showed that the AON/TT neurons make monosynaptic and polysynaptic connections onto NLOT neurons. Furthermore, in vivo fiber photometry revealed odor and/or sniff induced calcium signal elevation in the AON neuron axonal terminals in the NLOT of freely behaving mice. Finally, ablation of excitatory neurons in the NLOT not only impaired

olfactory guided food search and social discrimination but also disrupted aversive behavior to a synthetic predator odor. Taken together, these results indicate that the AON/TTàNLOT pathway plays a critical role in olfactory-guided behaviors.

44 **Structural Plasticity Of Peripheral Taste Axons**

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Cell turnover in the taste bud requires taste neurons to form new connections with new sensory cells over time. Do taste neurons alter their structure to accommodate taste bud cell renewal? To address this question, we developed an *in vivo* two-photon microscopy approach that allows for the observation of a single taste nerve arbor (portion of the axon innervating taste buds) at multiple time points for up to 100 days. We found that the terminal branches of taste arbors continuously and rapidly remodel – adding or subtracting terminal branches every 5.5±0.8 hours. The speed of this structural plasticity is faster than predicted by rate of taste bud cell renewal. The inhibition of new taste bud cell entry into the taste bud using Hh-inhibitors did not impact the rate of terminal branch change (U=312, p=0.61), and even complete taste bud loss did not prevent terminal branches from remodeling. These findings indicate that taste nerve arbor remodeling is not regulated by taste bud cell renewal. We also observed that adult taste branch retraction was typically predicted by a retraction bulb and/or fiber blebbing (86%), indicating that retraction uses conserved mechanisms. Furthermore, the rapid remodeling of individual arbors was coupled with limited loss and no addition in the total number of arbors per taste bud. Thus, arbors per neuron is stable and possibly dictated by the taste neuron type. Taste neurons appear to cope with the unstable cellular environment associated with taste bud cell renewal by maintaining a stable number of nerve arbors that are each capable of high-speed remodeling. The concurrent stability and plasticity of taste neuron arbors may explain how taste neurons remain functionally stable despite the challenge associated with continuous taste bud cell renewal.

45 **Regional Peak Mucosal Cooling Predicts Treatment Outcomes Of Nasal Valve Obstruction**

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Remodeling narrow nasal valve may effectively treat nasal obstruction symptoms, yet its precise mechanism is not fully understood. In this study, 20 patients with internal nasal valve obstruction underwent in-office radiofrequency (RF) treatment (Aerin Medical, Inc), based on the concept that RF energy creates a submucosa thermal lesion, induces fibrosis during wound healing that may result in the nasal valve expansion. Under local anesthesia, a small probe topically delivers 18s of less than 60°C RF energy at up to 5 positions along the upper lateral nasal valve region. Patients' Nasal Obstruction Symptom Evaluation score improved significantly at 90 days post-treatment (NOSE: pre 78.89±11.57; post 31.39±18.30, P=5e-7). Computational fluid dynamics (CFD) models were constructed based on the pre- and post-procedure CT scans to identify variables that may predict treatment outcome. There were no statistically significant changes in CFD computed nasal resistance (pre-0.096±0.065; post:0.075±0.026Pa/(ml/s); P=0.063) nor the measured peak nasal inspiratory flowrate (PNIF, pre 60.16±34.49; post 72.38±43.66ml/s; P=0.13). As validation, PNIF correlated significantly with nasal resistance (r=0.47, P=0.004). Among all the variables, only the peak mucosal cooling posterior to the nasal vestibule significantly correlated with the NOSE at baseline (r=-0.531, P=0.023) and with post-treatment improvement (r=0.659, P=0.003). Nasal airway volume in the nasal valve area increased only ~7% post-treatment, yet this minimal remodeling has a profound effect on perceived nasal obstruction, corroborating our previous hypothesis that subjective relief of nasal obstruction correlates with perception of regional mucosal cooling rather than nasal resistance or peak flow rate, a potential target for future effective, personalized therapeutic approaches.

46 **People With Obesity Experience More Pleasure From Food**

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Studies on obesity commonly report that people who are obese do not get more pleasure from food than do their thinner colleagues. However, the psychophysical measures of food liking in these studies (visual analogue or category scales) cannot provide valid comparisons across groups of individuals. We label such scales with descriptors of perceived intensity (e.g., weak, medium, strong). Those descriptors have relative meaning within an individual but cannot be used to compare hedonic sensations across individuals since we cannot share conscious experiences. However, we can come close to solving this problem for the assessment of food palatability by anchoring our hedonic scale to a hedonic experience not related to food palatability. The hedonic gLMS (hedonic general Labeled Magnitude Scale) does this by anchoring the top to the strongest liking "of any kind." Since liking food is rarely the most intense positive experience for most of us, the hedonic gLMS top label becomes an anchor against which we can see differences in food liking across groups. We asked a large sample of lecture attendees (N=3855) to rate their liking for 25 different foods on the hedonic gLMS (Bartoshuk et al, 2006). To the best of our knowledge, this was the first-time food liking had been assessed with a method that produces valid comparisons across people with varying BMIs. We present additional analyses here. Multiple regression showed that maximum palatability rose with BMI; the effect was greater for females. Factor analysis produced four food groups: sweet, bitter, salty and high fat foods. Multiple regression revealed that people with obesity reported significantly greater liking for the sweet, high fat, and salty foods and significantly less liking for the bitter foods.

47 **Association Of Taste-Related Genes With Fruit And Vegetable Intake Among Community-Dwelling Adults &Ndash; The Framingham Heart Study**

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Consuming a variety of fruits and vegetables (F&V) is a key component of healthy dietary patterns, yet, intake in the US remains low. One determinant of F&V intake may be genetic variants (SNPs) in genes related to taste perception or combinations thereof, represented as polygenic risk scores (PRS). Therefore, the aims of this study were to build taste-related PRS for the 5 tastes and determine their cross-sectional associations with prespecified categories of F&V intake (citrus/non-citrus fruits; cruciferous/red & orange/other vegetables). The cohort included Framingham Heart Study Offspring and Third Generation participants (N=6,230; mean age ± SD: 50 ± 14 y; 54% female). F&V intakes (servings/week) were estimated from food frequency questionnaires and log-transformed for analysis. Taste-related PRS were derived via a weighted approach for tastes with ≥2 SNPs identified in prior genome-wide association studies for taste perception (32 SNPs; 19 sweet, 9 bitter, 2 umami, 1 salt, 1 sour). Higher PRS indicated more alleles for higher taste perception. Overall, in linear mixed models adjusted for age, sex, population stratification and energy intake, higher umami-related PRS were associated with lower intakes of cruciferous and red & orange vegetables [β (95% CI) = -0.03 (-0.06, -0.01) and -0.05 (-0.08, -0.02), respectively; FDR <0.05]. No significant associations were found for bitter- or sweet-related PRS with any category of F&V intake. Exploratory analyses identified a single umami-related SNP (rs7691456_T) associated with lower intakes of all vegetable categories and citrus fruits (β range: -0.05, -0.03; all FDR <0.05). These data suggest an influential role of umami-related SNPs in determining F&V intakes, underscoring the importance of considering taste-related genes in precision nutrition.

48 **Distraction Effects On Chemosensory Perception In Lean And Obese Volunteers**

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Within this project, the neurocognitive mechanism of distracted eating was explored. Olfactory and gustatory performance under different levels of distraction was systematically compared between lean and overweight participants. An ecologically valid paradigm – Tetris game utilizing two difficulty levels (high vs. low) – was used as a distraction task. We observed that overweight participants rated intensity of taste and flavor stimuli as significantly diminished compared to lean participants within the high distraction condition. Thus, we assume changes on behavioral level induced by distraction are orchestrated by neural alternations that might be a unique biological marker of obesity. Noteworthy, the hedonic properties of gustatory stimuli were also significantly influenced by distraction. Both lean and obese participants perceived the stimuli as significantly less pleasant within high-distraction compared to low-distraction condition. Moreover, lean participants also perceived pleasantness of different food-associated odors as significantly decreased within condition of high compared to low distraction. Contrary to our assumptions, the intensity perception of olfactory stimuli was not affected by distraction. Within our sample, the overweight participants perceived intensity of olfactory and gustatory stimuli as significantly higher in comparison to the lean participants. This supports the assumption that overweight participants have higher sensitivity towards food-related chemosensory stimuli. In conclusion, our findings suggest that the neurocognitive mechanism of distracted eating is different in effect and structure between the lean and obese populations and distracted eating is a contributing factor to obesity.

49 **High-Throughput Sequencing Of Olfactory Single Neuron Projections Reveals Non-Random Spatial Organization**

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In most sensory modalities, neuronal connectivity reflects behaviorally relevant stimulus features, such as spatial location, orientation, and sound frequency. By contrast, the prevailing view in the olfactory system, based on the reconstruction of dozens of individual neurons, is that connectivity is random. Assuming random connectivity, models of olfactory learning were proposed to rely entirely on plasticity to construct meaningful representations. However, identifying any degree of spatial organization of distributed projections requires high-throughput projection mapping at cellular resolution, which is difficult to achieve using conventional optical tracing methods. Here we used high-throughput sequencing-based neuroanatomical methods, MAPseq and BARseq, to analyze the projections of 5,309 mouse olfactory bulb (415 neurons from two brains using BARseq, and 4,894 neurons from six brains using MAPseq) and 30,433 piriform cortex output neurons (from five brains using MAPseq) at single-cell resolution. Surprisingly, statistical analysis of this much larger data set revealed that the olfactory connectivity is spatially structured. Single olfactory bulb neurons targeting a particular location along the anterior-posterior axis of the piriform cortex also project to matched, functionally distinct, extra-piriform targets. Moreover, single neurons from the targeted piriform locus also project to the same matched extra-piriform targets, forming triadic circuit motifs. Thus, as in other sensory modalities, olfactory information is routed at early stages of processing to functionally diverse targets in a coordinated manner. Our results thus

suggest an alternative model in which olfactory information are processed along parallel, spatially segregated, functionally distinct streams.

50 **Adult Neurogenesis In The Mouse Accessory Olfactory System**

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In rodents, both sensory neurons in the vomeronasal organ and inhibitory interneurons (granule as well as periglomerular cells) in the accessory olfactory bulb are continuously replaced by adult neurogenesis, thereby (re)shaping the output of the accessory olfactory system (AOS). Notably, the precise physiological function of adult neurogenesis in the AOS remains unclear. Here, we begin to describe characteristics of neurogenesis in both peripheral and central AOS tissues. Using a novel genetic approach, we label newly generated vomeronasal sensory neurons as well as accessory olfactory bulb interneurons. After tamoxifen injection, neuronal stem cells in *Id2CreERT2*$^{+}$ *:: Rosa26R-tdTomato* mice express tdTomato upon coincident Id2 promoter activity. Descendants of these stem cells are thus labelled with tdTomato. Introducing the *Id2* stem cell marker as an AOS lineage tracer, we show (i) horizontal and tangential migration of sensory neuron precursors. We demonstrate that (ii) differentiated vomeronasal neurons appear two days after tamoxifen treatment. Finally, we provide insight into (iii) the migration of accessory olfactory bulb interneuron precursors into the accessory olfactory bulb.

51 **Don Tucker Finalist: Neural Circuit Basis For Generating Spindle Oscillations In The Developing Olfactory System**

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We recently demonstrated that the olfactory system in awake, neonatal rat pups exhibit highly stable activity patterns: starting at birth (P0) until around postnatal day 15 (P15), odor stimuli evoke spindle oscillations that are coherent between the olfactory bulb (OB) and the piriform cortex (PC). Here, we investigated the circuit-level mechanisms underlying the generation of these spindle oscillations. To determine the source of spindle oscillations, we recorded local field potential (LFP) activity simultaneously from the OB and PC in unanesthetized rat pups (P4-P9, n=30). Granger causality revealed a significant information flow from OB to PC, and PC to OB in the spindle frequency range, suggesting that the generation of spindle oscillations relies on feedback from PC to OB. To determine the circuit-level organization of potential cortical feedback projections to the OB, we applied current source density analysis (n=9) that revealed a sink in the glomerular layer, followed by a sink in the granule cell layer (presumably the target of cortical feedback projections). To investigate the causal effect of feedback projections on oscillatory activity, we recorded odor-evoked activity from OB before and after pharmacological inactivation of the lateral olfactory tract (n=9) or olfactory peduncle (OP, n=5) through lidocaine injection. Inactivation of either tract caused a significant increase in oscillation frequency in the OB. Thus, feedback modulates spectral characteristics of oscillations generated in the OB and may control different functional states of the neonatal olfactory system. Together, our results suggest that the neonatal olfactory system already exhibits adult-like circuit motifs, including net inhibitory feedback projections, capable of generating complex and flexible activity patterns.

52 **Models Of Inhibition In The Accessory Olfactory Bulb**

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Olfaction is not a structured sensory space, therefore the rules governing the topological organization of the olfactory bulb are still largely unknown. Investigating the logic behind olfactory inhibitory wiring could be the key to decoding odor maps and also shed light on how inhibitory interneurons participate in non-topographical neural coding in general. In the accessory olfactory bulb (AOB), we had previously observed that specific types of odorant-selective glomeruli are often juxtaposed in spatial clusters. When we delivered odors to GCaMP-labelled vomeronasal sensory neurons and imaged in the AOB for the responses in their axon termini, glomeruli fluoresced less if their neighbors were also activated, unless downstream glutamatergic signaling was blocked by D-AP5. This suggests that the observed clustering may be a feature of odor map organization, possibly to facilitate lateral inhibition. To test this hypothesis, we evaluated computational models for biologically plausible patterns in interglomerular inhibition. For each experimental recording, we modelled glomerular activity as excitatory nodes connected by various matrices of inhibitory weights, fitting the inhibition to account for the difference between control and glutamate blockade conditions. When we modelled inhibition as a function of interglomerular distance, we found little evidence of distance dependent decay, suggesting globally uniform rather than lateral inhibition. However, when we utilized a different model architecture that grouped glomeruli by their odor identities, we found some preliminary evidence of stereotypical inhibitory relationships which may suggest preferential inhibition between clustered glomeruli.

53 **Top-Down Feedback Enables Flexible Coding Strategies In Olfactory Cortex**

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In chemical sensation, multiple models have been proposed to explain how odors are represented by patterns of neuronal activity in the olfactory cortex. One hypothesis is that the identity of combinations of active neurons within specific sniff-related time windows are critical for encoding information about odors. Another model is that patterns of neural activity evolve across time and it is this temporal structure that is essential for encoding odor information. Interestingly, we found that top-down feedback to the olfactory bulb dictates what information is transmitted to the olfactory cortex by switching between these two strategies. Using a detailed model of the early olfactory system, we demonstrate that feedback control of inhibitory granule cells in the main olfactory bulb influences the balance between excitatory and inhibitory synaptic currents in mitral cells, thereby restructuring the firing patterns of piriform cortical cells across time. This resulted in performance gains in both the accuracy and reaction time of odor discrimination tasks. These findings lead us to propose a new framework for early olfactory computation, one in which top-down feedback to the bulb flexibly controls the temporal structure of neural activity in olfactory cortex, allowing the early olfactory system to dynamically switch between two distinct models of coding.

54 **How Fast Can A Human Sniff?**

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How fast can a human sniff? This question remains unanswered. Researchers have determined that rodents sniff at frequencies ranging from 4-12 Hz during odor sampling (Kepecs et al. 2007; Macrides et al. 1982; Rajan et al. 2006; Uchida and Mainen 2003; Youngentob et al. 1987), which is within the theta range. These bouts of fast sniffing in rodents are associated with expediting odor delivery to the olfactory bulb (Wesson et al., 2009) and with high cognitive effort including sensorimotor learning (Kay, 2005). Human olfactory research has shown that the sniff functions as more than an odorant delivery mechanism, also affecting odor intensity and perception, and driving activity in olfactory cortex (Mainland & Sobel, 2005). In this study, we aim to determine how fast humans are capable of sniffing. We will examine individual variation in the upper limit of human sniff speed and will determine whether sniff speed is correlated with olfactory performance. Participants will be asked to sniff as fast as they can while nasal airflow is recorded and will complete standard olfactory testing (UPSIT and sniffin stix). Preliminary results suggest that humans are able to sniff as fast as 4 Hz, within the theta range.

55 **A Biomimetic Olfactory Recognition System For The Discrimination Of Complex Odorant Stimuli**

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Despite various artificial noses that have been developed to mimic the human olfactory system, it remains challenging to sense and discriminate complex odorant stimuli efficiently and accurately. Here, a biomimetic olfactory recognition system combining an optimal panel of 10 mouse odorant receptors with back propagation neural network model was designed to discriminate the aromas of Chinese liquors, which are representative of complex odorant stimuli in real life. Our system shows an excellent predictive ability with an average accuracy of 96.5% to discriminate liquors of different aroma styles, as well as those of different brands and ageing years within the same aroma style. A total of 124 interactions between liquor aroma compounds and odorant receptors were further elucidated to understand odorant coding at the molecular level, including 14 newly deorphaned odorant receptors. Our work represents a proof of concept for combining receptors and machine learning in the discrimination of complex odorant stimuli.

56 **Quantity Has A Quality All Its Own: Mapping Odor Character Changes Across Intensities**

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Although most odor atlases describe the odor character of a given molecule using a single description, odor character can change across intensities. Other sensory modalities have similar phenomena, for example the Bezold-Brücke effect in color vision where hue varies with luminescence and the Zürmühl-Stevens effect in audition, where perceived pitch varies with sound pressure. Without a quantitative “odor space,” it is difficult to develop general rules describing how perception shifts with changes in intensity. To develop such rules, we asked 15 trained participants to rate the applicability of 51 odor labels to 100 odorants at two concentrations corresponding to low and high intensities. Several molecules exhibited concentration-dependent changes in character that were larger than typical character differences between different molecules. Odorants with a low detection threshold were more likely to undergo large perceptual shifts and these perceptual shifts tended to occur in consistent directions in odor space. Understanding how intensity and quality interact will provide an important constraint on models of olfactory perception.

57 ***Covid-19 Smell Symptom Screening Using Scentinel In The Workplace***

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Screening for COVID-19 symptoms can help to prevent outbreaks, particularly in vulnerable workplaces such as

nursing homes. Recent smell loss is a better predictor of COVID-19 than fever. Despite this, temperature checks are still frequently used to screen for COVID-19 symptoms to enter workplaces. Available smell tests are too costly and time consuming for population surveillance. To address this need, we developed *SCENTinel*, a rapid smell test, which evaluates 3 olfactory functions (detection, intensity, and identification) based on the exposure to one odor. Here, each *SCENTinel* test included one of four possible odors to allow for repeated testing while reducing learning effects. The purpose of this study was to assess the feasibility of employees completing *SCENTinel* prior to entering a nursing home facility. Data collection began on December 14, 2020, prior to widespread vaccination, and lasted until April 5, 2021. Participation was voluntary, and fifty-seven participants (70% female, 49% white, 44.7±12.7 years old) completed *SCENTinel* prior to their shift at least once, and 39 of the 51 participants (76%) completed *SCENTinel* before at least 2 shifts (average tests taken over the study period was 18±15). Odor detection, odor intensity ratings, and odor identification ability did not change over time. The majority of participants (97%) passed *SCENTinel* over the study period. These results support the feasibility of implementing *SCENTinel* as a workplace screening tool when smell loss is a sensitive indicator of viral infection.

58 **Patients With Parkinson&Rsquo;S Disease Share A Unique Olfactory Perceptual Fingerprint**

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Although the olfactory decline in Parkinson's disease (PD) precedes the motor symptoms by several years or decades, it has yet to provide for a specific early biomarker in PD. Typical olfactory tests probe olfactory performance, in tasks such as detection, discrimination, and identification. Because of the myriad possible causes for the decline in olfactory performance, such performance-based tests lack specificity. An alternative to performance-based tests is the olfactory perceptual fingerprint (OPF). OPFs characterize how the world smells to an individual. OPFs are related to genetic makeup (Secundo et al., PNAS 2015), and provide specificity where performance-based tests do not (Weiss et al., Neuron 2020). To test the hypothesis that PD is associated with a specific typical OPF, we tested 10 PD patients (9M, mean age = 66.3 ± 7.4 years, disease duration = 9.3 ± 7.9 years, MDS-UPDRS total score = 57.9 ± 21.6) and 10 healthy controls (9M, mean age = 64.9 ± 5.4 years) using 10 odors and 11 descriptors. We found that OPFs were similar within the two groups but significantly different between them (mean r = 0.3 ± 0.17 vs. mean r = 0.1 ± 0.22, paired t-test, t(8)=-4.22, p=0.002). Moreover, we could use OPFs alone to classify PD (unsupervised k-means clustering, 90% specificity, 70% sensitivity). These pilot data raise the possibility of a specific olfactory biomarker in PD.

59 **Prevalence Of Smell And Taste Loss In Youth With Covid-19**

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Chemosensory dysfunction is a common and early symptom of COVID-19, even in otherwise asymptomatic patients. In COVID-19-positive adults, the prevalence of smell loss is ~67% and of taste loss is ~42%. Despite the promise of tracking smell and taste as a discriminatory symptom of COVID-19, there has been little effort to quantify the prevalence of these symptoms in youth. Here we aim to examine the extent to which smell and taste have been assessed among youth with active COVID-19. To date, only 4.7% (N = 39/826) of studies including COVID-19 positive youth assessed smell and/or taste loss. We use random-effects meta-analysis to pool 39 studies including individuals younger than 18 years old, with confirmed or suspected COVID-19 diagnosis in which a measure of smell and/or taste was reported (24 secondary reports from medical records or parental reports, 13 self reports, 2 direct testing) and estimate the effect of chemosensory dysfunction due to COVID-19 in youth (age 0-17 years, 11 months, 29 days). Based on self-reports alone, the prevalence of smell loss is 14% (vs. 8% secondary reports) and the prevalence of taste loss is 18% (vs. 7% secondary reports). The only paper using a standardized direct test for smell loss (an adult version of the Sniffin' Sticks odor identification test) indicates a much higher prevalence of smell loss of 86% (N = 79). Prevalence increases from age 10, but no sex differences are revealed. We highlight the need for guidelines to assess chemosensory loss in children with suspected COVID-19. At a minimum, we recommend the use of self-reports to document the prevalence of chemosensory loss due to iCOVID-19 in youth, and possibly mitigate the burden of the COVID-19 pandemic in this age category.

60 **Scentinel 1.1 Rapidly Screens For Parosmia**

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After smell loss, many individuals develop parosmia, which is the distorted perception of the quality of some odors. Directly testing for parosmia would help understand its prevalence in the population, however there is only one test, which cannot be self-administered, that screens for parosmia. To fill this gap, in the 1.1 version of SCENTinel, a rapid smell test to screen for smell loss, we included a hedonic subtest to screen for parosmia in addition to the prior validated subtests detection, intensity, and identification. The hedonic subtest calculates a hedonic score, which is the difference between the pleasantness of the odor smelled minus the pleasantness of the imagined smell of unpleasant odor. Subtests are measured for one of four different odors (popcorn, coffee, flower, and bubblegum). The overall SCENTinel 1.1 score discriminates parosmia (n=77) from hyposmia (n=84; AUC=0.89) and from anosmia (n=51; AUC=0.82). Hedonic score and parosmia frequency were negatively associated (r=-0.2; p<0.001). Those with parosmia report a significantly lower hedonic score as compared to those without parosmia for all odors (p<0.001), but do not differ in the hedonic rating of an imagined unpleasant

odor (p=0.27). We conclude that SCENTinel 1.1 is a direct smell test that can rapidly and accurately screen for parosmia.

61 **Use Of 3D Printing To Optimize Nasal And Olfactory Drug Delivery**

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Background: High volume irrigation has served a critical role in management of sinonasal disease. Recent studies have investigated whether irrigation could serve a role in treating olfactory loss, with interest growing with the continued COVID-19 pandemic. Here we utilize 3D printing as a tool to visualize and optimize irrigation flow to the olfactory region. Method: Eight human models were 3D printed with a FormLabs Form3 printer based on individual CT scans. Irrigations were performed and video recorded with a squeeze bottle attached via silicon water-tight seal, at five head positions: 45° to-the-side, 90° to-the-side, 90° forward and 45° to-the-side and 90° forward. Along with head positions, nostril selection was varied with fluid entry through upper (conventional) or lower (backfill) nostrils. Results: Significant differences were observed across the different head positions. Complete irrigation of the olfactory region only reliably occurred for the 90° to-the-side head position, for both conventional and backfill filling. There was variation in 90° forward position but backfill provided complete irrigation for some models. 45° to-the-right and 45° forward allowed for around 50-75% irrigation through backfill, while most fluid flow went below the olfactory region for conventional filling. 45° to-the-side provided little irrigation for both nostril selections, however, backfill filling provided more irrigation overall for the nasal cavity. Conclusion: Variation in position and nostril selection was key to olfactory region saturation. 90° to-the-side or 90° forward provided the best results overall, with backfill filling providing more irrigation to the olfactory region and nasal cavity overall. This demonstrates that head position and nostril selection are important to olfactory region irrigation.

62 **Pathological Consequences Of Chronic Olfactory Inflammation On Neurite Morphology Of Olfactory Bulb Projection Neurons**

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Chronic olfactory inflammation (COI) in conditions such as chronic rhinosinusitis significantly impairs the functional and anatomical components of the olfactory system. Although chronic rhinosinusitis patients have smaller olfactory bulbs (OBs), the consequences of olfactory inflammation on OB neurons are largely unknown. Our previous study showed that COI induced by intranasal administration of lipopolysaccharide (LPS) in mice resulted in atrophy, gliosis, and pro-inflammatory cytokine production in the OB. In this study, we investigated the neurological consequences of COI on OB projection neurons, mitral cells and tufted cells. To induce COI, we performed repeated unilateral intranasal administration of LPS to mice for up to 10 weeks. Effects of COI on the OB were examined using RNA-sequencing approaches and immunohistochemical analyses. We found that COI upregulated immune-related biological pathways in the OB after 4 weeks. We also determined that the length of tufted cell lateral dendrites in the OB significantly decreased after 10 weeks of COI. The axon initial segment of tufted cells decreased in number and in length after 10 weeks of COI. The lateral dendrites and axon initial segments of mitral cells, however, were largely unaffected. In addition, dendritic arborization and axon initial segment reconstruction both took place following a 10-week recovery period. These results suggest that olfactory inflammation specifically affects tufted cells and their integrated circuitry, whereas mitral cells are potentially protected from this condition. Our findings demonstrate unique characteristics of the OB to undergo neuroplastic changes in response to inflammatory stress.

63 **Correlation Between Cognitive Performances And Olfactory Function In Patients With Mild Cognitive Impairment**

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BACKGROUND: Mild cognitive impairment (MCI) is a transient condition between cognitive health and dementia. The alterations in olfactory function may signal declines in cognitive functions associated with dementia. This study aims to analyze the correlation between olfactory function and five cognitive domains: attention, memory, language, visuospatial function, and frontal/executive function. METHODS: This study subjects were elderly people with MCI who use in an elderly welfare facility in city of D. A total of 68 subjects (16 men and 52 women) with mean age 79.22 ± 5.62 years were recruited. The Korean version of the Sniffin' stick test (KVSS-II) which is a modified type of Sniffin' stick recently developed for Koreans, was used for olfactory function testing in all participants. In the cognitive examination, the Seoul Neuropsychological Screening Battery-Second Edition (SNSB-II) was also performed. The SNSB-II is a test battery widely used for the clinical diagnosis of dementia in Korea. RESULTS: There was no significant correlation between olfactory function and attention and visuospatial function cognitive domain. KVSS-II discrimination score was a significant correlation with language cognitive domain (r=0.344, *p*=0.004) and frontal/executive function cognitive domain (r=0.388, *p*=0.001). KVSS-II Identification score was a significant correlation with memory cognitive domain (r=0.337, *p*=0.005) and frontal/executive function cognitive domain (r=0.304, *p*=0.012). CONCLUSIONS: Peason's correlations analysis results revealed that was statistically significant results in three

cognitive domains. These results suggest that a simple test of odor identification and discrimination is valuable in evaluating individuals at cognitive performances.

64 **Smell, Taste And Trigeminal Function: Similarities And Differences Between Results From Home Tests And Examinations In The Clinic**

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Background: This study aimed to examine an easy-to-conduct home chemosensory test as a screening tool prior to clinical testing and to investigate the associations between home and clinical tests. *Methods:* We examined 200 participants who performed a chemosensory test including subjective ratings as well as psychophysical smell, taste and trigeminal function tests at their homes. Following that, they were invited to the clinic for standardized testing using the Sniffin’ sticks test for assessment of olfactory function, taste sprays and strips for taste function, and a lateralization test for trigeminal function. *Results:* The home smell test correlated well with the Sniffin’ sticks test ($r = 0.75$, $p < 0.001$). The home test had a relatively high sensitivity for detecting smell loss (sensitivity of 67% at a specificity of 92%). The home test could distinguish between patients with olfactory loss and healthy controls. In contrast, the home tests for taste and trigeminal function did not provide valid results. When comparing home and clinical tests older age and olfactory loss were the most significant confounders in various models, while participants who had olfactory loss and admitted (!) to drink alcohol regularly were more likely to show consistency between home and clinical smell measurements. *Conclusions:* The present home chemosensory test allows individuals to screen their olfactory function in a simple way at home at any time. Results from smell tests obtained at home correlate with tests obtained at the clinic. However, tests conducted at home or in the clinic have different confounders.

65 **Well-Being In Patients With Olfactory Dysfunction**

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This cross-sectional, retrospective study aimed to investigate the differences in well-being (WB) among patients with olfactory disorder (OD) with quantitative and/or qualitative olfactory dysfunctions, and to identify factors associated with WB. We included 470 OD patients. WB (WHO-5 questionnaire), quantitative olfactory function (Sniffin Sticks) and qualitative dysfunction were assessed. Based on normative data, 44% of patients with anosmia, 31% with hyposmia, 36% with parosmia and 41% with phantosmia had poor WB, while the remainder showed good well-being. For quantitative function, anosmia patients showed lower WB scores than hyposmia and normosmia patients (all p's<0.03). For qualitative dysfunction, patients with severe parosmia showed lower WB scores than patients without and with less severe parosmia (p's<0.01). Regarding OD causes in hyposmic patients, post-viral patients showed poorer WB than idiopathic patients (p=0.01); sinonasal patients had lower WB than post-traumatic and idiopathic patients (all p's<0.04). The WB score positively correlated weakly, but significantly with the Threshold test score (r=0.11, p=0.02), and negatively with severity of parosmia (r=-0.10, p=0.03). Hierarchical regression analyses showed that sex, T and TDI scores significantly predicted WB score in OD patients. Our results implied that quantitative and qualitative dysfunction is associated with WB. However, only patients with severe dysfunction showed significantly lower WB. And for those with severe dysfunction, some of these patients indicated good WB. While this needs to be better understood, in order to improve WB, in these patients it appears to be highly important to improve olfactory function, and here especially olfactory sensitivity. Key words: anosmia, parosmia, well-being, olfactory dysfunction

66 **Chemosensory Losses In Active Probable Delta And Omicron Variants Breakthrough Covid-19 Cases**

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Chemosensory loss is a COVID-19 hallmark but it is unclear if the Delta (DL) and Omicron (OM) variants similarly impact smell and taste function, and whether vaccination results in less severe symptoms. 80 subjects with prior confirmed/clinical probable diagnosis of COVID-19 and 125 controls performed sensory tests via Zoom using the NIH toolbox 9-item scratch and sniff odor id test and bitter intensity ratings of 1mM quinine. 39 subjects had active COVID-19 (symptom onset <14d) at the time of testing, and most (36/39) were vaccinated. 25 of these active cases were likely infected by the DL variant with the rest as probable OM cases based on diagnosis dates. The other 41 positive cases occurred prior to the DL surge in the US with diagnosis >14d prior to sensory testing (x=6.5m). 9 of the 41 subjects reported smell loss (8 long-haulers); objective testing confirmed smell loss comparable to the active cohort in 7 (78%). 16 of the remaining 32 (50%) without reported smell loss still had objective losses. All probable DL-variant cases (100%) had objective smell loss based on age and gender adjusted normative cutoffs, although only 16/25 reported smell/taste loss. None of the likely OM cases reported smell/taste loss, yet 5/11 (45%) subjects had objective smell loss, higher than in controls (34%). For taste function, while COVID+ subjects with self-reported chemosensory loss rated quinine as less bitter, the difference was not significant (p>0.05). The results demonstrate (1) the DL variant may cause similar if not more severe impact on olfactory function while the impact of the OM variant is less profound and (2) vaccination does not fully prevent chemosensory loss. Results also add to evidence that self-reported chemosensory loss is useful but may not capture the full spectrum of losses from COVID-19.

67 **Ambient Air Pollution And Olfactory Sensitivity In 11 Locations Across The Globe**

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Over 90% of the global population live with air pollution above the WHO limit, and these numbers continue to increase. Exposure to outdoor air pollution has been linked to cardiovascular disease, stroke, chronic obstructive pulmonary disease and lung cancer, oxidative stress as well as an increase in the risk for acute respiratory infections. Although pollution is largely harmful to the respiratory system, we only have started to collect information about its potential effects on the sense of smell. Lifetimes of particles present in the atmospheric air has been confirmed to be strongly linked with olfactory sensitivity in humans (Williams, Ringsdorf, 2020), further supporting the notion that atmospheric conditions alter olfactory perception. A few reports including adults representing indigenous tribes suggest lower olfactory sensitivity to be related with lesser exposure to air pollution (Sorokowska, Sorokowski, Hummel, Huanca, 2013). In line with this, residents of the rural Tlaxcala characterized by low air pollution have been found more sensitive to odors than the inhabitants of highly polluted Mexico City (Guarneros, Hummel, Martínez-Góme, Hudson, 2009). In the present study, we compare the olfactory sensitivity of individual subjects (n=811) inhabiting 11 locations across the globe with the composition of atmosphere pollutants. The aim of this analysis is to estimate the overall relationship between air pollution and olfactory sensitivity, and further to point to the most harmful atmospheric pollutants for our sense of smell.

68 **Behavioral Chemosensory Interventions: Multiple Flavors Of Olfactory Training To Improve Diet Quality**

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Smell dysfunction is associated with several negative health outcomes, including unhealthy diet. The prevalence of persistent smell dysfunction has dramatically increased because of the COVID-19 pandemic. Critically, current recommendations for smell dysfunction are limited to orthonasal olfactory training (OT), which is effective in ~30% of patients with post-viral smell loss. The effects of OT on diet are lacking. A strong need exists to (a) improve OT efficacy, and (b) assess impact of olfactory improvement on diet quality. Here we describe a protocol for a multi-arm 3 (sensory method) by 2 (engagement) Randomized Clinical Trial (RCT) with OT using intent-to-treat analysis to test efficacy of six personalized, modular, multicomponent 12-week online interventions and 24-week follow-up on improving smell function and diet quality. Along with the standard OT exposure method (OrthoOT–), we propose two novel methods: RetroOT– and DietOT–. In two RetroOT arms, a polymeric matrix is chewed to deliver odorants retronasally. In two DietOT arms, ecologically relevant exposure occurs by enhancing dietary diversity, including fruits, vegetables, and whole grains. Each sensory-only training method (OrthoOT–, RetroOT–, DietOT–) will be paired with a parallel mindfulness arm (OrthoOT+, RetroOT+, DietOT+) to determine whether active cognitive engagement during exposure boosts efficacy. To monitor and improve adherence, all arms will include goal setting sessions, health coach guidance, online step-by-step instructions, and social media support. Findings from intent-to-treat analysis align with rigorous requirements to minimize bias in reporting OT efficacy and support evidence-based recommendations for health providers to use with patients with OD and address Healthy People 2030 goals for chemosensory disorders.

69 **The Best-Laid Plans Of Human Researchers Often Go Awry: Learning From Failure In The Third *Covid-19 Wave (Jul- Nov 2021)***

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During the first wave of COVID-19, the Global Consortium for Chemosensory Research (GCCR) successfully launched a large crowdsourced survey to investigate the self-reported loss of smell, taste, and chemesthesis. Using a similar approach, we investigated longitudinal changes in chemosensory function. Participants rated self-reported function and the intensity of common household and food items for smell, taste, and chemesthetic sensations over 12 weeks. Six months after launching the study, we discovered only 35 participants had completed the first day (38.4 ± 10.6yrs; 80% females). These data confirm that during COVID-19, participants report experiencing a significant loss in smell, taste, and chemesthesis function, but not nasal blockage (mean change on a 100 pt scale for smell: -75.1 ± 27.7; taste: -54.7 ± 36.0; chemesthesis: -38.3 ± 35.3). There was a positive association between self-reported chemosensory function and intensity rating for all sampled stimuli (smell ($r = 0.50$; $p = 0.02$), taste ($r = 0.48$; $p = 0.03$), and chemesthesis ($r = 0.58$; $p = 0.01$)). The severity of COVID-19 (estimated by the number of total symptoms reported) was significantly associated with the self-reported change in function for chemesthesis ($F1,25 = 7.17$; $p = 0.01$) but not for taste or smell. In addition, there was a significant negative correlation between severity and intensity ratings for salty ($r = -0.51$, $p = 0.02$) and

sour (r = -0.45, p = 0.04) but not for sweet and bitter. Despite the study being carefully designed and programmed, we were not able to engage participants repeatedly over time and could not directly answer our pre-registered questions. In the spirit of open science, we hope sharing our failure will allow the community to learn from this setback and utilize this knowledge and shared materials.

70 **Symptoms Of Depression Change With Olfactory Function**

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Olfactory loss is associated with symptoms of depression. The present study, conducted on a large cohort of mostly dysosmic patients, aimed to investigate whether improvement in olfactory performance would correspond with a decrease in depression severity. In 171 participants, we assessed olfactory function and severity of depression before and after an average interval of 11 months, with many patients showing improvement. Separate analyses were conducted for a) the whole group of patients and b) the group of dysosmic patients using both classic and Bayesian approaches. Student t-test demonstrated that the whole sample improved consistently, especially within the group of dysosmic patients in terms of odor identification. The dysosmic group also improved in odor threshold and overall olfactory function. Pearson correlation showed that increase in olfactory function corresponded with decrease in depression severity, particularly in dysosmic patients. To conclude, the present results indicate that symptoms of depression change with olfactory function in general and odor identification in particular.

71 **Perception Of Parosmias In Argentina, Germany, India, Japan, And The Philippines**

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Objective: To investigate possible regional differences in parosmic sensations with regard to verbal, and visual information using color and shapes. Methods: Patients with parosmia were recruited from Argentina, Germany, India, Japan, and the Philippines. The following epidemiological and clinical outcomes were studied: age, sex, duration of olfactory disorder, subjective quality of parosmia including frequency, intensity, pleasantness, effect on quality of life. Patients also answered questions with regards to the perception of a set of specific odorants. Finally, patients assigned shapes and colors to their parosmic perceptions. Results: A total of 502 patients (183 men and 319 women, 56 Argentinians, 370 Germans, 10 Indians, 48 Japanese and 18 Filipinos) with parosmia participated the study. There were significant regional differences in the perception of specific odorants. On the other hand, similarities were found when using colors or shapes as descriptors of parosmic sensation. Most patients associated parosmia with brownish, grayish, or greenish colors while much less selected reddish or purplish colors. In comparison, the shapes used for descriptions were more varied, with round/oval shapes being less frequently associated with parosmias than shapes with spikes, or square/rectangular or polygonal shapes. Conclusion: Parosmic sensations exhibit similarities across different culture. However, probably due to familiarity with and exposure to certain odors, the odors being mentioned within the context of parosmia vary from region to region. Overall, the similarities in the parosmic perceptions suggest that the mechanisms leading to parosmias are similar in regions.

72 **The Effects Of Vaping On Olfactory Function**

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Vaping using electronic nicotine delivery systems (ENDS) exposes the nasal epithelium and olfactory system to a complex array of potential toxicants while simultaneously stimulating the olfactory sensory neurons with high concentrations of odorants. The surge in vaping popularity motivates the assessment of potential olfactory dysfunction induced by vape exposure to assess potential public health consequences and inform government regulation. Olfactory function in over 100 participants was tested via Snap & Sniff ® kits to assess detection, discrimination, and identification performance. Subjects also underwent rhinomanometry testing to measure the relationship between pressure and airflow in the nose and answered a questionnaire about their vaping habits. Preliminary analysis of the data indicates that subjects who reported regular vaping of nicotine-containing products exhibited significantly worse olfactory discrimination performance than non-vaping control subjects. This impairment was no worse in subjects who had vaped shortly before assessment then those who had not, suggesting that it was not caused by short-term adaptation. It also did not obviously correlate with the nasal resistance to airflow, suggesting that it is not caused by conduction block. Approximately half of vapers indicated that they exhale vape aerosol through the nose when vaping between “sometimes” and “all the time,” demonstrating a previously underappreciated route of intranasal vape exposure. Additional analyses explore the role of nicotine concentration, effects of vaping device type, and a hypothesized interaction of vaping-associated olfactory impairment with COVID-19 history. Further research is needed to understand the mechanism of olfactory toxicity in vaping. Keywords: olfactory, vaping, nicotine, COVID-19

73 **Age-Related Changes In Resposiveness Of The Olfactory Epithelium**

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Olfactory impairment in aging adults is probably due to many causes, including structural changes at the level of the olfactory mucosa. Aim of the present investigation was to study age-related changes in response to olfactory and trigeminal stimuli at the level of the olfactory mucosa and the central nervous system, using electrophysiological responses. A total of 73 participants were examined (younger: n=40, 18-27 years; older: n=33, 50-78 years). All subjects received nasal endoscopy, a standardized medical history, and detailed olfactory testing (Sniffin Sticks). For intranasal stimulation olfactory (H2S, PEA) and trigeminal (CO2) stimuli were used. Stimuli of 500 ms duration were embedded in a constant airflow of ~8l/min using air-dilution olfactometry. Responses were recorded from the olfactory epithelium (electro-olfactograms, EOG). Simultaneously, EEG-derived olfactory event-related potentials (OERP) were analyzed. Results from psychophysical olfactory tests were negatively correlated with the age of the participants ($r=-0.42$, $p<0.001$). Although EOG amplitudes and latencies did not show an age-related difference, younger individuals showed significantly more EOG responses than older ones. For the central-nervous OERP response latencies were significantly shorter in younger participants than in older ones. OERP amplitudes were significantly different between age groups. However, the number of detectable OERP responses was smaller in older participants. In conclusion, the present results showed small age-related differences between a younger and an older group, both at peripheral and central-nervous levels. The present results indicate relatively small changes in the processing of olfactory information possibly pointing at redundancies in the system or the effectivity of compensatory mechanisms.

74 **Don Tucker Finalist: Adam17/Irhom2: Critical Regulators Of Olfactory Inflammation And Anosmia?**

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The cell surface metalloprotease ADAM17 and its obligate binding partner, iRhom2, are key regulators of inflammation in a number of acute and chronic diseases by their ectodomain shedding of inflammatory mediators such as tumor necrosis factor alpha (TNF-a). The objective of this study is to understand whether ADAM17/iRhom2 regulates the inflammatory response in the olfactory system resulting in anosmia. We discovered unexpected, prominent expression of iRhom2 in olfactory sensory neurons (OSNs), suggesting an inherent innate immune regulatory mechanism in OSNs necessitates iRhom2 expression. We found that iRhom2 is essential for the inducible shedding of overexpressed fractalkine (FKN) in mouse embryonic fibroblasts. It is known that OSNs utilize the chemokine FKN to communicate danger signals and recruit immune cells. We will now pursue studies of iRhom2's regulation of FKN shedding in *ex vivo* OSNs and the effect of iRhom2 *knock-out* on immune cell infiltration into the olfactory system *in vivo*. ADAM17/iRhom2 has a well-characterized role in controlling the shedding of TNF-α from myeloid cells. Previous work has shown that TNF-α is directly neurotoxic to OSNs and its ectopic overexpression in the olfactory epithelium results in anosmia in mouse models. By ELISA, we detected significantly lower levels of TNF-α in the olfactory mucosa of *iRhom2KO* compared to *WT* mice following intranasal LPS. We will assess the effect of iRhom2 *knock-out* on OSN damage and apoptosis *in vivo* following intranasal LPS by immunofluorescence and electron microscopy, and on the development of anosmia through olfactory behavioral assays. Our ultimate goal is to understand the potential for iRhom2 as a therapeutic target for the prevention of inflammation-associated anosmia.

75 **Microglia Responses In Nucleus Of The Solitary Tract Subnuclei Following Lingual Nerve Transection In Sprague-Dawley Rats**

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The anterior tongue contains fungiform papillae which are innervated by the somatosensory lingual nerve (LN) and each papilla contains a single taste bud that is innervated by the gustatory chorda tympani nerve (CT). Despite the CT and LN relaying different types of sensation, damage to either nerve can result in loss in both sensory systems, with both the taste bud and papillae degenerating during developmental injury. This sensory interaction is highly unusual in the peripheral nervous system. Both the CT and LN project to adjacent, but distinct, brainstem subnuclei within the nucleus of the solitary tract (NTS). Since the two nerves are interrelated in the periphery, and project to closely related subnuclei within the brain, we examined whether the CT and LN may share an immune response following damage to a single nerve. To that end, microglia, the primary indicators of a central immune response, were examined in both the lateral LN subnuclei and the medial CT subnuclei following unilateral LN transection at 65 days of age. Brains were extracted 2, 5, or 8 post-surgery and the NTS sectioned (40 μm). Every other section was stained with Luxol Fast Blue and cresyl violet to visualize nerve-specific subnuclei. Alternating sections were used to analyze microglia in respective NTS subnuclei using an Iba1 antibody. Our findings indicate that although microglia numbers increased in all transected-side NTS subnuclei at all time points, this increase in microglia density was significantly weaker in the medial subnuclei of the intact CT relative to the lateral subnuclei of the transected LN. Additionally, an increase in microglia density observed in the ventral subnuclei, which served as a control for specificity, indicates that it may instead be a diffuse microglia response occurring broadly in the NTS.

76 **Structural Brain Plasticity And Olfactory Function In A Mouse Model Of Congenital Blindness**

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Early sensory deprivation, such as early blindness, results in enhancement of the remaining non-visual sensory modalities associated with functional and anatomical brain plasticity. While auditory and tactile functions have been largely investigated, olfactory function remains less explored and consistent. A few studies showed enlargement of olfactory structures in the brain correlated with enhanced olfactory functions in early blind adults. The present study aims to assess olfactory performance and to examine anatomical changes in the whole brain in young (PND 9) and juvenile (PND 24) mice using ZRDBA a mouse model of congenital blindness. In this mouse strain, half of the littermates are born blind, and the other half are born normally sighted. Volumetric analyses were conducted on high resolution MRI images and these voxel-wise morphometric measures were compared between blind and sighted ZRDBA mice. One group (20 blind, 20 sighted) underwent two structural MRI scanning, at PND 9 and PND 24, while the second group (20 blind, 20 sighted) went through behavioral tests, such as odor attractivity and preference tests towards social odors. Based on a previous imaging study conducted on adult ZRDBA strain, we hypothesized that: 1) Structural MRI analyses will show brain plasticity in PND 24 blind pups, including olfactory areas and vision-related structures. The enlargement of these olfactory structures in the blind mouse brain would be associated with better olfactory function. 2) Brain plasticity will be observed only in vision-related structures with similar olfactory abilities in PND 9 blind pups. In line with our hypothesis, preliminary data show smaller vision-related areas, including the superior colliculus and the geniculate cortex, and bigger olfactory tubercule in PND24 blind mice. In addition, smaller superior colliculus was found in PND 9 blind mice compared with PND 9 sighted mice.

77 **Clustering And Classification Of Behavioral States In Mice Via Respiratory Patterns: A Machine Learning Approach**

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Breathing is both essential for survival and is dynamically modulated by metabolic need and autonomic tone associated with emotional states. In humans, physical activity and emotional experience are associated with distinct breathing patterns. However, current literature lacks an understanding of breathing dynamics across a broad spectrum of rodent behaviors. Here we demonstrate the diversity in breathing patterns across spontaneous, attractive odor-, stress-, and fear-induced behaviors. Using a Matlab toolbox, BreathMetrics, we find that more detailed respiratory features may be extracted from intranasal pressure recordings of breathing than from whole-body plethysmography recordings. Using k-means clustering based on principal component analysis of respiratory features, 11 well-defined behavioral and emotional states (a total of 938 behavioral bouts from 24 mice) are grouped into four clusters, revealing behaviors sharing similar breathing patterns and consistency of the breathing patterns within each behavior. We additionally identify key respiratory features that distinguish breathing patterns across the clusters. Finally, we implement a k-nearest neighbor classifier, a supervised machine learning model, to predict rodent behavioral states from breathing with an overall accuracy of nearly 70%. Taken together, our findings highlight the tight relationship between breathing and behavioral/emotional state, and the potential use of breathing patterns in classifying rodent behavior.

78 **Comparison Of Physicochemically Conserved Residues Of Human Odorant Receptors Within Two Classes**

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Human ORs included in class A GPCRs are phylogenetically divided into two clusters (Class I and Class II ORs) by their sequence identity. Interestingly, odorants that these classes each respond to seem to have different physicochemical features. Experimentally tested agonists of class I ORs tend to be more hydrophilic than those of class II ORs. Therefore, comparing the amino acid sequences of ORs within the classes may allow us to elucidate the structure-function relationships in ORs further. However, no study applied the physicochemical conservation analysis on the comparison of OR classes, which may provide more information about the structure. To extract factors that may distinguish the structures of ORs within the classes, we analyzed amino acid sequences of ORs within two classes in humans. Conserved residues from the analysis were classified into three different groups by conservation pattern in each class. We found that conservation profiles by structural regions in each class are different. To verify if these residues functionally distinguish the structures of ORs within two classes, two physicochemically differently conserved within the classes (PDCC) residues are experimentally tested by molecular dynamics simulations and expression on heterologous systems. The structure and the

response of ORs by their agonist were changed significantly when the physicochemical feature in the residues was mutated by the one in the other class.

79 **Nasal Exhaled Breath Proteome In Alzheimer&Rsquo;S Disease**

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Olfactory sensory neurons are unique in that their peripheral ends touch air in the nose, and their central ends touch the brain. When air moves through the nasal cavities during natural breathing, it comes into contact with olfactory sensory neurons. We hypothesized that during exhalation, turbulent air flowing past the olfactory neurons could volatilize proteins from the surface of the olfactory epithelium into the exhaled breath, which could then be captured and analyzed. Using a new nasal breath collection technique, we are analyzing nasal and oral breath samples collected from healthy young controls, healthy age-matched controls, and Alzheimer's disease patients. Preliminary results suggest differences in several proteins associated with mitochondrial function in Alzheimer's Disease patients compared to age-matched controls. Furthermore, we found differences in proteins associated with normal aging. These preliminary results will guide further analyses into specific proteins that change on breath in Alzheimer's Disease.

80 **Associations Between Taste And Smell Sensitivity, Preference, And Quality Of Life In Healthy Aging - The Nutriact Family Study Examinations (Nfse) Cohort**

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The chemical senses of taste and smell profoundly contribute to our everyday experiences including those related to food, danger and social interactions. Taste and smell function have been shown to decline with age, with robust impairment occurring only in the very old (80+ years). Much less is known about taste and smell function in young and middle old. Here, we investigated taste and smell sensitivity via thresholds in a sub-sample of the NutriAct Family Study (NFS), the NutriAct Family Study Examinations cohort (NFSE; N=251, age M=62.5 years). We examined different aspects that have previously been linked with taste and smell function: the degree to which taste and smell sensitivity relate to another and to taste and smell preferences, respectively, the role of gender and age, as well as effects on Quality of Life (QOL). We measured recognition thresholds for four different tastes (sweet, sour, salty bitter) and rose smell with an adaptive QUEST-based procedure and QoL with the Short Form-8 Health Survey (SF-8). Taste and smell preferences were self-reported. Thresholds for the four different tastes were highly correlated but no correlation was observed between taste and smell thresholds and also not between thresholds and their respective preference. Women were more sensitive for both taste and smell than men. We found no effect of age on sensitivity and no effect of sensitivity on QoL. All null-findings were corroborated with Bayesian statistics providing evidence for the null hypotheses. Together our results indicate the independence of taste and smell despite their overlap during sensorial experiences. Supporting previous findings of heightened chemosensory sensitivity in women. We found no evidence for age-related sensory decline, which could be due to our sample´s characteristics of non-clinical volunteers with good dental health and 93% non-smokers.

81 **Identification Of Potential Chemosignals For Individual Recognition In The Domestic Cat**

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Individual recognition using olfactory cues is essential for many social mammal species. The chemical profiles of scent marks change over time after deposition by animals because the more volatile molecules are lost first from the marks. The composition of scent marks from the same individual may also vary according to physical condition and diet. What might be the constant components in scent marks left by individuals? Here, we report a specific class of volatile molecules that symbolize the individuality of their urine marks to other conspecifics in domestic cats. Cats exhibited an innate behavior known as the flehmen response toward unfamiliar urine samples. This response was used as an indicator of bioassays to isolate individual recognition signals from cat urine. Chemical analysis shows that the isolated compounds are a subset of the 13 unusual branched fatty acids secreted from the kidneys. The urinary profiles of the branched fatty acids varied among individuals, show little interday variation in each individual, and stable for up to 24 hours after excretion in the environment. In behavior assays, cats distinguish between different compositions of branched fatty acids in the presence of unstable mixtures of other urinary volatile compounds by olfaction. In conclusion, a subset of the branched fatty acids is the potential chemosignal for individual recognition in cats. These findings provide the evidence that stable olfactory cues for individual recognition are present in unstable mixtures of hundreds of volatile compounds emitted from mammal scent marks.

82 **Confounding Associations Of Sex And Age On The Influence Of Blood Parameters And Body Weight On**

Odor Identification Performance In Healthy Adults

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Olfactory perception and nutrition are closely related and may influence each other via metabolic parameters. However, the relationship between metabolic blood parameters and olfactory performance is still unclear. Inconclusive findings exist for specific blood parameters. In this extensive analysis, we examined the relationship between olfactory performance, measured with MONEX-40, as well as intensity and pleasantness ratings with 38 metabolic blood parameters, age, sex, and the anthropometric measurements body mass index (BMI) and body fat percentage (BFP). Therefore, we included data of 418 healthy, well-phenotyped Caucasians of the *enable*-cohort. We replicated age-dependent olfactory identification scores ($p < 0.001$) and found slight evidence for a body fat dependence measured with BFP ($BF_{10} = 10.466$). We further identified a sex difference only in middle-aged adults ($p < 0.001$) that could be explained by environmental factors. Several blood parameters correlated significantly with the MONEX-40 score ($p < 0.05$ to $p < 0.001$). However, these effects diminished after adjusting for sex and age ($p > 0.9$) that were identified as confounders. The same applies for BFP. In addition, no parameters were identified to correlate significantly with perceived olfactory intensity or pleasantness score if controlled for sex and age ($p > 0.08$). Our results suggest that metabolic blood parameters are not related to olfactory identification performance in a relevant manner and highlight the importance of controlling for sex and age in chemosensory research.

83 **This Is The Title Of My Abstract: A Nasal Airflow Biomarker In Attentional Deficit Hyper Activity Disorder**

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Recent studies have uncovered complex interactions between patterns of nasal airflow and neurocognitive processing. These links raise the tantalizing possibility of altered nasal airflow in altered neurocognitive states. Here we test the hypothesis that such nasal airflow patterns are altered in Attentional Deficit Hyper Activity Disorder (ADHD). We developed a miniature wearable device, the Nasal Holter, that continuously measures and logs airflow in each nostril separately. We used this data to derive several measures, ranging from standard measures such as respiratory rate, to measures focusing on the periodic shift in nasal airflow across nostrils, a phenomenon known as the Nasal Cycle. In each measurement, participants wore the Holter for 24 hours continuously. We measured 34 participants with ADHD (17M, 26.7±3.1YO) and 37 controls (17M, 25.7±4.1YO). We observed that inhalations in ADHD are significantly larger (higher peak and volume), mean peak value ADHD= 0.69±0.28 arbitrary units (AU), mean control= 0.52±0.18, $t(69)=3.07$ $p= 0.003$, mean inhales volume ADHD= 0.41±0.18 AU mean control= 0.31±0.12, $t(69)=2.63$ $p= 0.01$). Moreover, we measured 28 of the ADHD participants twice, once "ON" and once "OFF" MPH (Ritalin). We observed a significant shift in the Laterality amplitude (LA) of the Nasal Cycle (i.e., the extent of difference across nostrils), that was reduced on MPH (LA "ON" = 0.3±0.14 AU, LA "OFF" = 0.35±0.15, $t(27)=2.8$, $p=0.008$). Finally, we observed a shift in dominant nostril, from Left off MPH to Right on MPH (LI "ON" = 0.04±0.2, LI "OFF" = -0.03±0.2, $t(27)=2.4$, $p=0.02$). These results suggest altered patterns of nasal airflow in ADHD. Further studies will reveal whether there is diagnostic, prognostic, and therapeutic value to these observations.

84 **Domestic Cats Damage Catnip And Silver Vine Plants Producing Iridoid And Enhance Chemical Pest Defense**

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Catnip (*Nepeta cataria*) and silver vine (*Actinidia polygama*) produce iridoids with repellent effects against arthropods. Domestic cats (*Felis silvestris catus*) benefit from these plant iridoids by the characteristic response comprising rubbing and rolling against the plants, which transfers iridoids onto feline fur where the chemicals repel mosquitoes. Cats also crumple and tear these plants by licking and chewing as a major part of this response, although the significance of this plant-damaging behavior has remained unclear. Here, we show the adaptive function of feline leaf-damage for chemical pest defense and the sensitivity to plant-specific iridoid production in cats. Physical leaf damage by licking and chewing increases iridoid emission substantially from both catnip and silver vine. Leaf damage also diversifies the iridoids in silver vine, which enhances cat self-anointing behavior. Although both the amount and types of iridoids differ between plant species, cats show a comparable duration of response to the relatively smaller amount of complex iridoid cocktail in damaged silver vine and to the much higher level of nepetalactone-only produced by damaged catnip. The diversification of iridoids in damaged silver vine increases their mosquito repellent activity at a low concentration, inducing a faster aversive behavior than

nepetalactol-dominated iridoids in intact leaves. In conclusion, physical damage of these plants in cats contributes to releasing more pest-repellent iridoids. Feline olfactory and behavioral sensitivity is optimized to plant-specific iridoid production, helping cats to maximize the mosquito repellency gained. Our findings highlight the benefit of the physical damage of insect repellent plants for chemical pest defense through a coordinated pattern of behavior in mammals.

85 **Combining The Best Of Both Worlds: Enhancing Physical Repellency Through Trace Amounts Of Topical Mosquito Repellents**

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Mosquito-borne diseases are posing an increasing threat to a wide range of people worldwide. Despite great efforts to control outbreaks, Malaria and Dengue fever see reoccurring waves of infection. Personal protection using mosquito repellents is a main defense for many affected people. Research of new repellents can contribute to broader acceptability and higher usage rates of mosquito repellent products, leading to a decreased risk of mosquito-borne infections. Following this, we previously reported the identification of a new repellence mechanism utilizing hydrophobic liquids to induce an escape response of mosquitoes after tarsal contact. Broadening our understanding of the effect of PDMS, we studied combinations with topical repellents like Citronella oil (*Cymbopogon Winteranius*) and DEET. We hypothesize that these repellent materials can effectively be transferred to the tarsi of *Aedes albopictus,* due to the effective wetting by PDMS. Through this direct transfer, the repellence potential can be greatly increased. We studied the landing behavior of *Ae. albopictus* on surfaces coated with PDMS and low concentrations of Citronella oil or DEET, respectively. When using repellent concentrations below the active concentration, in our assay, we observed a significant decrease in resting time of the mosquitoes on these surfaces. This observation was made for both evaluated repellent materials, indicating an underlying mechanism. Evaluating the effective concentration of Citronella on the mosquito tarsi we clarified a potent transfer of this repellent to the mosquito leg, which is mediated by the wetting ability of PDMS. We propose that the detection of repellent materials by mosquitoes mediated by tarsi is the main driving force of our observations of this synergistic effect.

86 **Taste Loss And Aging**

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The sense of taste has important biological functions. For example, sweetness suggests sources of energy, bitterness is an indicator for potential toxics. Although the human body has robust mechanisms to maintain stable whole-mouth taste sensations, the prevalence of taste loss increases with age. Age-related taste loss is considered to affect eating behavior and nutritional status, especially in frail or sick older people. As the world population is aging, numerous studies have addressed effects of aging on taste function. In this review, in Part 1 we elucidate reasons why older people are more vulnerable to taste loss. We conclude that biological aging and other factors that accompany aging, such as increased diseases and medication intakes, act in concert so that older people are more prone to taste loss. In Part 2, we summarize fifty-five studies investigating biological aging effects on taste function evaluated from various perspectives. At threshold level, many observations suggest that taste sensitivity in healthy older people is worse than in younger people. We found this age-related elevated taste threshold is most frequently reported for bitter and salty, followed by sour, and least for sweet. At suprathreshold level, the extent of age-related taste loss is concentration-specific but in general older people seem to experience a narrower “taste world” on perceived intensity compared to younger people. For the general taste identification ability, most studies show that older people are worse than younger ones. In Part 3, we summarize the attributes of age-related taste loss, which are related to taste quality, compound, concentration, stimulation site and gender.

87 **Detection Of Semiochemicals Through Biological Samples To Identify Behavioral Influence In Swine.**

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Previous studies have identified molecules unique to barrows. With this information, our study focused on identifying semiochemicals that are significant to sows, specifically at alternative stages of the estrous cycle: weaning, day of estrus, and 45d pregnant. Samples (n=10) were collected consecutively from the same sow during her entire estrous cycle. Saliva samples were analyzed through solid-phase microextraction (SPME) followed by gas chromatography-mass spectrometry (GS-MS). SPME employed polymer fibers to determine unique volatile molecules within the headspace of the saliva sample. No unique molecules were identified at weaning and 45d pregnant. Two molecules were found of interest in estrus samples and have also been identified as pheromones in insects. Tridecane (C13H28), with an average concentration peak of 16.12m/z, is found in Nymphs of the southern green shield bug as an aggregation pheromone to serve as a defensive mechanism against predators. Tridecane is also the main component in the defensive fluid produced by the stink bug *Cosmopepla bimaculate*. The second candidate molecule found, Dodecane (C12H26), had an average 13.44 m/z within samples. The *Glossina morsitans* uses dodecane to attract females while some male cockroaches use it as a sex pheromone. The purpose of this study is to identify semiochemicals found only in sows, that can be used as olfactory stimuli on boars during semen collection. Successful pheromones in swine are also found within insect species. The boar will be enticed due to the estrus volatile molecules that ruse the boar to mount the dummy. Further studies will demonstrate the behavioral response of boars to these estrus molecules and the concentration limit to identify these molecules through olfactory senses.

88 **Social Chemosignals Increase The Effects Of Mindfulness Treatment In Individuals With Social Anxiety: A Pilot Study**

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Previous studies have shown that individuals exposed to emotional chemosignals report a partial reproduction of the affective state of the sender. The present study aimed to investigate if emotional chemosignals can increase the benefits of a mindfulness-based intervention in individuals with social anxiety symptoms (SAD), a mental disorder characterized by intense fear and avoidance of social situations. Thirty women (mean age: 22.2) with SAD were recruited and divided into one of three odor conditions (happiness or fear chemosignals or clear air). The study was conducted over two consecutive days. Each day, participants performed the mindfulness intervention while being exposed to one of the three odor conditions. At the beginning of day 2, participants were subjected to a social stress induction. Photoplethysmography was used to measure heart rate variability (HRV) during the intervention. At the beginning and at the end of each day, anxiety level was measured with the STAI scale. Results on anxiety level showed a significant interaction of odor and time both for day 1 [$F(2,27) = 3.59$, $p = .041$] and day 2 [$F(2,25) = 6.04$, $p = .007$]. On day 1, only participants doing the intervention in the happiness condition reported a reduction of anxiety (happiness: $p = .003$, fear: $p = .08$, clean air: $p = .99$), whereas on day 2 both participants in the happiness and fear conditions reported a reduction of anxiety (happiness: $p = .002$, fear: $p = .002$, clean air: $p = 1.00$). Moreover, HRV analysis revealed a main effect of odor [$F(2,25) = 3.9$, $p = .033$]: HRV was higher during the intervention with happiness compared to fear odor ($p = .026$) indicating overall increasing well-being. The results give potential insight of how social chemosignals may be utilized to support positive outcomes of psychological therapy.

89 **How Does Covid-Related Masking And Social Distancing Affect Social Olfactory Communication? **

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Odor is an important component of human social interactions. Everyone has a unique body odor, and this odor can provide a wide variety of social information. Due to the current COVID-19 pandemic, there has been a shift in human interaction due to masking and social distancing, limiting our access to these important olfactory cues. Additionally, many people have struggled with COVID-related olfactory loss. In this study, we aimed to examine the influence of COVID safety protocols on perceptions of social olfactory information. Data collection is ongoing and results are preliminary (n=39). We developed a questionnaire to examine how people's perceptions of others' body odors have changed in the face of COVID-related masking and social distancing, including whether these measures interfere with the conscious perception of social odor cues, and whether others' body odor has become more intrusive since the start of the pandemic (*impact*, Cronbach's $\alpha = .64$). We also developed a brief questionnaire to assess participants' level of COVID risk in their social behaviors (Cronbach's $\alpha = .80$) and collected information on personality type and self-reported olfactory function. Participation in risky behaviors was significantly higher for unvaccinated than vaccinated individuals ($t(36) = -3.883$, $p < .001$) We found that covid impact scores were positively correlated with extraversion ($r(39) = .35$, $p = .03$) and body odor disgust sensitivity (BODS) scores ($r(39) = .37$, $p = .02$), but negatively correlated with odor awareness sensitivity (OAS) ($r = .52$, $p < .001$). Preliminary evidence suggests that the impact of social restrictions during the COVID pandemic influences individuals differently depending on personality factors and sensitivity to social odors.

90 **World Taste And Smell Day Association: Towards The Public Elevation Of The Chemical Senses**

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The COVID-19 pandemic demonstrated on a global level how all too often the chemical senses are not fully appreciated, until they are diminished. In 2020, a group of concerned individuals with science, industry, communication and direct experience with smell/taste loss came together to establish a day where the world could meet to celebrate the role of taste and smell. The mission of the World Taste and Smell Day (WTSD) Association is to create and sustain an international day devoted to elevating the senses of taste and smell. On September 14, 2021 WTSD hosted an online Exploratorium of the Joy and Science of Flavor (https://tasteandsmellday.tumblr.com/). This community-fueled online exhibition and related global social media efforts touched over 25,000 people. WTSD also featured a global panel discussion, several events in China and raised nearly $10,000 to support the three primary patient advocacy groups. Here is an opportunity for scientists to share their priorities and insights into what science has to offer to the public to raise awareness about these senses. The results of anonymous polls conducted at AChemS 2022 will be presented live at the poster session and made available to the community thereafter. The aim is to learn what messages the scientific community sees as key to communicate the importance of smell and taste to society.

91 **Cross-Cultural Comparison Of Waterless Empirical Taste Test (Wett&Reg;) Scores: Chinese Vs. American Subjects**

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Objectives: To determine whether scores on a novel taste test that requires no water differ between American and Chinese adults, and whether the test scores are influenced by gender and age. Methods: The 53-trial Waterless Empirical Taste Test (WETT®) was administered to 113 Chinese and 214 Americans. The subjects orally sampled monomer cellulose pads containing one of four concentrations of sucrose, citric acid, NaCl, caffeine, and monosodium glutamate and indicated whether a sweet, sour, bitter, salty, brothy, or no taste sensation was perceived. Separate gender by culture analyses of covariance with age as the covariate were performed on the total score and the scores for each taste stimulus. Results: No difference between American and Chinese subjects was found for the total WETT® score (p=0.129) or for sucrose (p=0.129) or NaCl (p=0.368). However, for monosodium glutamate, the scores were 28.40% higher for the Chinese than for the American subjects (p=0.024), and for citric acid and caffeine the scores were 24.12% and 21.79% higher for the American subjects (p=0.001 and 0.029). For all test qualities, women outperformed men and test scores declined with age. Conclusions: Although total scores on the WETT did not differ significantly between healthy adult Chinese and American subjects, individual taste qualities did differ, with better Chinese performance for monosodium glutamate and better American performance for citric acid and caffeine. Both age- and gender-related differences were noted. Future work is needed to determine the cause of these differences and whether the findings generalize to other Chinese and American samples.

92 **The Bitterness Of Antibiotics Is Associated With Genetic Variability In *Tas2Rs***

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For drugs to deliver their full benefits and have maximum efficacy, patients need to follow the recommended dosage and frequency. Unfortunately, the taste of drugs, specifically bitterness, can reduce patient compliance. Due to genetic differences in bitter taste receptor genes (*TAS2Rs*), some individuals may be at greater risk for low compliance due to heightened bitterness compared to others. Here we report on the sensory attributes of two antibiotics (ofloxacin and chloramphenicol) and investigate whether bitterness perception is associated with genetic variability in *TAS2Rs*. Participants (n=149) reported the intensity of suprathreshold concentrations of ofloxacin and chloramphenicol on the general Labeled Magnitude Scale. The dominant sensation reported from ofloxacin and chloramphenicol was bitterness, followed by drying. The mean bitterness from ofloxacin and chloramphenicol was 14.06 (±1.17) and 16.47 (±1.16), respectively, falling just below 'moderate.' For *TAS2R38*, A49P was significantly associated with the bitterness of chloramphenicol but not ofloxacin. The bitterness of ofloxacin was associated with SNP Val187Ala in *TAS2R9,* which confirms previous *in vitro* studies (Dotson et al., 2008). In summary, we observed individual differences in the bitterness perception of two bitter antibiotics, which are associated with genetic variability in *TAS2Rs*. Data on bitterness perception from drugs may help reformulate or innovate the delivery system, ultimately increasing compliance. Furthermore, a growing body of literature supports functional roles of T2Rs outside of the oral cavity; thus, relationships between T2R genotypes and taste perception may provide insight into efficacy or incidence of unintended side effects through interaction with extra-oral taste receptors.

93 **Investigating The Role Of Chemesthesis In The Behavioral Avoidance Of Sour Tastes In Mice**

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Taste sensory receptors enable animals to discriminate different tastes by sampling molecules in the oral cavity. Otopetrin1 (OTOP1), a proton-selective channel found in type III taste cells, is the sour receptor and plays a major role in the gustatory response to acids. Despite the absence of a functional OTOP1 sour sensor in *Otop1* KO mice, we previously found that *Otop1* KO and wildtype (WT) mice exhibit similar behavioral aversion to HCl and citric acid. One potential explanation is that sour taste is accompanied by chemesthetic sensations that arise from chemical activation of receptors such as the transient receptor potential (TRP) channels expressed in trigeminal afferents that innervate the oral cavity. Here, we generated an *Otop1*, *Trpa1*, *Trpv1* triple KO (TKO) mouse strain to investigate the role of chemesthesis in the behavioral avoidance of sour. Using a brief-access lickometer assay, we compared the preferences for bitter, pungent, and sour tastes in WT, *Otop1*, and TKO mice. For the control bitter solution, WT, *Otop1*, and TKO mice exhibited no significant difference in preference. For the pungent solutions that activate TRP channels, TKO mice exhibited a near indifference, as compared with the strong aversion observed in WT and *Otop1* KO mice, validating the TKO phenotype. Remarkably, tor the sour solutions, WT, *Otop1*, and TKO mice exhibited no significant difference in preference over a wide range of concentrations. Our results suggest a high degree of redundancy and that in addition to OTOP1, TRPV1 and TRPA1, other acid-sensing channels are involved in the behavioral aversion to sour tastes. Acknowledgements: We thank Sue Kinnamon, Courtney Wilson and Lindsey Schier for technical advice and Diana Bautista for generoursly sharing TRPA1/TRPV1 double knockout mice.

94 **Gustatory System Shifts In *Drosophila Suzukii*, An Emerging Threat To Global Fruit Production**

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Drosophila suzukii, commonly known as the spotted wing *Drosophila*, is a major agricultural pest of soft fruits, including strawberries, raspberries, and blueberries. It has invaded the United States and Europe at an unprecedented rate since 2008, causing severe economic losses for a wide variety of fruit industries. *D. suzukii* is destructive due to its unusual egg-laying preference for ripe, intact fruits. On the contrary, most other *Drosophila* species, including *Drosophila melanogaster*, prefer to lay eggs on fermenting fruits. The sensory mechanisms underlying the unusual egg-laying preference of *D. suzukii* for ripe fruits remain poorly understood. We found

that *D. suzukii* and *D. melanogaster* differ in sugar sensation. They also differ in mechanosensation and in the integration of taste and mechanosensory cues when making egg-laying decisions. Behaviorally, *D. melanogaster* shows a stronger preference than *D. suzukii* for higher sugar concentrations in two-choice egg-laying assays. Electrophysiological recording reveals that some taste sensilla have lost response to sugars in *D. suzukii*. RNA sequencing and RT-PCR results show that some *D. suzukii* sugar Gr receptors have reduced expression. Some mechanoreceptors had higher expression in *D. suzukii,* consistent with observations that *D. suzukii* prefers to lay eggs on stiffer substrates, while *D. melanogaster* prefers softer substrates. In addition, these two species respond differently to choices of different combinations of sweetness and stiffness. These differences between *D. suzukii* and *D. melanogaster* are likely to contribute to their natural preference for ripe and overripe fruits respectively.

95 **Effects Of Cranberry Polyphenol Extract (Cpe) Supplementation On Astringency And Flavor Perception As A Function Of Prop Taster Status And Other Individual Factors**

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We investigated if PROP taster status, age, gender, ethnicity, and BMI are markers of variation in perception of astringency and other flavor attributes of cranberry juice. Participants (n=125) evaluated cranberry juice cocktail samples supplemented with 0, 0.3, 0.5 and 0.75 g/L cranberry-derived polyphenol extract (CPE; Ocean Spray), and two controls (unsweetened cranberry juice and an aqueous solution of 0.75g/L CPE). Subjects evaluated the samples for sweetness, sourness, thickness, bitterness, astringency, cranberry flavor, overall flavor and liking using 15-cm end-anchored line scales. The data were analyzed using ANCOVA and machine learning tools (regression trees & random forest modeling) to examine if the latter approach would extract additional insights. ANCOVA revealed robust stimulus effects but no effect of PROP status on astringency perception. Instead, PROP status influenced cranberry flavor perception and liking, where super-tasters perceived more flavor and liked the samples less than non-tasters. Caucasian subjects generally perceived more bitterness and astringency from the samples and liked them less compared to Asian subjects. The visualized framework of regression trees showed that each sensory attribute was influenced by a different set of independent variables. Random forest modeling showed that each independent variable had a different explanatory power for each sensory attribute. These data show that PROP taster status did not specifically influence astringency perception when CPE was added to an ecologically relevant cranberry beverage but affected other key attributes. This study underscores the need to explore more complex stimuli that mimic real-world foods and to include personal factors, particularly ethnicity, which are important in shaping perceptual experiences.

96 **Theta-Beta Coupling In Orbitofrontal Cortex Underlying Olfactory Predictive Coding**

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The orbitofrontal cortex (OFC) plays a major role in multisensory integration and predictive coding. However, it is unclear how the OFC integrates information from other brain regions to achieve its function, especially in olfaction. Low and high frequency oscillations have been suggested to support communication between brain regions and local computations, respectively. We hypothesized that the OFC achieves olfactory predictive coding through cross-frequency coupling between low and high frequency oscillations. We used intracranial EEG recordings from a cued odor-sampling task and computed the strength of theta-beta coupling in the OFC. We first examined the overall theta-beta cross-frequency phase-amplitude coupling using the modulation index over a broad time window (0–2 s after the cue). To characterize the temporal dynamics of this coupling, we then calculated the modulation index using a sliding time window. These analyses revealed that cue-induced cross-frequency coupling is maximal in the lateral OFC. Furthermore, theta-beta coupling peaked at around 1 s following the cue, which is slower than oscillatory power increases previously observed in piriform cortex (PC). We found that low frequency power increases in OFC following cues differed significantly based on the specific odor being cued (Rose or Mint), whereas this was not the case in PC. Future analyses will include determining the frequency and direction of oscillatory coherence between OFC and PC following cues, and decoding of cue identity across oscillatory frequencies, prior to presentation of odor. These findings suggest that the orbitofrontal cortex might encode the identity of predictive codes in the olfactory system.

97 **Mixture Suppression Primarily Occurs In Broadly Tuned Neurons In The Geniculate Ganglion**

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Taste experience usually arises from a mixture of multiple taste stimuli. Frequently, mixtures produce a reduced response (mixture suppression). In this study, we examined the variation of mixture suppression between individual geniculate neurons to binary taste mixtures of citric acid with NaCl and sucrose by using calcium imaging. The degree of suppression to citric acid/NaCl and citric acid/sucrose mixtures varied considerably across neurons. Broadly tuned neurons had greater mixture suppression than narrowly tuned ones (r = -0.53, p

<0.001). Specifically, neurons responding to more taste qualities had greater suppression than those responding to fewer taste qualities (p <0.05). Mixture responses were suppressed to a greater extent in neurons that responded to both stimuli in the mixture compared to neurons that responded to only one of the stimuli in the mixture (142 mM NaCl/10 mM citric acid, p<0.001; 137 mM sucrose and 10 mM citric acid p<0.001). In fact, neurons that only responded to NaCl showed no mixture suppression to NaCl/citric acid mixture (p=0.72). Similarly, neurons that responded only to citric acid or sucrose did not show suppression to a citric acid/sucrose mixture (p=0.392 and p=0.337, respectively). This indicates that taste bud cells transducing both stimuli in the mixture must drive a functional response in the neuron for most mixtures to show suppression. One exception is that neurons responding only to citric acid were suppressed by the citric acid/NaCl mixture (p=0.007). Since Type III cells respond to both citric acid and NaCl, one explanation is that NaCl in the mixture inhibits citric acid responses of Type III taste bud cells. None of these results specifically support lateral cell-cell inhibition as a mechanism mediating mixture suppression.

98 **Comparison Of Unipolar And Bipolar Electrogustometric Threshold Measures For Clinical Applications**

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Electrogustometry has proven useful in clinical taste testing. Extant electrogustometers typically employ unipolar electrodes which require current movement between the tongue and distal body locations, such as the hand, forearm, or neck. While constant current circuits account for varied resistances due to different sectors of the body, concerns arise regarding the impact of even low electric current on non-taste tissues (e.g., the heart). Moreover, bipolar electrodes are more practical and patient compliant, since an indifferent electrode does not have to be held in the hand or attached to the skin, minimizing the likelihood of creating an open or discontinuous circuit. In this study of 16 healthy subjects, we compared bipolar and unipolar electrode detection thresholds, as measured by current density and a forced-choice staircase threshold paradigm. Threshold values were equivalent for bipolar and unipolar electrodes. Unipolar anodal thresholds were lower (greater sensitivity) than unipolar cathode thresholds (p <0.05). Age was inversely related to all threshold scores, and women outperformed men (all ps <0.001). No left:right tongue differences were apparent. This research indicates that taste testing using bipolar electrodes produces threshold scores equivalent to those using unipolar electrodes, validating the utility of such electrodes for clinical evaluations of the taste system.

99 **Sweet Desensitization Mediated By Glia-Like Taste Cells On The Tongue**

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Due to the lack of taste receptors, type-1 taste cells, also known as glia-like taste cells, have long been regarded merely as a supporter or a bystander in the central pathway of taste transduction. Although a recent *ex vivo* study demonstrated that type-1 taste cells can receive purinergic input from bitter-sensing type-2 taste cells, their functional role in transducing taste signals is yet speculative. Here, we pursue investigating the molecular-level functional crosstalk between glia-like type-1 and chemosensory type-2 cells during taste sensation by harnessing an *in vivo* functional imaging platform (μTongue) on subtype-specific taste cells. We found that sweet taste-elicited activation of type-2 taste cells gave rise to an increase in functional activity of adjacent type-1 taste cells, which was mediated by ATP released from type-2 taste cells. Synthetic activation or inhibition of type-1 taste cells led to modulation of central processing of sweet taste signals, from calcium activity in type-2 taste cells to neural activity in gustatory afferent nerves. We further investigated molecular mediators and receptor subtypes using functional screening and *in situ* hybridization. Taken together, our results propose that a functional interplay between type-1 and type-2 taste cells contributes to modulating sweet taste perception.

100 **Fat Versus Sugar Preferences In Knockout Mice Missing Lipid Sensors Gpr40 And Gpr120**

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The fatty acid sensors GPR120 and GPR40 have been implicated as lingual fat taste receptors, yet GPR120 knockout (KO), GPR40 KO, and GPR40/120 double KO (DKO) mice show normal preferences for Intralipid (soybean oil) in choice tests with water. Recent findings suggest that lingual GPR120 may function to distinguish fat from other tastants. Here we found that GPR120 KO mice did not differ from wildtype (WT) in their preference for isocaloric 3.2% Intralipid vs. 8% sucrose in 24-h choice tests. In contrast, GPR40 KO and GPR40/120 DKO mice showed reduced preferences for fat over sugar relative to WT mice. This reduced fat preference was attributed to impaired postoral fat appetite conditioning (appetition) rather than impaired fat taste. This is indicated by the findings that DKO and WT mice do not differ in their short-term (5-min) preferences for fat vs. sugar, and that GPR40 KO and GPR40/120 DKO mice, but not GPR120 KO mice, show impaired conditioning responses to postoral fat infusions. Also, DKO and WT mice are similar in their preference for Intralipid over a nonnutritive sweetener that has no postoral appetition actions in 24-h choice tests. Together, these results indicate that GPR120 taste sensors are not critical for the taste preference for fat relative to water or sweeteners and confirm that postoral GPR40 and GPR40/120 sensors mediate fat appetition.

101 **Taste Dysfunction In Covid-19: Specificity For Umami?**

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COVID-19 continues to impact the lives of most people throughout the world. The influences of this disease on quantitative taste tests remain enigmatic. We compared taste test scores of three groups on the 27-item version of the Waterless Empirical Taste Test (WETT®): 22 COVID-19 patients in the acute phase of the disease; 40 who had recovered from COVID-19 for 5 or more months; and 194 healthy controls who had never contracted COVID-19. An 8-item olfactory test was also administered. The overall taste test scores were equivalent among the three groups [ANOVA p = 0.33; respective means (95% CIs) = 14.9 (12.3-17.5); 16.1 (14.2-18.0); 16.7 (16.0-17.4)]. However, unlike the sweet, sour, bitter, and salty tasting stimuli, the acute subjects underperformed the other two groups in identifying umami [ANOVA p = 0.003; respective means (95% CIs) = 0.6 (0.1-1.1); 1.2 (0.7-1.6); 1.5 (1.4-5.7)]. The olfactory scores of the acute group were significantly lower than those of the other two groups, which did not differ significantly [ANOVA p <0.0001; respective means (95% CIs) = 5.8 (4.8-6.8); 6.4 (5.9-6.9); 7.2 (7.1-7.3)]. These findings are in accord with earlier studies suggesting that, on average, taste-bud mediated taste sensations of the four classic taste qualities are not meaningfully impacted by COVID-19, unlike olfactory sensations. However, the fifth taste quality, umami, appears to be impacted by this disease. Although there may be individual cases where true taste dysfunction occurs for all taste qualities, they appear to be rare or very transient. As in the general population, most complaints of taste loss in COVID-19 patients likely reflect loss of flavor sensations secondary to retronasal stimulation of the olfactory receptors during deglutition.