45th Annual Meeting of the Association for Chemoreception Sciences
April 19-22, 2023
Bonita Springs, FL

Printable Program & Abstracts

Wednesday, April 19, 2023

9:00 - 11:30 AM	Calusa EFGH
A Celebration of Gordon M Shepherd, M.D., D.Phil.	

This pre-meeting event is in memory of Professor Gordon Murray Shepherd, M.D., D.Phil. (1933-2022), a founding member and former president of AChemS. During his long, successful career (52 years at Yale University with full extramural funding), Gordon and his laboratory made seminal contributions in many areas of neuroscience research, and of course most notably in the chemosensory field. He published >300 research articles and 8 books, including The Synaptic Organization of the Brain in its 5th edition. He inspired and mentored so many AChemS members during his career, and we would like to get together in celebration of his life and achievements (see https://www.nature.com/articles/s41593-022-01141-2 for more details). Speakers include former trainees, colleagues and friends from all over the world and will cover a broad range of topics with a focus on chemosenses. These topics span from development, organization, function and computation of neural circuits to animal and human behavior, reflecting Gordon's broad interests and influence.

Chair(s): Charles Greer, Stuart Firestein, Minghong Ma

9:00 **Opening Remarks**
Charles Greer
Yale University

9:15 **Olfaction, A Sex Attractant, And Tsetse Flies**

John R. Carlson[1], Shimaa A.M. Ebrahim[1], Hany K.M. Dweck[1], Sebastian Chahda[1], Neeraj Soni[1], Brian Weiss[2]
[1]Dept. of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT, United States,
[2]Dept. of Epidemiology of Microbial Disease, Yale School of Public Health, New Haven, CT, United States

The work of Gordon Shepherd has inspired generations of researchers studying olfaction in a wide range of organisms. Chemical communication in insects is elegant in concept and sophisticated in design. Pheromones are used by many insects to recognize a conspecific in a habitat that may contain thousands of insect species. However, little is known about chemical communication in tsetse flies. Tsetse spreads disease across much of sub-Saharan Africa. They can carry African trypanosomes, which they transmit when they bite humans or animals. In humans the parasites cause African Sleeping Sickness, and in livestock they cause a disease called nagana, which has a devastating effect on the economic development of Africa.

The antenna of the tsetse fly *Glossina morsitans* contains four classes of olfactory sensilla. Sensilla in one region of the antenna were found via electrophysiology to respond to a variety of odorants, via both excitatory and inhibitory responses. Certain odor receptors of tsetse respond to host odors in an *in vivo* expression system. Screening of odorants that activate one receptor has identified 2-propanol as a candidate for an environmentally friendly and practical tsetse attractant. We recently found that *G. morsitans* produces compounds that elicit strong behavioral responses from males. Methyl palmitoleate (MPO), methyl palmitate (MP), and methyl oleate (MO) elicit male behavioral responses in two paradigms. Perfuming a virgin female of another *Glossina* species with MPO elicits mounting behavior from a *G. morsitans* male. All three compounds elicit electrophysiological responses from ORNs of *G. morsitans*. In addition, we found that infection with trypanosomes modulated the chemical profile in mated flies, and that infected females had reduced receptivity to mating.

9:45 **Video Tribute**
Kensaku Mori
Riken Center for Brain Science, Japan

9:50 **Molecular Mechanisms Regulating Olfactory Bulb Formation**

Fumiaki Imamura, Claire Miller, Ayako Ito
Penn State College of Medicine, Hershey, PA, United States

The olfactory bulb (OB) evaginates from the anterior end of the telencephalic vesicle during brain development. However, the cellular and molecular mechanisms regulating OB formation are unique and different from those of the cerebral cortex. Indeed, OB dysplasia can occur without significant defects in the cerebral cortex, which results in congenital olfactory defects. For example, more than 90% of children with Kallmann syndrome are born with anosmia or hyposmia associated with the absence or dysplasia of the OBs. However, the molecular mechanisms regulating OB formation are largely unknown. In this study, we first investigated the role of FGF signaling in OB formation, since *fgf8* and *fgfr1* are causative genes of Kallmann syndrome. To locally inhibit FGF signaling, a dominant-negative form of FGFR1 was introduced into the anterior end of the telencephalon by *in utero* electroporation. We found that inhibition of FGFR1 signaling aberrantly promoted the neuronal differentiation of radial glial cells (RGCs) in the OB (OB RGCs), which resulted in OB hypoplasia, suggesting

that FGF signaling regulates normal OB formation by controlling the development of RGCs. Furthermore, we found that inhibition of FGF signaling downregulated noggin expression in the OB. Since noggin is an antagonist of BMP receptors, we also examined the role of the BMP signal in OB formation. Interestingly, the overactivation of BMP signaling in OB RGCs resulted in OB hypoplasia. These results suggest that crosstalk between FGF and BMP signaling pathways in the OB RGCs is important for proper OB formation during development. I will present our research results and discuss how Dr. Gordon Shepherd's research shaped the trajectory of my career.

10:20 **Video Tribute**

Pierre-Marie Lledo
Institut Pasteur de Paris, France

10:25 **Cognitive Functions And Dysfunctions Emerging From A Large-Scale Data-Driven Model Of The Olfactory Bulb**

Michele Migliore
Institute of Biophysics, National Research Council, Palermo, *, Italy

Understanding the neural basis of brain functions and dysfunctions has a huge impact on a number of scientific, technical, and social fields. Experimental findings have given and continue to give important clues at different levels, from subcellular biochemical pathways to behaviors. However, most of the multi-level mechanisms underlying the cognitive architecture of the involved brain regions are still largely unknown or poorly understood. This mainly depends on the practical
impossibility to obtain detailed simultaneous in vivo recordings from an appropriate set of cells, making it nearly impossible to decipher and understand the emergent properties and behavior of large neuronal networks. With Gordon Shepherd, we have addressed this problem using a large-scale computational model of the olfactory bulb. I will present and discuss the main results and techniques we used to design and exploit this model, implemented following its natural 3D structure, with the main general aim to uncover the mechanisms underlying higher brain functions and helping the development of innovative therapies to treat brain diseases.

10:55 **Video Tribute**

Doron Lancet
Weizmann Institute of Science, Israel

11:00 **Local Circuit Function In The Olfactory Bulb**

Ben Strowbridge
Case Western Reserve Univ, Cleveland, OH, United States

The olfactory bulb serves as a relay region for sensory information transduced by receptor neurons in the nose and ultimately routed to a variety of cortical areas. While the molecular organization of different olfactory sensory inputs in this region has been well described, understanding how synaptic inputs interact to generate an efficient output code for specific odors remains challenging. Unlike principal cells, which receive glutamatergic input from one class of sensory neuron, GABAergic interneurons, like granule cells, integrate excitatory inputs from many different sensory modules. And unlike most CNS neurons, granule cells lack an axon and instead generate postsynaptic output onto principal cells via reciprocal dendrodendritic synapses that were first described by Gordon Shepherd and colleagues in the 1960s. Through their dendritic connections with mitral and other principal cells, GABAergic granule cells sculpt the output of the olfactory bulb and facilitate odor discrimination. Less is known about how granule cell interneurons are activated during sensory processing. Our group recently reported the first paired intracellular recordings of dendrodendritic excitation between glutamatergic mitral cells and granule cell dendrites. Surprisingly, we also found both spontaneous and evoked excitatory postsynaptic potentials in granule cells that are far larger and faster than dendrodendritic inputs, suggesting that granule cells may be excited by two distinct glutamatergic local circuits. Using computational simulations, we show that the large, non-dendrodendritic EPSPs more reliably encode the duration of principal cell discharges than dendrodendritic EPSPs. Together these excitatory pathways determine much of the timing of the local inhibitory synaptic responses that shape the output of the olfactory bulb.

11:30 **Tributes**

Paul Trombley[1], Chiquito Crasto[2], Fuqiang Xu[3], Arjun Masurkar[4]
[1]Florida State University, [2]Texas Tech University, [3]Shenzhen Institute of Advanced Technology, China, [4]New York University

12:00 - 2:30 PM	Great Egret
AChemS Executive Committee Meeting (Invite Only)	

1:00 - 4:00 PM	Calusa EFGH
A Celebration of Gordon M Shepherd, M.D., D.Phil.	

This pre-meeting event is in memory of Professor Gordon Murray Shepherd, M.D., D.Phil. (1933-2022), a founding member and former president of AChemS. During his long, successful career (52 years at Yale University with full extramural funding), Gordon and his laboratory made seminal contributions in many areas of neuroscience research, and of course most notably in the chemosensory field. He published >300 research articles and 8 books, including The Synaptic Organization of the Brain in its 5th edition. He inspired and mentored so many AChemS members during his career, and we would like to get together in celebration of his life and achievements (see https://www.nature.com/articles/s41593-022-01141-2 for more details). Speakers include former trainees, colleagues and friends from all over the world and will cover a broad range of topics with a focus on chemosenses. These topics span from development, organization, function and computation of neural circuits to animal and human behavior, reflecting Gordon's broad interests and influence.

Chair(s): Charles Greer, Stuart Firestein, Minghong Ma

1:00 **Opening Remarks**
Stuart Firestein
Columbia University

1:10 **From Signal Transduction To Sensory Disorders**
Frank Zufall
Center for Integrative Physiology and Molecular Medicine (CIPMM), Saarland University, Homburg, *, Germany

This lecture will highlight some of our work that began decades ago with investigations of signal transduction mechanisms in various olfactory and chemosensory systems and now has evolved into a systems-oriented approach that is directed at understanding mechanisms of sensory disorders. One example of this strategy is our work on the role of the sodium channel gene *SCN9A* (Nav1.7) which has linked anosmia with analgesia. This work now shows that inhibition of neurotransmitter release at the first synapse in the olfactory and pain pathways is the principle mechanism of pain insensitivity and anosmia in both mice and humans with loss-of-functions in Nav1.7. Another example for this strategy is our work on the sensing of danger-associated metabolites and the execution of appropriate defense programs. These studies involve the interaction of microbiota-derived chemical signals with host cells in multiple epithelia including those of the vomeronasal organ, the main olfactory system, and the respiratory system. We will also highlight how our work with Gordon Shepherd shaped the trajectories of these studies and, in fact, our entire careers. Supported by Deutsche Forschungsgemeinschaft (DFG) grants SFB 894 and SFB-Transregio 152.

1:40 **Neurogastronomy And The Odorant Receptors**
Timothy S. McClintock[1], Hiroaki Matsunami[2], Claire A. de March[2,3]
[1]University of Kentucky, Lexington, KY, United States, [2]Duke University, Durham, NC, United States, [3]CNRS, Université Paris-Saclay, Gif-sur-Yvette, *, France

Dr. Gordon M. Shepherd coined the term "neurogastronomy" to encompass the intersection of flavor science with the chemistry and physics of food preparation. He envisioned smell and taste science enhancing the pleasure of eating and improving the formulation of healthy diets. His revolutionary ideas expand opportunities for practical use of chemosensory science and are a natural outlet for public outreach. Neurogastronomy, by its very nature, expands links between the chemical senses and the other fields of science invested in the study of food. It has become a multidisciplinary field of science with its own annual meeting and a strong public education mission. Dr. Shepherd encouraged and supported work on the odorant receptors (ORs), viewing their essential role in generating food odor images as one avenue toward manipulating food preferences. This has motivated our studies of OR response patterns, done in freely behaving mice and followed-up by heterologous expression of ORs in cultured cells. We find that general odorants evoke concentration-dependent responses from numerous ORs. In nearly all cases the responsive ORs are diverse in sequence, arguing that natural selection for OR divergence and expansion have been more important than selection for sensitive detection of specific odorants. OR response patterns to highly similar odorants are surprisingly diverse, suggesting that odor percepts tolerate some variation in OR response pattern. When testing odorant mixtures, we find that interaction effects are common. These are most often antagonism of ORs by an odorant, but additive effects involving multiple agonists are also observed. These data begin to inform us about some of the opportunities Dr. Shepherd envisioned as leading to a more logical and mechanistic science of flavor.

2:10 **The Impact Of Odor Familiarity On Piriform Cortex Odor Response**
Ian F. Chapman[1,2], Max L. Fletcher[1]
[1]University of Tennessee Health Science Center, Memphis, TN, United States, [2]Monell Chemical Senses Center,

Philadelphia, PA, United States

The piriform cortex (PC) plays a key role in the processing of olfactory information in the brain. To explore how this region encodes olfactory experience, we exposed awake, freely moving mice to multiple presentations of a variety of odors every day for five days. Using miniscope calcium imaging, we recorded the activity of neurons within the PC during each session. We could reliably identify and record from a large population of these neurons across all sessions, allowing us to directly compare odor coding across days. Overall, we find that while PC population responses to individual odors become less consistent across days, responses within a session accurately predict odor identity. As the mice became familiar with odors, they investigated the odors less (as measured by behavioral scoring), particularly towards the end of sessions. This decrease in attention during these odor trials coincided with a decrease in response correlation to the initial day presentations, suggesting that an animal's behavioral state plays a role in response consistency. We are currently exploring how novelty impacts PC responses to familiar odors by including a novel odor into the panel or by placing the animal in a new context. Overall, these findings provide new insights into the neural mechanisms underlying the encoding of odor familiarity within the PC of awake, behaving mice.

2:40 **Gordon Shepherd: Flavor And Retronasal Smell**

justus verhagen[1,2]

[1]The John B. Pierce Laboratory, New Haven, CT, United States, [2]Yale School of Medicine, New Haven, CT, United States

Gordon M. Shepherd made exceptionally large contributions to neuroscience. His continuous curiosity and scholarship touched a wide range topics and approaches, although olfactory neuroscience remained at its center. He also touched an exceptionally large group of neuroscientists, leaving behind a hugely positive legacy. One such areas is the field of flavor. Gordon indeed was hugely influential on the emerging field of flavor behavioral neuroscience. He contributed to, and guided, many diverse projects and was inspiring to many in the field. He held strong working relations with several core research groups, in particular at Yale. He furthermore established “Neurogastronomy” and “Neuroenology”, supported by his two popular recent books. I will summarize several key enduring contributions made by Gordon to flavor neuroscience and retronasal smell in particular. His excitement, guidance and scholarship will be highlighted.

3:10 **Tributes**

Trese Leinders-Zufall[1], Shin Nagayama[2], Haiqing Zhao[3], Michael Singer[4], Minghong Ma[5]

[1]Saarland University, Germany, [2]University of Texas Houston, [3]Johns Hopkins University, [4]Cartesian Therapeutics, [5]University of Pennsylvania

3:30 **Miscellaneous Musings**

GMG Shepherd
Northwestern University, Chicago, IL, United States

I will try to say a few words about topics relevant to Gordon M Shepherd's life and career, from my mostly filial perspective. Time allowing, I'll mention some of my lab's current work on how mice handle food, which is threatening to derail my hitherto successful efforts to entirely avoid the olfactory system.

4:00 - 4:30 PM	Great Egret
AChemS 2023 Codefest	

The goal of the AChemS 2023 Codefest is for you to apply your data analysis skills, learn from others, get feedback, explore others' work, and connect with the larger AChemS community. This year's Codefest will focus on data from the National Health and Nutrition Examination Survey (NHANES). We will provide starter code, orient you to the available data, and provide a team of teachers as support for working in both R and Python. We welcome coders of all skill levels and from any chemosensory system!

4:30 - 5:00 PM	Calusa Terrace
Diversity Fellowship Meet and Greet	

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

6:00 - 7:00 PM	Calusa EFGH
Keynote Lecture Sponsored By ADM	

6:00 **Improving Global Health Through (Meta)Genomic Studies Of Neglected Parasites**
Makedonka Mitreva
Washington University

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

Thursday, April 20, 2023

7:30 - 9:00 AM	Estero Foyer
Breakfast With Industry	

ADM NUTRITION SCIENCE & TECHNOLOGY
At ADM, we unlock the power of nature to provide access to nutrition worldwide. With industry-advancing innovations, a complete portfolio of ingredients and solutions to meet any taste, and a commitment to sustainability, we give customers an edge in solving nutritional challenges from seed to fork.

SENSONICS
Sensonics® International provides the medical, scientific, and industrial communities with the highest quality smell and taste tests available. The UPSIT®, the most widely used olfactory test in the world, is available in 45 languages. Recent offerings include an advanced electrogustomer, the self-administered Waterless Empirical Taste Test, and an extended smell training system.

8:00 - 11:00 AM	Estero Ballroom
Poster Session I	

100 **Paracrine Mode Of Sweet Adaptation Mediated By Glia-Like Type I Cells**

Gha Yeon Park[1,2], Geehyun Lee[1,2], Jisoo Han[3], Pyonggang Choi[1,2], MinJae Kim[1,2], Chaeri Park[1,2], Zhaofa Wu[4], Yulong Li[4], Myunghwan Choi[1,2]

[1]School of Biological Sciences, Seoul National University, Seoul, *, South Korea, [2]The Institute of Molecular Biology and Genetics, Seoul National University, Seoul, *, South Korea, [3]Korean Brain Research Institute (KBRI), Daegu, *, South Korea, [4]School of Life Science, Peking University, Beijing, *, China

As present in diverse levels of sensory information processing, perceived sweetness to prolonged exposure of sweet compounds declines over time. This so-called sweet adaptation has long been known to be mediated by the internalization of sweet receptors at the apical tip of taste buds. Here, we report that there is an alternative mode of sweet adaptation occurring within the taste bud, possibly mediated by intercellular interaction between glia-like type I and sweet-sensing type II cells. First, exploiting volumetric microscopy on fungiform taste buds *in vivo*, we revealed that the downstream afferent nerve terminals exhibit a higher degree of sweet adaptation compared to the upstream type II cells. Next, we identified that purinergic crosstalk from type II to type I cells is mediated by a specific subtype of P2Y receptor. Pharmacological activation of type I cells resulted in significant attenuation of taste-evoked ATP release from type II cells and neural calcium activity in the afferent nerves, indicating that type I cells provide inhibitory modulation to the peripheral transduction of sweet information. Taken together, our results substantiate that peripheral sweet adaptation is not only mediated by receptor internalization, but also by intercellular crosstalk between type II and type I cells.

102 **Effect Of *Kokumi* Taste-Active Casr Agonists In Htrpv1 And Mutants Expressed In Hek293T Cells**

Yiseul Kim[1], Vijay Lyall[2], Mee-Ra Rhyu[1]

[1]Korea Food Research Institute, Jeollabuk-do, *, South Korea, [2]Virginia Commonwealth University, Richmond, VA, United States

Extracellular calcium sensing receptor (CaSR) agonists, such as γ-glutamyl peptides, are involved in *Kokumi* taste perception and are also suggested to enhance salt taste in sensory analyses. Salt detection is mediated by at least two pathways. One is Na^+-selective and uses the amiloride-sensitive epithelial Na^+ channel (ENaC), the other is cation nonselective and amiloride-insensitive. Recent patch-clamp study using rat fungiform taste cells expressing ENaC provides direct evidence that CaSR agonists have no effect on ENaC activity. Here, we investigated if *Kokumi* taste substances can modulate salt response via amiloride-insensitive pathway. We monitored temporal changes in $[Ca^{2+}]_i$ in HEK293T cells expressing the human vanilloid receptor 1 (hTRPV1) cation channel which has been suggested as one of the potential amiloride-insensitive salt mediator. In wild type TRPV1, glutathione (GSH) and γ-Glu-Val-Gly (γ-EVG) induced concentration-dependent responses similar to that of capsaicin. TRPV1 antagonist capsazepine markedly attenuated these responses, but a calcimimetic NPS R568 did not exert agonistic behavior. Two known capsaicin-insensitive mutants, Y511A and S512Y were used to verify direct binding of *Kokumi* taste substances to the agonist binding pocket on TRPV1. Both capsaicin mutants, Y511A and S512Y, significantly reduced the apparent affinity of hTRPV1 for GSH and γ-EVG. This indicates that *Kokumi* taste-active CaSR agonists share at least two binding positions Y511 and S512 with capsaicin in hTRPV1.

104 **Trpm4 Contributes To Sour-Evoked Taste Signaling In A Subset Of Taste Receptor Cells And Is Required For Normal Sour-Driven Behaviors**

Yifeng Guo[1], Laura Martin[2,3], Ann-Marie Torresgrossa[2], Kathryn Medler[1]

[1]Dept. of Biological Sciences, University at Buffalo, Buffalo, NY, United States, [2]Dept. of Psychology, University at Buffalo, Buffalo, NY, United States, [3]Dept. of Food Science and Technology, Oregon State University, Corvallis, OR, United States

Growing evidence suggests that the signaling processes in taste receptor cells (TRCs) are more complex than previously appreciated. It is important to understand TRC signaling events since these cells initiate all subsequent taste processes. TRCs are comprised of different cell populations that use distinct signaling pathways to generate specific output signals for taste stimuli. Type II TRCs use G protein-coupled receptor signaling to detect sweet, bitter and umami tastants. We previously reported that transient receptor potential melastatin 4 (TRPM4) is required for the normal cellular responses to these stimuli in conjunction with TRPM5 in Type II TRCs. In that study, we observed that TRPM4 expression is not restricted to Type II TRCs but is also present in a subset of Type III cells. Since Type III cells are known to detect sour and salt, we performed short term lick assays to determine if the absence of TRPM4 affected taste-driven behaviors to these stimuli. Salt driven behaviors in TRPM4-KO mice were not different from wild type, while sour driven behaviors were significantly different. We then used live cell imaging to determine how TRPM4 contributes to sour-evoked taste responses. Sour taste depends on the activity of the proton channel, OTOP1 which initiates a signaling cascade that activates voltage-gated calcium channels (VGCCs) to cause neurotransmitter release. To date, this signaling pathway has not been completely characterized. We found that TRPM4 activity affects the sour-evoked calcium responses in a subset of sour-sensitive TRCs. Taken together, our live cell imaging and behavioral data indicate that TRPM4 is required for normal sour taste and identifies a complexity in sour signaling that has not been characterized.

106 **Sex Differences In Bitter Diet Intake But Not Brief Access Taste Test**

Kimberly F. James[1], Verenice Ascencio Gutierrez[1], Samantha L. Brooker[1], Laura E. Martin[2], Ann-Marie Torregrossa[1, 3]

[1]Department of Psychology, University at Buffalo, Buffalo, NY, United States, [2]Department of Food Science and Technology, Oregon State University, Corvallis, OR, United States, [3]Center for Ingestive Behavior Research, University at Buffalo, Buffalo, NY, United States

The influence of the estrous cycle on food intake is well documented and there are data suggesting that bitter sensitivity may change with hormone status. Our laboratory is interested in mechanisms of acceptance for bitter foods. In females, we first need to 1) determine if our proposed mechanism, salivary proteins (SPs), is altered by estrous and 2) to determine if stage of estrous changed bitter acceptance. First, saliva was collected from rats on a control diet (n=16) for 2 estrous cycles. There were no differences in SP expression (p's > 0.05). We then tracked the estrous cycle of female rats (n=32) and switched groups of rats from control to a quinine containing diet on different days of the cycle (i.e. diestrus 1, diestrus 2, proestrus, or estrus). All rats decreased 24hr intake while on quinine diet ($p < 0.001$), however, there were no group differences ($p = 0.87$). Since estrous did not appear to be contributing to SP expression or diet acceptance we then directly compared male and female profiles of diet-induced SP upregulation and expression. Male and female rats were given a control diet followed by 6 weeks on the quinine diet. Females were not cycle synchronized. There were two significant results: 1) there are baseline sex differences in the expression of SPs and 2) upregulation of diet-induced SPs takes twice as long in females. These animals were also tested in brief access taste tests for NaCl and quinine before and after protein upregulation. There were no group differences in licking a NaCl solution (0-2M) ($p > 0.05$). Both males and females significantly increased licking to intermediate concentrations of quinine solution following exposure to the bitter diet ($p < 0.001$). These data suggest that SPs that overlap in males and females are sufficient to alter diet acceptance.

108 **Stimulus Duration Effects On Electrogustometeric Thresholds And Chemogustometry Test (Wett&Reg;)**

Toshi Matsuda[1], Robert Brown[1], Vince Grosso[1], Richars/L Doty[2]

[1]Sensonics International, Haddon Heights, NJ, United States, [2]University of Pennsylvania, Philadelphia, PA, United States

Electrogustometry (EGM) is a practical way to test taste. However, the effects of stimulus duration on electrogustometric thresholds and the relationship to all the basic tastes are not well known. In this study of 28 healthy subjects, we compared anodal detection thresholds using a counterbalanced design, with 4 different stimulus durations (0.5, 1, 1.5 and 2 seconds), from a unique bipolar electrode in which the anode and cathode are contiguously located, on one side of the anterior tongue. A bipolar electrode produced 2 anodal stimulations separately using a stainless-steel central disk (0.39 cm^2 area) and a 1 cm^2 annular disk by a 2 mm wide nylon separator. After thresholds were determined using a single staircase procedure, all participants were employed 27 items-WETT® for each side of the tongue as a chemogustometry test. Non-parametric analyses were performed. Four different thresholds on a central disk electrode and an annular (donut-shaped) electrode were assessed. Even though the median thresholds of 0.5 and 1 sec. durations on a central disk were lower (3.36 μA and 2.83 μA) than other thresholds (4 μA and above), no significant differences were found among the thresholds of 4 stimulus durations. No significant sex, age, or tongue side effects were apparent. Correlations among EGM thresholds were higher for central disk (from 0.61to 0.73) than for annular disk (from 0.43 to 0.64; all ps<0.001).

There were weak correlations between 2 sec. stimulus thresholds and total WETT scores (-0.43 for central disk and -0.41 for annular disk) on both disks, and between 2 sec. thresholds on central disk and sweet WETT scores (-0.47), and between annular disk's 2 sec. thresholds and sour WETT scores. The results indicate that different perception mechanisms between electric and chemical tastes.

110 **The Method Of Delivering A Sucrose Solution To B6 Mice Influences The Number And Distribution Of Fos-Immunoreactive Neurons In Several Taste-Related Brain Areas**

Michael S King[1], Lianyi Lu[2], John D Boughter Jr[2]

[1]Department of Biology, Stetson University, DeLand, FL, United States, [2]Department of Anatomy and Neurobiology, The University of Tennessee Health Science Center, Memphis, TN, United States

Although ingestion of a solution requires oromotor activity and stimulates gustatory, somatosensory and visceral sensors, the relative effect of these inputs on the number of active neurons in the brain is unknown. Therefore, we used immunohistochemistry for the Fos protein (Fos-IR) to identify active neurons in several taste-related brain regions in B6 mice following licking (free-access, 15 min, n=5), intra-oral (IO) infusion (0.1 ml/min, 15 min, n=4), or esophageal gavage (0.5 ml, 5 s, n=5) of 1.0M sucrose (S). Compared to IO infusion, licking reduced the number of Fos-IR neurons in the external medial (EM) parabrachial nucleus (PBN) and decreased the percentage of neurons in the lateral (RL) rostral nucleus of the solitary tract (rNST), EM and dorsomedial (DM) PBN, and ventral orbitofrontal cortex (OFC) while increasing the percentage of neurons in the central (RC) rNST, central lateral (CL) PBN, and medial OFC ($p's<0.05$). By comparing the groups that received S either by licking or IO infusion to those receiving it via gavage, several areas were identified to have more neurons that respond to viscerosensory than orosensory input. Specifically, the RL rNST, dorsolateral and DM PBN, agranular gustatory cortex (GC), basolateral and central lateral amygdala (AMYG), the core and shell of the nucleus accumbens (NAc), dorsal bed nucleus of the stria terminalis (BNST), and ventral OFC contained more, or a higher percentage of, Fos-IR neurons following delivery of S via gavage than after licking or IO infusion. On the other hand, the RC NST, CM, waist and CL PBN, and medial OFC contained a higher percentage of neurons activated by orosensory input. These results highlight that some NST, PBN, GC, AMYG, NAc, BNST and OFC subareas contain more neurons that respond to viscerosensory than orosensory input.

112 **Inhibitory Neuron Subpopulations In The Rnst**

Charlotte Klimovich, Emma Gutarts, Keerat Sandhu, Susan Travers
Division of Biosciences, College of Dentistry, Ohio State University, Columbus, OH, United States

The rNST contains many GABA neurons. In other brain regions, inhibitory neurons are heterogeneous with defined populations employing different synthetic enzymes or peptides. The present study used fluorescent in situ hybridization (RNAscope) to begin to assess the degree to which there are distinct inhibitory neuron subpopulations in rNST. Paraformaldehyde-fixed, cryostat-cut (15μm) sections from C57BL/6 mouse brains (N=5F, 5M) were triple-labeled for different combinations of synthetic enzymes for GABA (GAD65), glutamate (VGLUT2), the vesicular GABA/glycine transporter, VGAT, and for somatostatin (SST) or preproenkephalin (PENK), a precursor for the enkephalins. There was nearly complete overlap between VGAT and GAD65. Because VGAT is ubiquitous in GABA neurons, this suggests that, in contrast to regions like the olfactory bulb, GAD65 does not define a limited subset of inhibitory neurons. However, as observed using co-staining with VGAT (Kalyanasundar et al., '22), less than 1/3 of rNST inhibitory neurons expressed SST. Additional inhibitory rNST neurons were PENK+. The pattern of co-expression suggested that rNST neurons are comprised of distinct subpopulations since more GAD65 neurons co-expressed only SST or ENK than both peptides, and others expressed neither. Although a majority of SST (~60%) and PENK (~70%) rNST neurons were inhibitory, other SST and PENK cells express the excitatory marker, VGLUT2. These data emphasize the complexity of cell types in the first-order gustatory relay. Moreover, the existence of distinct inhibitory cell types could help explain the varied effects of global activation of GAD65 rNST neurons in suppressing behavioral responses to multiple, distinct taste qualities and in altering both the gain and tuning of neurophysiological responses (Travers et al., '22).

114 **Neural Basis Of Defensive Freezing Behavior Induced By Predator Threat Via The Accessory Olfactory System**

Quynh Anh/ T. Nguyen[1,2], Ricky Chhor[1], Andrea Rocha[1], Yuna Yamashita[1], Christian Stadler[1,2], Sachiko Haga-Yamanaka[1,2]

[1]UCR Department of Molecular Cell Systems Biology, Riverside, CA, United States, [2]UCR Neuroscience Graduate Program, Riverside, CA, United States

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. 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 was reduced toward older odors. The freezing behavior was observed only when mice had direct contact with the cat odor and abolished in mice lacking functional Trpc2, indicating that the accessory olfactory system is necessary for this behavioral output. Interestingly, neural activation in some brain areas were with behavioral outputs in the fresh saliva-exposed mice but not in the ones exposed to old saliva. These results suggest that fresh cat saliva contains

chemical cues that signal imminence of predator threat, and the signal is processed through a specific population of neurons in the accessory olfactory system. Our research aims to identify the neural mechanism of imminence of threat detection and shed light on the neural basis underlying the defensive behavioral decision making in prey animals.

116 **Structural Covariance Of The Olfactory Tubercle-Brainstem Network In Humans**

Guangyu Zhou, Gregory Lane, Christina Zelano
Department of Neurology, Feinberg School of Medicine, Northwestern University, Chicago, IL, United States

As a component of the ventral striatum, the olfactory tubercle has been suggested to constitute an important brain region within reward circuitry. Previous animal studies have shown that the olfactory tubercle receives dopamine projections from the ventral tegmental area as well as the brainstem, including the periaqueductal gray. In humans, recent neuroimaging studies have provided evidence of the involvement of the olfactory tubercle in reward processing. However, the functional and structural connectivity of the olfactory tubercle within the context of reward pathways in humans—particularly the brainstem-reward circuitry—are not well understood. Structural covariance, which can be derived from the correlation of gray matter density between brain regions, provides a method to examine structural connectivity non-invasively. Here, we used seed-based structural covariance analysis to examine the structural connectivity between the primary olfactory subregions, including the olfactory tubercle, in a large population of young adults from the Human Connectome Project. Our findings revealed a strong structural covariance present uniquely between the olfactory tubercle and the periaqueductal gray, which is a major brain region of the dopamine reward pathway. Moreover, voxel-wise structural covariance analysis indicated a pattern of lateral-medial organization of the olfactory tubercle. These findings provide evidence of structural connectivity between the olfactory tubercle and the midbrain central gray in humans.

118 **Orexin Neurons In Lateral Hypothalamus Contribute To Cortical Responses And Taste-Related Behavior**

Kathleen Maigler, Maya Dinero, Donald Katz
Brandeis University, Waltham, MA, United States

Primary taste cortex (gustatory cortex, GC) is important for taste decisions and related learning. Evidence from our lab has strongly suggested that GC does not function alone. For instance, my optogenetic data suggest that lateral hypothalamus (LH) is one of the structures that cooperates with GC to process the hedonic value of a taste (palatability). The current research aims to unravel whether this across-region interaction is driven by a specific subset of LH to GC projection neurons. Unlike GC, LH contains a diverse population of peptide-expressing neuronal subtypes. Among them, orexin+ cells appear to be designed to influence feeding via axons linking them to both reward areas and cortex. To test how LH orexin+ neurons are involved in the GC taste response, I selectively perturbed this orexin+ pathway (using transgenic rats and optogenetics) and examined whether this manipulation alters 1) the GC taste response and 2) palatability-driven licking behavior. When disrupting orexin signaling, we found a significant impact on GC taste responses as well as the licking performance in the brief access task, indicating that the orexin projection between LH and GC is an important contributor in maintaining the functionality of the taste system.

120 **Quantitating The Effect Of Sample Size On The Outcome Of Affective Sensory Tests**

Robert Pellegrino[1], Sara Burns[2], Martin Tchernookov[3], Vijay Singh[4], Curtis R Luckett[2,5]
[1]Monell Chemical Senses Center, Philadelphia, PA, United States, [2]University of Tennessee, Knoxville, TN, United States, [3]University of Wisconsin, Whitewater, WI, United States, [4]North Carolina Agricultural and Technical State University, Greensboro, NC, United States, [5]New Age Eats, Berkeley, CA, United States

The dichotomy between the certainty of results and resources is exemplified in sensory affective testing. Furthermore, the data produced by using 9-point hedonic scales has a unique underlying distribution that is dependent on consumer scale usage and the product itself. We use a combination of resampling real sensory hedonic data with simulations to characterize how the outcome of affective sensory tests change across a range of participants and product space. We found that most data is multimodal and non-parametric in nature, limiting the usefulness of conventional measures of statistical power and sample size assessments. Through a novel method of pseudo-simulation that uses the actual underlying distribution, we were able to find sample sizes and statistical power across a wide range of sample sizes. By contrasting this method to conventional methods of sample size estimation, we were able to see how the gaussian sample size estimates consistently underestimated the number of participants needed for a particular statistical power. Our novel method compared similarly to sample size estimates generated from nonparametric methods. Our findings highlight the unique underlying distributions of data collected from 9-point hedonic scales and show how that distribution thwarts conventional methods of sample size estimation. Lastly, we introduce a novel method that can be implemented to estimate sample size in hedonic sensory testing.

122 **Combined Transection Of The Glossopharyngeal And Chorda Tympani Nerves Alters Food Choice, Macronutrient Intake, And Meal Patterns In Male Rats**

Carolina R. Cawthon, Alan C. Spector
Department of Psychology and Program in Neuroscience, Florida State University, Tallahassee, FL, United States

There is considerable research into the functions of gustatory nerves as they relate to basic discriminative and hedonic responding to prototypical taste stimuli but little attention has been given to the role these nerves may play in selection from multiple food choices during *ad libitum* feeding. Therefore, we used our 5-item Food Choice Monitor to examine food intake, choices, and meal patterns in 32 male rats after recovery from sham

surgery (SHAM) or combined transection (2Nx) of the glossopharyngeal and chorda tympani nerves, which together innervate taste buds of the tongue. Rats had access to standard chow plus 4 custom rodent diets which were high (H) or low (L) in fat (F) and/or sugar (S) and half of each surgical group experienced the foods prior to surgery. Surgery affected food choices, meal parameters, and macronutrient intake ($p \leq .05$). Specifically, after 2Nx (histology in progress), rats consumed more of the HF choices and less of the LF choices resulting in 2Nx rats having greater intake of fat and reduced intake of carbohydrates compared to SHAM rats. The 2Nx rats ate at a similar rate to SHAM rats but took longer to satiate and thus consumed significantly larger meals. Since 2Nx rats ate fewer meals, total daily energy intake was similar between groups. We found only minor effects of presurgical diet experience. The elevated fat intake, meal duration, and meal size suggest 2Nx increased the palatability and/or reduced the satiating potency of the fatty food choices; the similar within-meal eating rates favors the latter. Whatever the mechanism, these results demonstrate that lingual taste nerve transection can significantly alter the relative macronutrients ingested as well as the manner in which the food is consumed even when there are only minor differences in total energy intake.

124 **Nutrigenomic Regulation Of Sensory Plasticity**

Hayeon Sung[1], Anoumid Vaziri[1], Daniel Wilinski[1], Riley Woerner[1], Peter Freddolino[2], Monica Dus[1]
[1]University of Michigan, Ann Arbor, MI, United States, [2]University of Michigan Medical School, Ann Arbor, MI, United States

Diet profoundly influences brain physiology, but how the nutritional information is transmuted into neural activity and behavior changes remains elusive. Here we show that the metabolic enzyme O-GlcNAc Transferase (OGT) moonlights on the chromatin of the *D. melanogaster* gustatory neurons to instruct changes in chromatin accessibility and transcription that underlie sensory adaptations to a high sugar diet. OGT works synergistically with the Mitogen Activated Kinase/Extracellular signal Regulated Kinase (MAPK/ERK) *rolled* and its effector *stripe* (also known as EGR2 or Krox20) to integrate activity information. OGT also cooperates with the epigenetic silencer *Polycomb Repressive Complex 2.1* (PRC2.1) to decrease chromatin accessibility and repress transcription in the high sugar diet. This integration of nutritional and activity information changes the taste neurons' responses to sugar and the flies' ability to sense sweetness. Our findings reveal how nutrigenomic signaling generates cell-specific responses to global nutrient variations.

126 **Postoral Appetition Actions Of Isomaltulose And Sucrose In C57Bl/6 Mice**

Anthony Sclafani[1], Alexander Castillo[2], Ion Carata[2], Rachel Pines[2], Eli Berglas[2], Serena Joseph[2], Joymin Sarker[2], Merna Nashed[2], Matthew Roland[2], Sebastian Arzayus[2], John I. Glendinning[3], Richard J. Bodnar[2]
[1]Brooklyn College of CUNY, Brooklyn, NY, United States, [2]Queens College of CUNY, Queens, NY, United States, [3]Barnard College, Columbia University, New York, NY, United States

Isomaltulose is a sucrose analog with a similar taste profile but is less sweet to humans. Isomaltulose (ISO) is equicaloric to sucrose (SUC) but is considered healthier because of its slower rate of absorption. This study compared the postoral preference conditioning actions (appetition) of 8% ISO and 8% SUC in C57BL/6 (B6) mice. Exp. 1 revealed that B6 mice preferred SUC to ISO in 2-day choice tests, but their relative preference could be reversed by adding a mixture of nonnutritive sweeteners (sucralose and saccharin, SS) to the ISO. B6 mice preferred ISO + 0.05% SS to SUC but did not differ in their preference for ISO + 0.01% SS and SUC. In Exp. 2, B6 mice initially preferred both ISO + 0.01% SS and SUC to 0.1% SS by 67-75%. After separate 2-day sweetener vs. water tests, they increased their ISO + 0.01% SS and SUC preferences to 87% and 84%. This indicates that ISO, like SUC, has postoral appetition actions that enhance its taste-mediated intake. Exp. 3 directly compared the appetition effects of the two sugars using ISO + 0.05% SS and SUC solutions. Initially, the mice preferred ISO + 0.05% SS to SUC by 78%, presumably due to the sweeter taste of the mixture. However, after separate 2-day sugar vs. water tests, the mice preferred SUC to ISO + 0.05% SS by 66%. This preference reversal indicates that SUC has a stronger postoral appetition effect than ISO. This is likely due to the more rapid digestion of SUC and activation of intestinal glucose sensors (SGLT1) that mediate sugar appetition. Thus, the attractive taste of ISO can match or exceed that of SUC with the addition of nonnutritive sweeteners, but SUC has a more potent postoral appetite stimulation action. This does not negate, however, the potential health benefits of ISO.

128 **A Bayesian Framework For Understanding Multisensory Flavor Preference Decisions**

Joost X Maier, Megan N Garrison, Timothy V Dong, Alex Hua, Tyler Horiuchi
Wake Forest School of Medicine, Winston Salem, NC, United States

Flavor is a major determinant of consumption. Although commonly referred to as “taste”, flavor is a multisensory experience: drawing from gustatory, olfactory and somatosensory inputs, each sourced from separate peripheral senses. Previous work in humans demonstrates that multisensory flavor cues are integrated to inform perceptual decisions. However, the computations underlying multisensory flavor interactions and their role in food choice remain poorly understood. Here, we used rats as a model system to obtain preferences in a series of daily two-alternative free choice tests, in which they drank from two bottles containing taste, odor or taste+odor mixture solutions. Mean preference and variance over repeated presentations of the same condition (“reliability”) were used to evaluate validity of the maximum likelihood estimation framework. This framework predicts that judgments of multisensory stimuli are a weighted average of the unisensory component judgement; that the weight components carry is proportional to their relative reliability; and that multisensory judgements are more reliable than their component judgements. Results from naïve rats (raised on standard chow) confirm these predictions. Results from rats raised with a limited set of specific taste-smell mixtures further show that the ability to weight taste and smell components is unaffected by flavor congruency. Finally, results from rats raised on a diet consisting of a wide variety of real foods show that sensory enrichment increases the overall weight

animals place on odor components. This work provides a quantitative framework for understanding the multisensory interactions underlying hedonic evaluation of flavor and the factors that shape them, and suggest specific hypotheses regarding their neural underpinnings.

130 **Achems Undergrad Finalist: Some Anthocyanins Trigger Appetitive And Attractive Behaviors In Drosophila Melanogaster, Suggesting These Compounds Play An Important Role In Plant-Insect Interactions.**

Isabella B Allar[1], Claire H Welp[1], Ashley C Moyer[1], Joshua Ekanem[2], Zane Zobejana[2], Morgen Story[2], Erik C Johnson[1], Nicole M Hughes[2], Jackson T Sparks[2], Cecil J Saunders[3], Michael J Rizzo[1]

[1]Wake Forest University, Department of Biology, Winston-Salem, NC, United States, [2]High Point University, Department of Biology, High Point, NC, United States, [3]Kean University, Department of Biological Sciences, Union, NJ, United States

Anthocyanins are molecules that contribute to the bright red and blue colors of some plants and are often concentrated in their fruits. Previous studies on anthocyanins have mostly focused on the protection they provide plants or their potential health benefits as antioxidants. A few reports suggest that anthocyanins may stimulate the olfactory or gustatory system of insects that feed or lay eggs on anthocyanin-rich plant tissues. In this study, we test the hypothesis that anthocyanins are an important chemical cue in triggering feeding and egg-laying behaviors of the frugivore insect *Drosophila melanogaster*. To better understand the role anthocyanins play in this plant-insect interaction, we investigate the chemosensory reaction of *Drosophila melanogaster* adults and larvae to isolated anthocyanins through the Proboscis Extension Response (PER) and Larval Substrate Preference (LSPA) Assays. The PER Assay has shown appetitive responses to anthocyanins with significantly higher rates of proboscis extension in response to anthocyanins Cyanidin-3-glucoside (C3G) ($p<0.001$) and Petunidin-3-glucoside (Pt3G) ($p<0.01$) than water. These results are echoed in the LSPA in which larvae show greater preference for anthocyanins C3G and Malvidin-3-glucoside (Mv3G) over agar, than 5% sucrose or red dye over agar. Negative Geotaxis assays have also shown that anthocyanins have positive effects on locomotion, Mv3G and C3G fed flies having a higher success rate in climbing 8 cm in test containers than those fed on the control diets. Additionally, anthocyanins have shown to have positive developmental effects with eggs lain in a Mv3G-rich environment emerging 9 hours faster than control diet conditions ($p<0.05$). These data suggest that anthocyanins are a potent chemosensory stimulus for at least one insect species.

132 **Does Emotional Overeating Mediate The Relationship Between Sweet Liking And Added Sugars Intake? A Pilot Study**

Safi Melanie, Enrique R Pouget, Xin Yi Lin, Alia Salvador Coronel, Lily Dionisio, Abeir Anasseri, May M Cheung
City University of New York - Brooklyn College, Brooklyn, NY, United States

The appeal of sweetness is a major driver of added sugars consumption, but eating behaviors also influence a person's food choices. In particular, the emotional overeating behavior trait has been associated with the overconsumption of highly palatable foods (e.g., energy-dense sugary foods). However, whether emotional overeating may influence the relationship between sweet liking and added sugars consumption requires further investigation. We collected information on sweet liking (using a validated Food Liking Survey), added sugars intake (using the short Healthy Eating Index), eating behavior (using the Adult Eating Behavior Questionnaire), and self-reported body weight and height in 52 adult participants. We were able to replicate the relationship between age and sweet liking ($r = -0.46$, $p < 0.001$) as well as added sugars intake ($r = -0.33$, $p = 0.018$). We also detected a significant association between sweet liking and added sugars intake ($r = 0.39$, $p < 0.004$) and body mass index ($r = 0.32$, $p = 0.021$). The interaction between sweet liking and emotional overeating was small but approaching significance ($\beta = -0.02$, $p = 0.089$); however, this trend disappeared when adjusted for age. Overall, the data supported the hypothesis that sweet liking is associated with added sugars consumption and obesity. Whether emotional eating behaviors mediate the relationship between sweet liking and added sugars consumption requires a larger sample size to be determined. Identifying methods to modify sweet liking (e.g., through low-sugar diets) may be a promising strategy to lower added sugars intake to promote better health.

134 **Orobehavioral Separation Of Cooling From Warming Requires Trpm8**

Kyle T. Zumpano, Jinrong Li, Christian H. Lemon
University of Oklahoma, Norman, OK, United States

The role of trigeminal thermoreceptors in oral temperature recognition and preference is unexplored. Prior data from our group show distinctions between mild oral cooling and oral warming in trigeminal neural responses depend on input from the cold and menthol sensor transient receptor potential melastatin 8 (TRPM8). Here we studied the role of TRPM8 in behavioral responses to oral cooling and warming. A custom thermo-lickometer measured brief-access licking responses by thirsty TRPM8 gene deficient (n=9) and C57BL/6J (B6; n=10) mice to water at a reference temperature and a set of comparison temperatures. Daily comparison temperatures were drawn from an eight-temperature series (35°C to 1°C), with series for different references tested over sequential blocks. Data were collected blind to mouse genotype. When offered a warm (35°C) reference and a cool (<30°C) comparison temperature, both TRPM8 deficient and B6 control mice avoided licking warm and favored cool water. Cooling preference increased as comparison temperatures fell further below 35°C and peaked at 15°C ($p<0.01$) in both mouse lines (no interaction, $p=0.6$). But when offered water at a mild reference of 30°C and a lower comparison temperature, B6 mice showed indifference whereas TRPM8 deficient mice avoided 30°C in

favor of lower temperatures (interaction, p=0.01). TRPM8 deficient mice gave reduced sampling of 30°C (p<0.01), implying active avoidance of mild oral cooling. Finally, tests in water-replete B6 (n=4) and TRPM8 deficient (n=4) mice revealed similar licking avoidance in TRPM8 mutants of mild oral cooling with sucrose solutions. These data show orosensory preference for mild oral cooling is context dependent in TRPM8 deficient mice and imply TRPM8 ion channels are required to establish the boundary between cooling and warming.

136 **Impact Of Aldehyde Dehydrogenases And Aldo-Keto Reductases In Human Olfactory Peri-Receptor Events.**

Valentin Boichot[1], Franck Menetrier[1], Jean-Michel Saliou[2], Frederic Lirussi[3,4,5], Francis Canon[1], Mireille Folia[6], Jean-Marie Heydel[1], Thomas Hummel[7], Susanne Menzel[7], Maria Steinke[8,9], Stephan Hackenberg[10], Mathieu Schwartz[1], Fabrice Neiers[1]

[1]Université de Bourgogne, INRAE, Centre des Sciences du Goût et de l'Alimentation (CSGA), Dijon, *, France, [2]University of Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, UAR CNRS 2014 - US Inserm 41 - PLBS, Lille, *, France, [3]UMR 1231, Lipides Nutrition Cancer, INSERM, F-21000 , Dijon, *, France, [4]UFR des Sciences de Santé, Université Bourgogne Franche-Comté, F-25000 , Besancon, *, France, [5]Plateforme PACE, Laboratoire de Pharmacologie-Toxicologie, Centre Hospitalo-Universitaire Besançon, F-25000, Besançon, *, France, [6]Department of Otolaryngology-Head and Neck Surgery, Dijon, *, France, [7]Smell and Taste Clinic, Department of Otorhinolaryngology, TU, Dresden, *, Germany, [8]University Hospital Wuerzburg Chair of Tissue Engineering and Regenerative Medicine Roentgenring 11 , Wuerzburg, *, Germany, [9]Fraunhofer Institute for Silicate Research ISC, Roentgenring 11, Wuerzburg, *, Germany, [10]Department of Otorhinolaryngology – Head and Neck Surgery, RWTH Aachen University Hospital, Aachen, *, Germany

Xenobiotic metabolizing enzymes (XMEs) are key enzymes in the detoxification of toxic compounds. In oral and nasal cavities, they protect chemosensory tissues and olfactory sensory neurons by metabolizing exogenous compounds in order to eliminate them more easily. Some XMEs have been shown to modify the pattern of activated olfactory receptors by metabolizing new molecules with sensory properties that differ from the initial odorant molecule. Aldehyde dehydrogenases and aldo-keto reductases, two oxidoreductase families belonging to XMEs are well represented in oral and nasal cavities and can metabolize a wide range of components, especially aldehydes. Using a proteomic study of the human nasal mucus performed by mass spectrometry, the most abundant members of these two families were selected for the rest of this study: aldehyde dehydrogenase family 1 member A1 (ALDH1A1), aldehyde dehydrogenase family 3 member A1 (ALDH3A1), and aldo-keto reductase family 1 member B10 (AKR1B10). Their precise locations in human olfactory/respiratory epithelium and turbinate were examined using immunohistochemistry techniques. The enzymatic activities of the recombinant enzymes towards a panel of 20 odorant molecules with aldehyde function were assessed and demonstrate the capacity of these enzymes to interact with some odorant molecules. Finally, our results obtained by X-ray structure resolution of ALDH3A1 in complex with octanal support the role of the catalytic Cys 243 in the active site of the enzyme and add new elements on how the substrate metabolization takes place with the probable implication of the Asn 114.

138 **Individual Differences In The Taste Of Excipients And An Over-The-Counter Pediatric Medicine**

Julie A. Mennella[1], Mengyuan Kan[2], Elizabeth D. Lowenthal[3], Joel Mainland[1], Blanca E. Himes[2], M. Yanina Pepino[4]

[1]Monell Chemical Senses Center, Philadelphia, PA, United States, [2]University of Pennsylvania, Department of Biostatistics, Epidemiology and Informatics, Philadelphia, PA, United States, [3]University of Pennsylvania Perelman School of Medicine, Department of Pediatrics, Philadelphia, PA, United States, [4]University of Illinois at Urbana-Champaign, Urbana, IL, United States

Objective. Our research program aims to systematically determine the sources of variation in the flavor of pediatric medicines and their excipients. The present study focused on a popular, over-the-counter, non-steroidal anti-inflammatory medicine (Berry-flavored Children's Motrin™ Oral Suspension) of which ibuprofen is the active pharmaceutical ingredient and Acesulfame K (Ace K) and sucrose are sweetening excipients. Methods. Trained adult panelists (n = 154) used the general Labelled Magnitude Scale (gLMS) and hedonic gLMS after tasting 5mL of 0.012M Ace K and 0.6M sucrose and after swallowing 5mL of Motrin. Genotypes for 141 panelists were obtained by using Illumina Infinium Global Screening Array v3.0 Beachip, and association analyses with sensory phenotypes were conducted on several candidate single nucleotide polymorphisms (SNPs) near or within genes that encode selected taste receptors. Results. Hedonic ratings for Motrin ranged from -64 (strong dislike) to 98 (strongest imaginable like). Consistent with prior findings, SNP rs3741845 within the *TAS2R9* bitter receptor gene was associated with the bitterness of Ace K (p <0.001). While SNP rs35744813 within the *TAS1R3* sweet receptor gene was associated with the sweetness of Motrin (p = 0.01), the association with the sweetness of sucrose did not reach significance (p = 0.07). Overall, hedonic ratings of Motrin were positively related to hedonic ratings of both sweetening excipients (correlation efficient > 0.3; p <0.001). Conclusions. Investigations into the taste of excipients provided insights into sources of individual difference in the palatability of the complex flavor of a pediatric medicine. Distinct variations in how a given medicine tastes and whether a child is willing to ingest it support a precision medicine approach.

140 **How Bitter Is It? Bitterness Perception Of Theobromine And Common Over-The-Counter Medications**

Stephanie Okoye[1], Leah Hall[2], Jessica Nicanor-Carreon[1], Masha Niv[3], Yanina Pepino[1,2,4]

[1]Division of Nutritional Sciences University of Illinois Urbana-Champaign, Urbana, IL, United States, [2]Food Science and Human Nutrition University of Illinois Urbana-Champaign, Urbana, IL, United States, [3]Food Science and Nutrition, Institute of Biochemistry, The Hebrew University, *, Israel, [4]Carle Illinois College of Medicine, Urbana, IL, United States

Bitter taste is aversive to many consumers, presenting a problem for the food and pharmaceutical industry. There is a need to broaden the repertoire of bitterness suppressors methods, ideally in a consumer-tailored manner. However, the mechanism of bitterness reception is complex. There are 25 bitter receptors in humans. Although the field has significantly advanced by identifying ligands to cognate bitter receptors, most response profiles for these bitterants are derived from cell-based assays. The goal of this pilot project is to generate suprathreshold dose-responses for bitter ligands known to activate one or two TAS2R subtypes in cell-based assays (e.g., Theobromine, Naringin, Acetaminophen, Dextromethorphan, Diphenhydramine) using a trained panel of adults. To date, 19 participants (8 men/11 women) had completed sensory visits. We presented each compound in duplicate at three concentrations in randomized order on two blocks: once with and once without noseclips. Using a sip-and-spit method, participants rated taste, smell, and irritation intensity using generalized magnitude scales. Preliminary analyses show a significant effect of concentration on bitterness and irritation ratings for all compounds (except for naringin for which concentrations were suboptimal). There were negligible ratings for smell intensity, and bitterness and irritation ratings were unchanged by the presence of noseclips (all P>0.16). These preliminary results extend the few reported compounds that elicit both bitterness and chemesthetic responsiveness in humans. Future studies will determine association of genetic variations in these particularTAS2R subtypes with individual sensory phenotypes to feed computational models and help predict bitterness of additional compounds as well as potential suppressors of bitterness.

142 **Machine Learning Identifies The Most Important Parameters To Predict Prop Phenotype And Genotype, And The Overall Taste Status In Healthy Subjects And Patients With Taste Disease.**

Melania Melis[1], Lala Chaimae Naciri[1], Mastinu Mariano[1], Thomas Hummel[2], Iole Tomassini Barbarossa[1]

[1]Department of Biomedical Sciences, Cagliari University, Monserrato (CA), *, Italy, [2]Department of Otorhinolaryngology, Smell and Taste Clinic, Dresden University of Technology, Dresden, *, Germany

Taste sensitivity is mediated by taste receptors that detect chemical molecules in the oral cavity and in numerous extra-oral tissues. The sensitivity of these receptors, which is associated with their genetic variants, can affect physiological functions. We used the Supervised Learning (SL) algorithms to identify the sensitivity for the prototypical stimulus, 6-n-propylthiouracil (PROP), the genetic variants of the bitter receptor TAS2R38 and to identify which parameters best predict the overall taste status (OTS) of healthy controls (HC) or patients with chemosensory loss (PCL). SL algorithms automatically identified the objectives, by exploiting subject biological parameters which were used as predictive variables in the data set. The CatBoost model best identified the PROP phenotype and TAS2R38 genotype of subjects, while Random Forest Regressor best predicted the OTS in HC and PCL. The intensity ratings for a different amount of PROP were the most important parameters in training the model and understanding the difference among PROP phenotypes and TAS2R38 genotypes, while the scores of the lowest concentration of salty and of the higher concentration of sweet were the most important ones to predict the OTS of HC and PLC, respectively. These results, by showing that the SL approach allows obtaining an automatic and high-precision identification of PROP phenotypes, TAS2R38 genotypes, and OTS in HC and PCL, suggest that it may represent an objective and reliable tool for taste physiology studies, with applications varying from basic science to medicine.

144 **Gastrin Releasing Peptide Receptors Modulate Gustatory Cortex Circuits Excitability And Function**

Maria Isaac[1,2], Carlo T. Fontanini[1], Arianna Maffei[1,2]

[1]Department of Neurobiology and Behavior, Stony Brook, NY, United States, [2]Graduate Program in Neuroscience, Stony Brook University, Stony Brook, NY, United States

Eating is a dynamic process driven by internal states, sensory and visceral information, and experience-based associations. The study of appetite regulation primarily focuses on hypothalamic circuits, although recent evidence points to the gustatory insular cortex (GC) as a fundamental component of feeding behavior. Gastrin releasing peptide (GRP), a neuropeptide highly expressed in the gustatory cortex (GC), induces meal termination in human and animal studies, when injected systemically. GRP signals through gastrin releasing peptide receptors (GRPRs), but the neurons expressing GRPRs in the gustatory system have not been identified. Here we used a transgenic mouse line labeling GRPR-expressing cells and report that they are enriched in GC. GRPR-GC cells comprise a histologically heterogenous population of neuronal and non-neuronal cells. We found that GRPR expression colocalizes with the non-neuronal marker Glial Fibrillary Acid Protein (GFAP), and with inhibitory markers Glutamic Acid Decarboxylase 67 (GAD67), Somatatostatin (SST) and Parvalbumin (PV). The heterogeneity was further verified by in situ hybridization and analysis of membrane properties using patch clamp recordings in acute slice preparations. Comparison of the prevalence of GRPR cells between male and female mice revealed a larger prevalence of GRPR expressing cells in males, unveiling possible sex differences in GRP signaling. Bath application of GRP during electrophysiological recordings showed that GRP substantially increases inhibitory drive onto GRPR expressing cells. These results show that satiety signals like GRP exert a powerful influence on GC by engaging diverse populations of cells in a sexually dimorphic fashion. Future experiments will assess the effects of GRPR signaling locally, in GC, on eating behaviors.

146 **Anatomy Of Tongue Innervating Mechanoreceptors**

Thomas A Myers, Robin F Krimm
University of Louisville, Louisville, KY, United States

Somatosensory innervation of the oral cavity enables us to detect the various textures of the foods we consume. To date, little is known about the genetic identify and anatomy of mechanoreceptors innervating the oral cavity. Taste buds within fungiform papillae are surrounded by nerve fibers that express the mechanosensitive channel, Piezo2. Fungiform papillae also express neurotrophin-3 (NT-3), so we asked if neurons innervating fungiform papillae express TrkC, the receptor for NT-3. We found that TrkC+ neurons innervate fungiform papillae and fibers are Piezo2+. We sought out more specific genetic labels by examining Pvalb and VGLUT3 expressing neurons. These neurons have overlapping expression with TrkC, but not with each other. We then compared three genetically identified putative mechanosensitive neuron types: TrkC, Pvalb, and VGLUT3 across postnatal ages. In P120 mice, an average 79% of fungiform papillae are innervated by TrkC+ neurons, 80% by Pvalb+ neurons, and 30% by VGLUT3+ neurons. We found that the number of fungiform papillae innervated by TrkC+ fibers decreased from 100% to 79% by P120. Pvalb+ fibers first innervate fungiform papillae at P30 and the number of innervated papillae increases with age. Interestingly, VGLUT3+ fibers first innervate fungiform papillae at P60. We found that Pvalb+ neurons are NFH+/CGRP-, indicating that the Pvalb+ population consists of A-fibers (NFH+), but not c-fibers (CGRP+). This was corroborated by our finding that Pvalb+ nerve fibers are myelinated. VGLUT3+ neurons consist of two populations: one population that is NFH+ and another that is NFH-. This indicates that VGLUT3+ neurons range from C-fibers to A-fibers. These data provide the anatomical underpinnings for interpretation of future functional experiments

148 **Machine Learning Models To Evaluate Potential Toxicity Of Flavors And Fragrances And Identify Safer Substitutes**

Joel Kowalewski[1], Anandasankar Ray[2]

[1]Sensorygen Inc, Pasadena, CA, United States, [2]University of California Riverside, Riverside, CA, United States

Flavor and fragrance ingredients remain largely unregulated. Recent toxicological initiatives like Tox 21 have nevertheless emerged, leading to high-throughput screens to evaluate ingredients for health hazards. While these efforts are a promising step, they are limited by the size of the chemical space tested, the high number of relevant human proteins to test and the associated testing costs. These constraints can be addressed through computational screening which could generate prioritized subsets for further toxicological evaluation. Here,we trained Machine Learning (ML) models on the Tox21 database, containing ~10,000 chemicals and ~70 activities from in vitro assays for toxicologically relevant protein targets, including human proteins where activity may indicate developmental and endocrinological toxicity. Our trained ML models were accurate and allowed us to screen large databases containing most flavors, fragrances, and cosmetic ingredients (Cosing and GoodScents), ranking the chemicals and physicochemical features of potential concern. We prioritized compounds with predicted activity on human hormone receptors to identify safer substitutes, using our SensoryAI platform. This platform predicts the sensory profiles for ~150 olfactory and gustatory characters. Using this we screened ~1 million chemicals in a library that has been filtered based on low *in silico* toxicity values, for similar smelling and tasting compounds. We create a database of plausible substitutes for compounds that may be health risks. Our approach helps integrate toxicological analysis into early R&D or help reformulate products with substitutes for consumers that are increasingly safety-aware.

150 **A Thiazolidinedione Is A Partially Effective Bitter Blocker**

Ha Nguyen, Cailu Lin, Ivona Sasimovich, Katherine Bell, Amy Huang, Nancy Rawson, Danielle Reed
Monell Chemical Senses Center, Philadelphia, PA, United States

The bad bitter taste of medicines is a barrier to overcoming non-compliance with medication use, especially life-saving drugs given to children and the elderly. Compounds that potentially block bitterness screened from chemical libraries with cell-based assays, and a class of drugs, thiazolidinediones (TZDs), were identified as potential blockers. In this study, a TZD was tested, rosiglitazone (ROSI), using a high-potency sweetener as a positive control, neohesperidin dihydrochalcone (NHDC). We tested their bitter-blocking effect on the bitter drugs tenofovir alafenamide fumarate (TAF) and praziquantel (PRAZ) by conducting behavioral taste testing with two separate sensory panels: a limited-diversity panel (n=97) and a diversity panel (n=159). Participants rated the bitterness intensity of the solutions on a 100-point generalized visual analog scale. As expected, participants in both panels rated the bitter drug TAF and PRAZ as less bitter on average when mixed with NHDC than the drug alone. ROSI partially suppressed the bitterness of TAF and PRAZ, but the suppression effect differed between the two panels. ROSI significantly reduced the bitterness of PRAZ but not TAF in the first panel and reduced the bitterness of TAF but not PRAZ in the second panel. However, there was a group of participants in the diversity panel (n=41) who reported moderate average suppression of TAF (44%) or PRAZ (37%) with ROSI. These results suggest that ROSI is a partially effective bitter blocker, and other TZDs should be tested with more drugs and on different populations to define which one works best with which drugs.

152 **Adiponectin Enhances Fatty Acid Signaling In Human Taste Cells By Increasing Surface Expression Of Cd36**

Fangjun Lin[1], Trina Rudeski-Rohr[1], Timothy A. Gilbertson[2]

[1]Burnett School of Biomedical Sciences, College of Medicine, University of Central Florida, Orlando, FL, United States, [2]Department of Internal Medicine, College of Medicine, University of Central Florida, Orlando, FL, United States

Adiponectin is an adipose-derived hormone that plays critical roles in stimulating glucose and fatty acid metabolism and enhancing insulin sensitivity. Adiponectin exerts its action by activation of three independent receptors, AdipoR1, AdipoR2, and T-cadherin. Each of these receptors is highly expressed in the murine taste system, however, their effects and mechanisms of action in the modulation of gustatory fat responses, if any,

remain unclear. Our earlier work showed that an immortalized human fungiform taste cell line (HuFF) is functionally comparable to primary rodent taste cells and could serve as a model for investigations of fatty acid signaling. In this study, we utilized HuFF cells to investigate the effect of AdipoRon (an adiponectin receptor agonist) on fatty acid-induced responses. Calcium imaging studies showed that AdipoRon administration (0.1-10 μM) enhanced HuFF cell responses to fatty acids in a concentration-dependent fashion, but did not affect responses to a mixture of sweet, bitter, and umami tastants. This enhancement was inhibited by an irreversible CD36 antagonist (sulfosuccinimidyl oleate; 400 μM) and by an AMPK inhibitor (Compound C; 10 μM), but was not affected by an antagonist for GPR120 (AH-7614; 10 μM). Moreover, AdipoRon had no effects on GW9508 (an agonist for GPR40 and GPR120)-induced calcium responses. In addition, AdipoRon increased the phosphorylation of AMPK and the translocation of CD36 to cell surface, which was eliminated by blocking AMPK with compound C. These results indicate that AdipoRon acts via the activation of AMPK to increase cell surface expression of CD36 in HuFF cells to selectively enhance their responses to fatty acids and is consistent with the ability of adiponectin receptor activity to alter taste cues associated with dietary fat intake.

154 **Distinct Fatty Acid Signaling Pathways In Type Ii And Type Iii Taste Cells**

Emeline Ward[1,2], Fangjun Lin[1,2], Naima Dahir[1,2], Ashley Calder[1,2], Trina Rudeski-Rohr[1,2], Timothy A. Gilbertson[1,2]

[1]Burnett School of Biomedical Sciences, College of Medicine, University of Central Florida, Orlando, FL, United States, [2]Department of Internal Medicine, College of Medicine, University of Central Florida, Orlando, FL, United States

The continued increase in obesity, along with its associated diseases, establishes the need for a better understanding of the physiological mechanisms of fat taste transduction in the gustatory system. While obesity is a multifactorial disease, one of the major contributors to this disease is the intake of excess fat. Our recent data indicate that both Type II and Type III taste cells contribute to peripheral gustatory fatty acid signaling, though the specific role of fatty acid signaling in these cell types is not completely understood. Calcium imaging and electrophysiological assays on genetically identifiable Type II & III cells suggest that different transduction pathways are involved in the response to polyunsaturated fatty acids (PUFAs) in the two cell types. Utilizing a variety of immunocytochemical, biochemical, and cell-based approaches, we will provide evidence that Type II cells respond to PUFAs via activation of Na-permeable TRPM4/5 channels, similar to the transduction pathway of other GPCR-mediated taste pathways in this cell type. In contrast, Type III cells that lack TRPM4/5 also show robust responses to PUFAs. Instead PUFA responses in Type III cells are dependent upon Ca^{2+} and display inward Ca^{2+} currents during PUFA application in patch clamp recording. Our preliminary data indicate the source for Ca^{2+} influx in Type III cells may be mediated by influx through TRPC channels along with CD36 signaling, and involves the downstream activation of the STIM1/Orai1/3 complex and subsequent opening of Ca-release activated Ca (CRAC) channels. Our data indicates that Type II and Type III cells both respond to fatty acids but do so via different transduction pathways that may reflect a different functional role for fatty acid signaling in each of these cell types.

156 **Conditional Deletion Of *Ace2* In Taste Buds Alters Peripheral Taste Function In Male Mice**

Emma M Heisey[1], Guangkuo Dong[2], Schuyler Kogan[1], Yang Shi[1], Lin Gan[1], Lynnette P McCluskey[1]

[1]Medical College of Georgia / Augusta University, Augusta, GA, United States, [2]University of North Carolina at Chapel Hill, Chapel Hill, NC, United States

Severe acute respiratory syndrome coronavirus 2 (Sars-CoV-2) infection is characterized by the loss of taste, smell, and chemesthesis. The virus infects its host by binding to the host angiotensin-converting enzyme 2 (Ace2) receptor, which also regulates fluid homeostasis and inflammation in other tissues through the renin-angiotensin-aldosterone system. Currently, there is little known about the role of Ace2 in the taste system, preventing an understanding of mechanisms underlying viral-induced taste loss. Our preliminary studies show that Ace2 is robustly expressed in anterior and posterior taste buds. We developed a novel *Ace2$^{fl/fl}$:K14-Cre* mouse strain ("*Ace2* cKO") to conditionally delete Ace2 from taste receptor cells and the surrounding lingual epithelium. We tested potential effects of *Ace2* cKO on peripheral taste function by recording responses from the chorda tympani (CT) nerve, which transmits activity from taste buds on the anterior tongue to the brain. Changes in CT responses were specific to sweet and sour stimuli in male *Ace2* cKO mice. Specifically, responses to sucrose and the artificial sweetener, acesulfame-K, were significantly enhanced in male cKO mice while citric acid responses were diminished compared to female *Ace2* cKO animals and *Ace2$^{fl/wt}$:K-14-Cre* controls of either sex. Responses to monosodium glutamate, quinine, hydrochloric acid, sodium acetate, sodium chloride, and sodium chloride in the presence of the epithelial sodium channel antagonist, amiloride, were similar among strains. These results indicate that *Ace2*, an X-linked gene, regulates taste input to the brain in males under normal conditions. Ongoing studies focus on potential morphological and molecular mechanisms for functional changes in *Ace2*-deficient taste buds under homeostatic and inflammatory conditions.

158 **The Responsiveness Of Human Taste Buds Cells Is Affected By Treatment With Npy Family Peptides.**

Satya Iyer , Jean-Pierre Montmayeur, C. Shawn Dotson
Georgia State University , Atlanta, GA, United States

Obesity and related metabolic disorders have been linked to the dysregulation of food intake. Several gut peptides have been implicated in feeding modulation and body mass accumulation. These circulating peptides influence appetite through their actions on the hypothalamus, the brain stem, and the autonomic nervous system. The influence of these peptides on food intake and thus body mass accumulation underscores the potential value

of such hormones as treatments for obesity and related metabolic disease. In previous reports, we have shown that the hormone peptide YY (PYY) is present in the saliva of both humans and mice and that the augmentation of salivary PYY_{3-36}, using either genetic or pharmacological approaches, affects taste responsiveness, as well as food intake and body weight in diet-induced obese mice. However, it is unknown exactly how these peptides impact upon this responsiveness. A significant number of metabolic polypeptides have been shown to be expressed in taste bud cells (TBCs). The expression of these peptides signaling systems in the peripheral gustatory system suggest that these compounds may play a role in modulating TBC function and thus the communication of taste information. We assessed whether metabolic peptides *directly* impact the functioning of TBCs by measuring the response properties of human TBCs in culture, stimulated with prototypical taste stimuli, in the presence or absence of these peptides. Data from these experiments suggest that the response of human TBCs themselves are modulated by the presence of metabolic peptides like PYY and Neuropeptide Y and provide compelling new evidence supporting the hypothesis that the gustatory periphery can be dynamically modulated, potentially, in the context of an animal's metabolic state and/or environmental circumstance.

160 **Correlative Intravital And Histological Imaging On Intact Taste Buds**

Sungho Lee[1,2], MinJae Kim[1,2], Gha Yeon Park[1,2], Jubeen Yoon[3,4], Junsuk Lee[3,4], Chang Ho Sohn[3,4], Myunghwan Choi[1,2]

[1]School of Biological Sciences, Seoul National University, Seoul, *, South Korea, [2]The Institute of Molecular Biology and Genetics, Seoul National University , Seoul, *, South Korea, [3]Center for NanoMedicine, Institute for Basic Science, Seoul, *, South Korea, [4]Department of Nano Biomedical Engineering, Yonsei University, Seoul, *, South Korea

Understanding the physiology of taste cells requires multifaceted cellular information from gene regulation to functional responses. A variety of experimental approaches for obtaining each biological information is available, such as in situ hybridization for gene transcription and microfluidics-integrated intravital microscopy (μTongue) for functional responses. However, the acquisition of genetic and functional information correlatively at a single-cell level has yet to be realized for taste cells, hampering a comprehensive understanding of the causal interaction between gene and function. Here, we report a novel data acquisition pipeline providing correlated information on tastant-evoked functional responses of taste cells *in vivo* and their transcriptional regulation. In this pipeline, *in vivo* functional data is firstly acquired from several taste buds using μTongue and then the vicinities of the taste buds of interest are marked by using near-infrared branding. Using the branding as a landmark, the same taste buds are re-identified in a sliced tissue and processed for in situ hybridization. As a proof-of-principle, we performed *in vivo* imaging of sour-responsive cells in fungiform taste buds, and correlatively performed in situ hybridization targeting OTOP1, resulting in single-cell-level correspondence. Our proposed pipeline is broadly compatible with recent spatial transcriptomics and proteomics approaches for generating large-scale correlative datasets.

162 **Death In The Taste Bud: Morphological Features Of Dying Taste Cells And Implications For A Novel Role For Type I Cells**

Courtney E Wilson[1], Robert S Lasher[1], Ernesto Salcedo[1], Yannick Dzowo[1], Ruibiao Yang[1], John C Kinnamon[2], Thomas E Finger[1]

[1]University of Colorado School of Medicine Department of Cell and Developmental Biology, Aurora, CO, United States, [2]University of Denver, Denver, CO, United States

Taste buds comprise 50-100 taste cells that turn over repeatedly throughout the life of an organism. Progenitor cells below the taste bud give rise to the three mature taste cell types: Type I, II, and III cells. The lifespan of each of these cell types differs but is mostly in the range of 1-4 weeks. Although the phenomenon of cell death in a taste bud is well documented, little is known about the process of taste cell death and degradation. Here we present anatomical evidence of dying taste cells in murine circumvallate taste buds, using datasets acquired by Serial Block Face Scanning Electron Microscopy (sbfSEM), which allows for the digital reconstruction of cellular and subcellular objects in 3D computer space. In these datasets, we have identified cells that exhibit anatomical hallmarks of cell death: cell shrinkage, cell fragmentation, swollen endoplasmic reticulum, lysosomal expansion, swollen Golgi bodies, nuclear membrane degradation, and heterochromatin expansion. The majority of dying cells we identify are Type II cells, however a few may be dying Type III cells and several degenerating cells cannot be assigned a type. This is noteworthy in that Type I cells have the shortest lifespan. The absence of obvious necrotic Type I cells suggests that the process of cell death for Type I cells is very rapid and so is not well-represented in these data. Interestingly, the parts of healthy Type I cells contacting neighboring dying cells contain larger lysosomes as compared to parts of the same Type I cell not bordering a dying cell, suggesting a role for Type I cells in the engulfment and degradation of material from dying cells.

164 **Leveraging Multi-Echo Epi To Improve Olfactory Fmri Bold Sensitivity**

Sichen Ludwig Zhao[1,2], Clara U. Raithel[2,3], M. Dylan Tisdall[4], John A. Detre[2,4], Jay A. Gottfried[2,3]

[1]Department of Bioengineering, School of Engineering and Applied Science, University of Pennsylvania, Philadelphia, PA, United States, [2]Department of Neurology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, United States, [3]Department of Psychology, School of Arts and Sciences, University of Pennsylvania, Philadelphia, PA, United States, [4]Department of Radiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, United States

Olfaction plays a critical role in learning, memory, and emotion across phylogeny, though studies on human olfaction are limited. Functional MRI (fMRI) using blood-oxygenation-level dependent (BOLD) contrast has been successfully used to investigate human olfactory function, but key olfactory regions - namely the orbitofrontal cortex, piriform cortex, amygdala, and entorhinal cortex - are challenging to image. These regions are located ventrally near the air/tissue interface of the sinuses, which leads to distortions and signal dropout in the respective areas (susceptibility artifacts) during conventional single echo echo-planar imaging fMRI. Olfactory fMRI paradigms are further degraded by sniff-related susceptibility artifacts. Multi-echo echoplanar imaging (ME-EPI) fMRI has the potential to address these challenges. Here, we developed a ME-EPI fMRI protocol, specifically optimized for ventral olfactory regions. By sampling across a range of echo time, this protocol mitigates signal dropout and reduces noise. Using a simple olfactory discrimination task, we demonstrate that our optimized protocol increases sensitivity to BOLD activation in olfactory regions and significantly reduces the number of subjects required to detect group effects compared to conventional methods. Our protocol also allows us to detect BOLD activation in regions, where the conventional method sees signal dropout due to the artifacts, including the orbitofrontal cortex. As the standard fMRI data analysis pipelines can be directly applied to a ME-EPI dataset with only minimal modifications, our newly developed protocol can be readily adopted and therefore promises to rapidly advance the study of human olfaction.

166 **Construction Of Olfactory Database And Utilization For Food Aroma Design**

Yusuke Ihara, Yasuko Nogi, Yayoi Kawato, Chiori Ijichi
Ajinomoto Co., Inc, Kawasaki, *, Japan

Humans percept billions of odors by the combinatorial activity of 400 olfactory receptors (ORs). It is important for digitizing smell to elucidate the peripheral olfactory coding. For that purpose, we have constructed a three-dimensional database containing 2703 odorants, namely (i) activity profile of 400 human ORs, (ii) molecular structure, and (iii) verbal description of odor. The database enabled us to selectively extract data and analyze it from different points of view. In this poster, we will report overviews of our database and examples of applications in food flavor designing. [OVERVIEWS] There was a bias in the number of odorants that showed significantly high activity among ORs and 15 ORs showed particularly broad responses. To investigate the relationship between odor recognition by ORs, we visualized the distribution of odorants in the OR activity space. It showed intersection and subset relationship between ORs, suggesting a hierarchical structure of odor recognition. [EXAMPLES] (1) We identified ORs responsible for retort-sterilized milk off-flavor and screened antagonists for them. The identified antagonist showed a significant decrease in odor intensity of the off-flavor compound in milk ($n = 5$, $p < 0.01$). (2) We built mathematical models to predict the applicability of verbal descriptors for an odorant from its OR activity profile ($r = 0.927$, $p < 0.001$ for new rubber). (3) We obtained OR activity profiles for aromas of 23 gummy candies of various flavors. We conducted principal component analysis. The first (OR2L8 – OR1A1) and the second (OR51B5 – OR8K3) principal components explained 12.6% and 8.4% of variance, respectively. By utilizing these technologies, we will realize digital food flavor design and contribute to the offering of sustainable, healthy, and delicious food products.

168 **Olfactory Redux: A Plausible Role For Temporal Processing In Human Olfactory Perception?**

Brianne M. Linne[1], Jay A. Gottfried[1, 2]
[1]Department of Neurology, University of Pennsylvania, Philadelphia, PA, United States, [2]Department of Psychology, University of Pennsylvania , Philadelphia, PA, United States

Research in animals has demonstrated a role for both spatial and temporal processing in manifesting an odiferous percept. The precise mechanism through which temporal coding is incorporated into olfactory perception remains a subject of debate. In recent work, a “primacy” model has been proposed as an update to the longstanding Hopfield latency model (Hopfield, 1995), whereby the glomeruli activated earliest in time will play an outsized role in shaping the odor code and guiding perception and behavior (Wilson et al., 2017). The existence of a key early temporal window and its role in odor identification has been demonstrated in rodent models (Bolding & Franks, 2017; Chong et al., 2020), highlighting new compelling directions in olfactory theory. However, in the human olfactory system, the possible implications of a temporal primacy model have received scant attention, with only a single recently published study exploring the relevance of temporal processing in the context of primacy in humans and concluding such mechanisms to have negligible impact (Perl et al., 2020). In the present work, we explored this question further, using multiple distinct temporally manipulated odor pairs, and with a robust number of subjects ($n = 30$). By employing a signal detection framework, as well as a confidence assessment to account for unstable response criteria, we find that our results suggest a more dynamic role. Here, we demonstrate that at both the individual and group levels, humans are capable of meaningfully discriminating temporally inverted odor pairs. These findings help bolster a conceptual framework of temporal processing in odor perception and lend credence to more fine-grained investigations of the role of temporal coding and the validity of the “primacy” model in human olfaction.

170 **Title: Determinants Of Inhalation-Linked Response Dynamics In Odorant Representations By Glomeruli Of The Mouse Olfactory Bulb**

Elvis F. Acquah, Shaina Short, Matt Wachowiak
University of Utah School of Medicine, Salt Lake City, UT, United States

In the mammalian olfactory system, odorant-evoked activity is temporally patterned by inhalation and plays an important role in encoding odor information. However, the determinants of distinct temporal patterns remain unclear. The ‘primacy’ model hypothesizes that inhalation-linked response latencies reflect relative sensitivity to an odorant, with the most sensitive neurons responding earliest. We tested this model by defining the relative sensitivities of glomeruli in the mouse olfactory bulb (OB) to specific odorants and characterizing their response dynamics across concentrations. We also tested alternate models by relating responses to odorants with diverse

chemical structures. We used two-photon imaging from the OB of awake, head-fixed mice expressing genetically encoded calcium or glutamate reporters in olfactory sensory neurons (OSNs) or mitral/tufted (MT) cells, and built on recent work establishing 'primary' glomeruli for particular odorants (Burton et al., doi: 10.7554/eLife.80470). While increasing odorant concentration recruited activation in 'non-primary' glomeruli, initial response latencies and timing of responses to subsequent inhalations did not correlate with relative sensitivity, inconsistent with the primacy model. In many cases, inhalation-linked glomerular responses were weak or undetectable. Instead, distinct temporal patterns were well-predicted by a combination of odorant chemistry and glomerular identity; these effects persisted across concentrations and at pre-and postsynaptic levels. These results suggest that while response latencies can encode odor information, relative timing does not robustly reflect relative sensitivity to an odorant, limiting the ability of the earliest-responding glomeruli to represent odor identity in a concentration-invariant manner.

172 **Hippocampus And Piriform Cortex Involvement In Odor Mixture Discrimination Tasks Of Varying Cognitive Demand**

Huibo Li[1,2], Abigail Stuart[1], Jamie Zeng[3], Nasya Becton[3], Leslie M. Kay[1,2,3]

[1]Institute for Mind and Biology, The University of Chicago, Chicago, IL, United States, [2]Department of Psychology, The University of Chicago, Chicago, IL, United States, [3]The College, The University of Chicago, Chicago, IL, United States

Neural oscillations in the olfactory system play functional roles in sensory processing and cognitive processes. In the rat olfactory bulb (OB), gamma (60–90 Hz) and beta (15–40 Hz) band Local Field potential (LFP) oscillations are markers of specific behavioral states. Elevated gamma oscillations in the OB are necessary and sufficient for fine odor discrimination across rats, mice, and honeybees. However, we still don't know what cognitive elements of the odor discrimination task drive increased gamma and the underlying network contributions. Previous work suggests that cognitive load (tasks of various demands) is a factor. We hypothesize that not only higher olfactory discrimination difficulty but higher cognitive load in an odor discrimination task also induces elevated gamma. Further, we hypothesize that the interaction between odor similarity and cognitive demand involves the piriform cortex and hippocampus. To test our hypothesis and characterize network input in fine odor discrimination, we trained rats to discriminate a pair of coarse odorants and a pair of fine (very similar) odorants in a two-alternative choice (TAC) task and asked them to discriminate both pairs in one session, with and without associated context cues, in block or interleaved manner. We recorded LFPs from the OB, piriform cortex, and hippocampus while the rats performed this task. We show that rats can successfully learn and perform a difficult variation of the TAC task and that a context cue changes neural oscillations and performance in fine odor discrimination.

174 **Utilizing An Insect Or As A Detection Mechanism For Disease Associated Volatiles**

Rhodry J. Brown[1], Gyu Rie Lee[2], Hiroaki Matsunami[1]

[1]Duke University, Durham, NC, United States, [2]University of Washington, Seattle, WA, United States

Odorant receptors (ORs) are extremely sensitive receptors that can detect thousands of volatile chemicals. Even at very low concentrations, ORs have been shown to activate in the presence of ligands they are specific for. Scientist's current abilities to measure trace chemicals do not compare to the natural abilities of organisms that rely on olfaction for survival, such as insects. Previous literature found that there are many volatile chemicals that can be associated with prevalent diseases such as Malaria, Tuberculosis, and COVID-19. The ability to detect these diseases by their volatile chemicals at the same sensitivity as insects would be useful, yet no known ORs are activated by these ligands without activation from other chemicals. To build a system that utilizes ORs to detect specific disease associated ligands, we decided to use an OR from the Jumping Bristletail (*Machilis hrabei*) known as MhOR5, as it has several distinct advantages. The structure of MhOR5 has been experimentally elucidated, allowing for more accurate analysis of ligand binding, also, MhOR5 expresses well in cell culture, and does not rely on an OR co-receptor, which is common in most insect ORs. We have tested MhOR5 against a panel of volatiles associated with disease and discovered several disease-associated volatiles that robustly activate MhOR5 at low concentrations. These volatiles underwent docking analysis to identify resides to be mutated. With the functional analysis of mutants, and the interactions predicted by docking analysis, some of the underlying mechanisms of ligand selectivity may be elucidated. This will open the door to engineer ORs to detect diseases.

176 **Quantity Has A Quality All Its Own: How Odor Quality Changes With Intensity**

Fernanda M. M. Ocampos[1], Robert Pellegrino[1], Jennifer E. Margolis[1], Matthew Andres[1], Britney B. Nguyen[1], Emily J. Mayhew[1], Richard C. Gerkin[2], Joel D. Mainland[1,3]

[1]Monell Chemical Senses Center, Philadelphia, PA, United States, [2]Arizona State University, Tempe, AZ, United States, [3]University of Pennsylvania, Philadelphia, PA, United States

Although most odor atlases describe the odor character of a given molecule using a single description, odor character can change across intensities. [1,2] 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.[3] 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 153 odorants at two concentrations corresponding to low and high intensities. Approximately 10% of the tested odors exhibited concentration-dependent changes in character that were larger than typical character differences between different molecules.

Odorants with higher shifts in perceived intensity were more likely to undergo large perceptual shifts (R = 0.51). Understanding how intensity and quality interact will provide an important constraint on models of olfactory perception. References Gross-Isseroff, R., & Lancet, D. (1988). Concentration-dependent changes of perceived odor quality. *Chemical Senses*, *13*(2), 191–204. Laing, D. G., Legha, P. K., Jinks, A. L., & Hutchinson, I. (2003). Relationship between Molecular Structure, Concentration and Odor Qualities of Oxygenated Aliphatic Molecules. Chemical Senses, 28(1), 57–69. Stevens, S. S. (1934). The Attributes of Tones. Proc National Acad Sci 20, 457–459 Lee, B. K., Mayhew, E. J., Sanchez-Lengeling, B., Wei, J. N., Qian, W. W., Little, K., Andres, M., Nguyen, B. B., Moloy, T., Parker, J. K., Gerkin, R. C., Mainland, J. D., & Wiltschko, A. B. (2022). A Principal Odor Map Unifies Diverse Tasks in Human Olfactory Perception. BioRxiv.

178 **Glomeruli Mapping And Receptor Selections Are Governed By The Same Self-Organization Process Through Chance Encounters With Behaviorally Relevant Complex Mixtures In The Wild: A Simulation Study**

Asma Djehiche, Ruslan Garifullin, Reşad Mammadov, Mecit Yaman
University of Turkish Aeronautical Association, Ankara, *, Turkey

It is often repeated that olfactory receptors are non-specific and cross-responsive, and only their combinatorial differential response, as accrued at the olfactory bulb, is a signature of the odorant stimuli. Granted that evolutionary selection of these receptors in the wild must have followed chance encounters with spatially/temporally complex archetypal mixtures, we can infer about the bulbar representations of natural odorants these two premises: (1) Phylogenetically similar receptors coalesce at the olfactory bulb giving rise to map-like multi glomerular representations. (2) These representations ultimately reflect the behaviorally relevant odor space of an organism. Still, one question remains: How is the 2D organization of the olfactory bulb determined from the high dimensional receptor space, and is this mapping unique? To study this question we simulated a set of 50 mixtures containing tens of volatile organic compounds from the Bruker Mid-IR Volatile Organics Library. We assume these mixtures are found in the wild with set archetypal characteristics, and have normal variance. The stimuli space is modeled as the discrete wavenumbers of an FTIR spectrum (about 1000 wavenumbers, similar to the receptor types of a rodent). Using a self organizing Kohonen map, we mapped the mixtures set onto a 2D space. The mapping showed three essential features that correlate to glomerular representations of natural odorants: (1) Archetypal odorants have map-like representations, (2) components, partial mixtures, and unknown relative concentrations do not produce map-like representations, (3) self organization process govern both mapping and receptor selection. We validate our simulations using recently published nearly full olfactory receptor phylogenetic tree of a rodent.

180 **Ribosomal Profiling Identifies Alk As Critical For The Development Of Oral Sensory Neurons Of The Geniculate Ganglion**

TAO TANG, Brian Pierchala
Department of Anatomy, Cell Biology & Physiology Stark Neurosciences Research Institute, Indiana University, Indianapolis, IN, United States

To identify signaling pathways necessary for oral sensory neuron development and maintenance, the translatome of oral sensory geniculate ganglion (GG) neurons was selectively profiled using the RiboTag method. From this analysis, Anaplastic Lymphoma Kinase (ALK) was identified as one of the most highly enriched tyrosine kinase receptors. To confirm that Alk is enriched in *Phox2b*+ GG neurons, fluorescence in situ hybridization (FISH) was performed on GG from adult mice. *Alk* was expressed in nearly all *Phox2b*+ neurons and was essentially absent from *Phox2b*- neurons. Evaluation of a developmental time course of *Alk* expression revealed that *Alk* was robustly expressed in the GG at all ages examined, from E14.5 into adulthood. To determine whether Alk is necessary for development or maintenance of the peripheral gustatory system, GG and taste buds (TBs) from P3, P14 and P30 $Alk^{-/-}$ and $Alk^{+/+}$ mice were examined. Neither oral sensory neurons (PHOX2B+) nor total GG neurons (TUJ1+) were lost in $Alk^{-/-}$ mice at any age. However, TB number, volume and innervation were all significantly decreased in $Alk^{-/-}$ mice, but not Alk+/+ mice. Gene rearrangements in ALK are responsible for a portion of non-small cell lung cancers, and treatment of lung cancer patients with selective ALK inhibitors, such as Ceritinib, frequently results in dysgeusia. To evaluate whether chemotherapeutic ALK inhibitors impact the peripheral gustatory system, adult mice received Ceritinib injections for 30 days. TB volume and TB innervation (TUJ1+ and P2X3+) were significantly reduced in Ceritinib-treated mice. Interestingly, the somal diameters of oral sensory neurons were smaller in Ceritinib-treated mice, and there was a loss of PHOX2B+ neurons. In conclusion, ALK is critical for development and maintenance of oral sensory neurons.

182 **The Role Of Rnf43/Znrf3 In Taste Tissue Homeostasis**

Ranhui Xi, Chanyi Lu, Jiang Xu, Darsaan Khanna, Peihua Jiang
Monell Chemical Senses Center, Philadelphia, PA, United States

Taste bud cells turn over continuously throughout life. The Wnt and Hedgehog signaling pathways are involved in this process. The E3 ubiquitin ligases Rnf43 and Znrf3 are Wnt targeted genes and constitute a negative Wnt feedback loop. Our previous work showed that Rnf43/Znrf3 depletion or systemic provision of exogenous R-spondin, the ligand for Rnf43/Znrf3, results in taste cell and taste bud hyperplasia. Here, using $Rnf43^{fl/fl}$; $Znrf3^{fl/fl}$; Krt5-CreERT2; ROSA-tdTomato mice, we further show that one week after tamoxifen induction, the number of taste buds in Rnf43/Znrf3 double knockout mice rises significantly and continues to rise over time, reaching a striking number at 8 weeks. This increase is observed across taste fields, including the anterior tongue, circumvallate papilla, soft palate, and epiglottis. The newly generated ectopic taste buds are composed entirely of tdTomato+ cells, suggesting de novo generation of these taste buds. Furthermore, we find that ectopic taste buds are rarely innervated by $P2X3^{+}$ fibers. Yet, these buds are surrounded by synapsin-1^{+} nerve fibers, which

suggests that these ectopic taste buds may attract somatosensory fibers to relay such information to the central nervous system. Additionally, rather than growing haphazardly throughout the epithelium, newly generated taste buds in the anterior tongue and soft palate epithelium appear to be somewhat adjacent to existing ones during early days and more broadly distributed at later stages, suggesting cell fate conversion of non-taste epithelial stem cells to taste stem cells. Overall, our work demonstrates a critical role of Rnf43/Znrf3 in taste tissue maintenance, with ablation of Rnf43/Znrf3 resulting in a long-term expansion of taste buds and taste cells as well as re-patterning of sensory nerve endings.

184 **Mesenchymal Alk3-Bmp Signaling Regulates Epithelial Cell Differentiation Through Governing The Production Of Previously Unappreciated Secretory Proteins**

Mohamed Ishan[1], Zhonghou Wang[1], Peng Zhao[2], Yao Yao[1], Steven Stice[1], Lance Wells[2], Yuji Mishina[3], Hong-Xiang Liu[1]

[1]Regenerative Bioscience Center, Department of Animal and Dairy Science, College of Agricultural and Environmental Sciences, University of Georgia, Athens, GA, United States, [2]Department of Biochemistry and Molecular Biology, Complex Carbohydrate Research Center, University of Georgia, Athens, GA, United States, [3]Department of Biologic and Materials Sciences, School of Dentistry, University of Michigan, Ann Arbor, MI, United States

The differentiation of taste cells from their progenitors is a complex and fundamental process important for both the development and maintenance of taste buds. In mice, the homogeneous epithelial cells of tongue swellings (~ E11.0) give rise to early taste bud cells in embryos and to the non-gustatory epithelial cells that are maintained as progenitors for taste cell renewal later in life. Here we report that mesenchymal bone morphogenetic signaling (BMP) signaling via receptor ALK3 (ALK3-BMP) regulates epithelial cell differentiation through regulating the production of secretory proteins and epithelial Wnt/β-catenin activity. Our studies showed that mesenchyme-specific (*Wnt1-Cre* and *Sox10-Cre* driven) conditional knockout of type I BMP receptor *Alk3* (*Alk3 cKO*) resulted in an absence of taste papilla placodes in the E12 mouse tongue. Bulk RNA-Seq analyses in separated epithelium and mesenchyme showed that mesenchyme-specific *Alk3 cKO* led to many more differentially expressed genes (DEGs) in the tongue epithelium than in the mesenchyme, including a down-regulation of multiple Wnt/β-catenin signaling components. The addition of LiCl in the medium to activate Wnt/β-catenin signaling rescued taste papilla development in *Wnt1-Cre/Alk3^{cKO}* tongue cultures. Biochemical and cell differentiation analyses revealed that ALK3-BMP signaling in the mesenchyme governs the production of secretory proteins that regulate epithelial cell differentiation to taste cell progenitors, i.e. suppressing inhibitors and promoting promotants. These secretory proteins were not those previously recognized in regulating epithelial cell differentiation. Collectively, our data demonstrated the crucial roles of tongue mesenchyme in epithelial cell differentiation through molecular signaling such as ALK3-BMP.

186 **Determining The Role Of Sox9$^+$ Epithelial Cells In Circumvallate Taste Papilla/Von Ebner&Rsquo;S Salivary Gland Complex Homeostasis**

Trevor J. Isner[1,2,3], Eric D. Larson[1,3,4], Linda A. Barlow[1,2,3]

[1]Department of Cell & Developmental Biology, University of Colorado Anschutz Medical Campus, Aurora, CO, United States, [2]Cell Biology, Stem Cells and Development Graduate Program, University of Colorado Anschutz Medical Campus, Aurora, CO, United States, [3]Rocky Mountain Taste & Smell Center, University of Colorado Anschutz Medical Campus, Aurora, Aurora, CO, United States, [4]Department of Otolaryngology - Head and Neck Surgery, University of Colorado Anschutz Medical Campus, Aurora, CO, United States

The murine circumvallate taste papilla (CVP) in the posterior tongue houses hundreds of taste buds, each containing ~60 taste receptor cells (TRCs) that are constantly renewed. The CVP epithelium connects ventrally with ducts of the Von Ebner's salivary glands (VEGs) forming the CVP/VEG complex; prompting a question regarding the lineage relationship in these functionally distinct but anatomically connected tissues. The transcription factor SOX9 is expressed by stem cells across adult epithelia, hence we explored if SOX9 is also expressed in CVP/VEG, as well as the relationship of SOX9$^+$ cells to known adult CVP taste stem cells, marked by LGR5 (10.1002/stem.1338). SOX9 immunofluorescence (IF) of LGR5-GFP$^+$ CVP sections revealed SOX9$^+$/LGR5$^+$ as well SOX9$^+$ only epithelial cells at the CVP-VEG junction – a location compatible with cell contributions to both tissues, implying SOX9$^+$ and LGR5$^+$ populations are distinct. Pseudotime analysis of single cell RNA sequencing data from adult CVP/VEG epithelium in fact suggests CVP and VEG epithelia arise from a common SOX9$^+$ stem population, which gives rise to LGR5$^+$ progenitors upstream of the taste and salivary lineages. We are now testing the stem potential of SOX9$^+$ progenitors *in vitro* and *in vivo*. We find that isolated SOX9$^+$ cells generate organoids, and are using morphological criteria and marker expression to determine if organoids are taste and/or salivary gland-like. In vivo, *Sox9^{CreERT2};Rosa26^{tdTomato}* mice assessed 2 days post-tamoxifen induction reveal Tomato expression mirrors SOX9 IF at the CVP/VEG junction, establishing the validity of the genetic model for long term lineage tracing experiments. Our early findings suggest a model wherein SOX9 marks bipotent progenitors of the CVP/VEG complex.

188 **Tracing The Terminal Nerve Of Rodents**

Enrico Amato[1,2], Ed Zandro M Taroc[1,2], Paolo E Forni[1,2]

[1]Department of Biological Sciences, University at Albany, State University of New York, Albany, NY, United States, [2]The RNA Institute, University at Albany, Albany, NY, United States

Neuronal migration is a crucial aspect of development. In mammals, prior to brain neurogenesis, the nasal placode (NP) gives rise to various populations of neurons. This includes the Gonadotropin-releasing hormone-1 (GnRH-1) neurons, which migrate from the nose to various regions of the developing brain. Once in the brain, the GnRH-1 neurons play a key role in controlling the hypothalamic-pituitary gonadal (HPG) axis. Sets of placodal progenitors give rise to a ganglionic structure called the terminal nerve (TN). The GnRH-1 neurons migrate from the nose to the brain along the axons of the TN. The lack of specific genetic markers in rodents has made it difficult to characterize TN development and function. Although a selective molecular marker for this nerve has not yet been identified, the gene Tag1 (CNTN2) has been previously described to be expressed, at high levels, at the early stages of terminal nerve development. Additionally, we found that the cells of the TN express the gene Prokineticin receptor 2 (Prokr2) gene. Notably, Prokr2 loss of function is associated with disruption of the GnRH neuron migration and olfactory bulb morphogenesis in some human cases of Kallmann syndrome. Using our newly generated Tag1CreERT2 mouse line and a previously generated Prokr2iCre mouse line, we were able to genetically trace cells of the TN in rodents. Moreover, using single-cell-RNA-sequencing we were able to identify genes enriched in the developing terminal nerve. In this study, we described the early development of the TN and relationships the TN has with other populations of neurons within the developing nose of mice.

190 **Olfactory Epithelial Neurogenesis Increases Following Acute Inflammation Through Cntf Signaling**

Joe Oliver, Chiharu Lovins, Theo Hagg, Cuihong Jia
East Tennessee State University, Johnson City, TN, United States

Olfactory sensory neurons (OSNs) in the olfactory epithelium are continuously replaced by basal cell-mediated neurogenesis to maintain the sense of smell. Failure to regenerate OSNs after injury causes olfactory dysfunction. Acute inflammation leads to the death of OSNs but leaves the basal cells intact. Defining signaling pathways that regulate basal cell proliferation would reveal new therapeutic targets to improve olfactory neurogenesis and deficits. Our previous study showed that ciliary neurotrophic factor (CNTF) is highly expressed in horizontal basal cells (HBCs), while the CNTFRα receptor is expressed in globose basal cells (GBCs). CNTF is suppressed by focal adhesion kinase (FAK) and, conversely, intranasal instillation of a FAK inhibitor in mice promotes GBC proliferation and neurogenesis via CNTF. Here, we investigate whether CNTF contributes to OE neurogenesis following methimazole-induced acute inflammation and whether FAK inhibition could enhance it. Methimazole increased CNTF and GBC marker Mash1 expression, suggesting that HBCs produce more CNTF to promote GBC proliferation following injury. This was confirmed by the fact that methimazole increased basal cell proliferation in wildtype mice, but not CNTF-/- littermates. Importantly, methimazole did not affect the levels of phospho-FAK, suggesting that it increases CNTF not via FAK. FAK inhibitor treatment following methimazole further enhanced CNTF, and we are now testing whether FAK inhibition can enhance methimazole-induced basal cell proliferation. FAK inhibitor did not affect TNF expression, suggesting that it does not interfere with methimazole-induced acute inflammation. These data will help to define the role of FAK and CNTF and validate the therapeutic potential of FAK inhibitors to improve olfactory neurogenesis after injury.

192 **Achems Undergrad Finalist: Insights Into Adult Neurogenesis Of Vomeronasal Sensory Neurons**

Lena Terlau[1], Friederike D. Seifert[1], Christoph Hamacher[1], Andres Hernandez-Clavijo[1], Stefanie Kurth[1], Kristin Seré[2,3], Martin Zenke[2,3], Marc Spehr[1]
[1]Department for Chemosensation, Institute for Biology II, RWTH Aachen University, Aachen, *, Germany, [2]Department of Cell Biology, Institute for Biomedical Engineering, Medical Faculty, RWTH Aachen University, Aachen, *, Germany, [3]Helmholtz-Institute for Biomedical Engineering, RWTH Aachen University, Aachen, *, Germany

Neurogenesis in olfactory epithelia is crucial for an animal's capacity to continuously adapt to its environment. Thus, adult neurogenesis in chemosensory epithelia continues throughout the lifetime of a rodent. However, the precise processes underlying adult neurogenesis in the vomeronasal organ remain unclear. Here, we begin to describe characteristics of neurogenesis in the vomeronasal sensory epithelium. We aim to label newly generated vomeronasal sensory neurons (VSNs) using a genetic approach: upon tamoxifen injection, VSN progenitor cells in *Id2CreER^{T2} :: Rosa26R-tdTomato* mice express tdTomato upon coincident *Id2* promoter activity. Descendants of these cells are permanently labeled by red fluorescence. Using the Id2 proliferation and differentiation marker as a VSN lineage tracer, we describe (i) the proportion of new-born neurons within the VSN population. We identify (ii) the epithelial position and morphology of individual new-born neurons and characterize their age-dependent migration patterns within the sensory epithelium. Furthermore, our results provide first insights into the lifespan of VSNs. Finally, by analysing marker protein co-labeling of tdTomato-positive cells, we investigate the differentiation and maturation state of new-born neurons after 1, 3, 7, 11, 14, 21, and 57 days post injection.

194 **Selective Vulnerability And Circuit Integration Of Olfactory Bulb Dopaminergic Neurons After Olfactotoxic Sensory Deprivation.**

Tenzin Kunkhyen, Jordan D. Gregory, Alyssa A. Lauer, Taryn R. Brechbill, Alex N. Rangel, Claire E. J. Cheetham
University of Pittsburgh, Pittsburgh, PA, United States

One of the hallmarks of neurogenerative diseases is selective loss of specific sub-populations of neurons. 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 to lack of sensory input. Furthermore, OB DA neurons are continuously generated throughout life, enabling them to fully repopulate after sensory input blockade. This makes OB DA neurons an ideal model system to determine selective vulnerability and how newborn DA neurons are functionally integrated to replace previously lost neurons. Injection of an olfactotoxic drug, methimazole, enables us to see the impact of rapid elimination followed by gradual restoration of sensory input to the OB. CA chronic *in vivo* 2-photon imaging in DAT-cre;Ai9;Ai162 and THcre;Ai9 mice that express red fluorescent morphological marker to track survival and integration, and a green genetically encoded calcium indicator to track the odor response properties of individual neurons over weeks. We found that loss of DA neurons was significantly elevated during the first week after ablation of OB sensory input and in contrast, there was no effect on DA neuron loss 7-14 days after OSN ablation. We are analyzing whether there are differences in the vulnerability of the previously described large embryonically generated and small postnatally generated DA neurons. We are also quantifying the rate of newborn DA neuron integration before and after ablation and whether there are differences in odor response characteristics between neurons that are vulnerable or resilient to sensory disruption. Distinguishing vulnerable neurons from their resilient neighbors will help with novel targeted therapeutic strategies for the treatment of neurodegenerative diseases.

196 **Vomeronasal Horizontal Basal Stem Cells From Development To Regeneration.**

Raghu Ram Katreddi[1,2], Noah M. LeFever[1,2], Ed Zandro Taroc[1,2], Paolo E. Forni[1,2]
[1]Department of Biological Sciences, Albany, NY, United States, [2]The RNA Institute , Albany, NY, United States

The Vomeronasal organ (VNO) is a part of the accessory olfactory system, which is responsible for detecting pheromones, chemical factors that trigger a spectrum of sexual and social behaviors. The vomeronasal epithelium (VNE) shares several features with the epithelium of the main olfactory epithelium (MOE). However, it is a district neuroepithelium populated by chemosensory neurons that differ from the olfactory sensory neurons (OSNs) in cellular structure, receptor expression, and connectivity. Sox2-positive cells have been previously identified, in the VNE, as the stem cell population that gives rise to neuronal progenitors and vomeronasal sensory neurons. On the other hand, the MOE also comprises p63+ve horizontal basal cells (HBCs), a second pool of quiescent stem cells that become active in response to injury. Here we investigated the existence and potency roles of HBC-like cells in the development of the VNO (vHBCs). Constitutive and conditional Cre tracing and single-cell sequencing suggest the existence of pools of p63+/Keratin-5+ multipotent stem cells, able to form neuronal and non-neuronal cells both at perinatal and adult stages. Moreover, lineage tracing experiments on p63 KO adult animals revealed that sets of p63+/Keratin-5+ stem cells in medial regions of the VNO share a similar regenerative ability to that described for the HBCs of the MOE.

198 **Fine-Tuning Of Cxcl12 In The Olfactory Stem Cell Niche Is Required For Neurogenesis**

André Dietz, Katja Senf, Julia Karius, Eva Neuhaus
Pharmacology and Toxicology, Jena University Hospital, Friedrich Schiller University Jena, Jena, *, Germany

Olfaction depends on lifelong production of sensory neurons from CXCR4+ neurogenic stem cells. Here, we use several genetic models to investigate how regulation of CXCL12, the chief CXCR4 ligand, in the olfactory stem cell niche adjusts adult neurogenesis. We identify subepithelial tissue and glial cells within the olfactory epithelium as main CXCL12 sources. Quiescent gliogenic stem cells bind and present lamina propria-derived CXCL12 via heparan sulfate to neighboring CXCR4+ stem cells. In addition to basal enrichment, CXCL12 derived from apical glia cells is also required for CXCR4 activation. Over-stimulation is prevented by ACKR3, a high affinity CXCL12 scavenger, which is present in mature glial cells and titrates CXCL12. Finally, we show that accurate adjustment of CXCL12 is critical for proper lineage progression of neuronal stem cells. Overall, these findings establish precise regulation of CXCL12 as prerequisite for CXCR4-dependent neurogenesis and implement ACKR3 as a scavenger influencing tissue homeostasis beyond embryonic development.

8:30 - 10:30 AM	Calusa EFGH
Learning and memory in the chemical senses	

Chair(s): Kathrin Ohla and Qi Yuan

8:30 **Learning And Memory In The Chemical Senses**

Kathrin Ohla[1,2], Qu Yuan[3]

[1]Münster University, Münster, *, Germany, [2]Firmenich, Satigny, *, Switzerland, [3]Memorial University, St. John's, NL, Canada

Learning and memory are amongst the most fundamental mental processes, yet they remain understudied in the chemical senses. This is surprising given that taste is a potent primary reinforcer and smell has close connections to brain regions linked to memory. Research on chemical senses bears hence the potential in advancing knowledge of the brain mechanisms of learning and memory at large. In this symposium, we will present the mechanisms of learning and working memory in both olfaction and gustation in rodents and humans. We will start off with the latest research on chemosensory learning in rodents including the consequences of incidental taste learning (Flores) and odor fear learning mechanisms in ageing (Yuan). Because the existence and relevance of working memory in the chemical senses has long been challenged as it stores information at a time scale that seems too short to be compatible with chemosensory processing, we will present evidence from human research on the organization and mechanisms of gustatory (Ohla) and olfactory (Yang) working memory.

8:40 **Taste Experience Alters Associative Learning-Related Cortical Responses**

Veronica Flores[1,2], Jian-You Lin[2]

[1]Furman University, Greenville, SC, United States, [2]Brandeis University, Waltham, MA, United States

Sensory experience modulates perception and learning of new and familiar stimuli. Benign experience with a single taste protects that same taste from future association with a negative consequence; one notable example being latent inhibition of conditioned taste aversion (CTA). Our work has expanded upon this phenomenon showing that benign experience with salty and sour tastes (TE) strengthens later CTA learning towards novel sucrose. Here, we present a set of experiments that investigate how TE impacts CTA learning circuits with focus on Gustatory Cortex (GC). Optogenetic silencing of GC during TE blocked the enhancement of CTA, confirming GC's role in the TE phenomenon. One interpretation of *how* TE enhances learning is that it changes GC processing of the later-presented novel taste. Using *in-vivo* electrophysiology in female Long Evans rats, we test this hypothesis and show that TE modulates GC response dynamics underlying novel taste processing. TE increases the discriminability of GC ensemble and single-unit responses to familiar and new tastes. Currently, we test the hypothesis that potentiation in discriminability primes the rat for stronger learning. More specifically, we evaluate how TE changes GC taste responses to a novel taste before and after CTA learning and how these changes track behavior. We show that TE alters the impact of CTA learning on GC responses to novel sucrose such that GC response magnitude and length are increased post-CTA relative to pre-CTA. In contrast, only modest enhancements in response magnitude were observed in the taste naïve group. Single-neuron responses recorded across multiple sessions reveals a similar trend. Together, the results of these experiments suggest that taste experiences, even those deemed benign, can facilitate future associative taste learning.

9:10 **Extinction Of Olfactory Fear Memory Is Impaired In Aged Rats**

Tayebeh Sepahvand, Negar Nazari, Vishaal Rajani, Qi Yuan
Memorial University, St. John's, NL, Canada

Aging is associated with a decline in cognitive function and flexibility. Long-term depression (LTD) has been associated with behavioral flexibility such as learning extinction. Using a classical odor and shock-conditioning model, we test how the extinction of the olfactory fear learning is altered in aged rats, and how it relates to LTD in the piriform cortex (PC). Young (3-6 months old) and aged (19-22 months old) rats underwent olfactory fear conditioning in which a conditioned odor was paired with shock. Following odor conditioning, rats underwent 7 days of odor-only extinction. Our results showed impaired extinction in aged rats, despite of comparable learning acquisition to young rats. Using ex vivo recording in PC slices, we compared LTD in the layer Ib of the PC following the extinction. We observed robust LTD in aged PCs following the extinction protocol, whereas litter LTD was observed in young rats. LTD was inducible in young rats without extinction training. Thus extinction is accompanied by decreased LTD in young rats, whereas impaired extinction in aged rats is associated with inducible LTD. PC LTD may be a neural correlate for olfactory extinction learning and exclude further LTD induction ex vivo. We have previously shown a shift from NMDAR- to L-type calcium channel (LTCC)-dependent LTD in the PC with aging. Here we tested the NMDAR vs LTCC dependence of the olfactory fear extinction. In young rats, NMDAR blockade with either MK801 systemic injection, or APV PC infusion, prevented odor fear extinction. In parallel, PC LTD became inducible. In the aged rats, enhancing NMDAR function with a partial agonist d-cycloserine, resulted in successful extinction of the odor fear. These results suggest NMDAR-LTD in the PC accounts for odor extinction learning, which is lacking in aged rats.

9:30 **The Capacity And Organisation Of Gustatory Working Memory**

Kathrin Ohla[1,2]

[1]Münster University, Münster, *, Germany, [2]Firmenich, Satigny, *, Switzerland

Working memory (WM) is an active, short-term information storage. Its organization has been subject to

extensive investigation. However, research into the generality of WM across types of information and sensory systems has neglected the sense of taste. This is surprising given the relevance of taste information processing to identify nutrients and maintain a homeostatic balance. Here I present evidence for the existence of a gustatory WM. I will show that taste can be dynamically encoded, maintained, and retrieved on short time scales consistent with WM. Using novel single and multi-item taste recognition tasks, we found that a single taste can be reliably recognized despite repeated oro-sensory interference suggesting active and resilient maintenance (Experiment 1). When multiple tastes were presented (Experiment 2), the resolution with which these were maintained depended on their serial position, and recognition was reliable for up to three tastes suggesting a limited capacity of gustatory WM. Lastly, stimulus similarity impaired recognition with increasing set size, which seemed to mask the awareness of capacity limitations. Together, the results advocate a hybrid model of gustatory WM with a limited number of slots where items are stored with varying precision.

10:00 **Oscillatory Working Memory Mechanisms In The Human Olfactory System**

Andrew I. Yang[1], Jay A. Gottfried[2,3]

[1]Department of Neurosurgery, Emory University, Atlanta, GA, United States, [2]Department of Psychology, University of Pennsylvania, Philadelphia, PA, United States, [3]Department of Neurology, University of Pennsylvania, Philadelphia, PA, United States

Neural oscillations have long been postulated to contribute to mnemonic representations. We investigated working memory representations of novel sequences of sensory stimuli, using local field potentials recorded from the human hippocampus and piriform while subjects experienced sequences of three distinct odors. In both regions, coupled theta and gamma oscillations emerged throughout encoding and maintenance. During encoding, odors elicited greater gamma at distinct theta phases that depended on their sequence position, regardless of odor type. Both the consistency of phase preference, and separability across sequence positions were predictive of subsequent temporal order memory. This theta phase code of the “when” of sequences was behaviorally relevant in both regions. In contrast, theta-gamma oscillations in piriform, but not hippocampus, also disambiguated the ‘‘what’’ of sequences. Theta-gamma patterns represented individual odor types, independent of sequence position, using a higher-dimensional code based on both frequency and phase. During maintenance, odor-specific patterns of theta-coupled gamma were spontaneously reactivated in piriform. Importantly, individual odors were sequentially reactivated, preserving the order of the initial percept. Such memory replay was time compressed across contiguous theta cycles, and their prevalence was correlated with successful sequence memory. Interestingly, replay events, observed in piriform, coincided with periods of enhanced cross-structural theta synchrony with hippocampus. Our data suggest that nested theta and gamma are oscillatory mechanisms for mnemonic representations multiplexing both the “what” and “when” of odor sequences. Taken together with the literature, neural oscillations may be a physiologic mechanism to coordinate multiple streams of complex memories, generalizing across modalities and sensory systems.

10:30 - 10:45 AM	Calusa Foyer
Coffee Break	

10:40 - 12:00 PM	Calusa EFGH
STANDING ON THE SHOULDER OF GIANTS, A TRIBUTE SYMPOSIUM	

Chair(s): Susan Travers

10:40 **Standing On The Shoulders Of Giants, A Tribute Symposium Introduction**

Susan Travers
Ohio State University , San Diego, OH, United States

This symposium celebrates and pays tribute to the research and impact of three Giants of chemosensory science. Dr. James Byron Snow, as the first official Director of the National Institute on Deafness and Other Communication Disorders, sculpted the research strategy of a nascent institute and created countless opportunities for the advancement of chemosensory research. Dr. Donald Leopold was the consummate physician/scientist, advancing clinical research and practice and improving the lives of patients with chemosensory disorders. Dr. Gordon Shepherd, a pioneer in neuroscience, used innovative techniques from computational neuroscience and bioinformatics to make stunning contributions to our understanding of the functional organization of and mechanisms of information processing in the brain, using mammalian olfaction as a model system. Their innovations and insights have shaped current and future research in the chemical senses. As we seek a future of transformative research, discovery and therapeutics in the chemical senses, we stand on the shoulders of Giants.

10:45 **A Tribute To James Byron Snow, Jr., M.D.**

Debara L. Tucci
Director, National Institute on Deafness and Other Communication Disorders , Bethesda, MD, United States

James Byron Snow, Jr., M.D., passed away on May 28, 2022. He made significant contributions to research and clinical practice in otolaryngology and is considered a pioneer in the field. As the first official director of the National Institute on Deafness and Other Communication Disorders (NIDCD), he created significant opportunities for scientists and trainees alike to perform impactful research in the NIDCD's mission areas of hearing, balance, taste, smell, voice, speech, and language. Dr. Snow paved the way for the immense research contributions of internationally known NIDCD intramural scientists and grantees. Dr. Debara L. Tucci, the current director of NIDCD, will present a tribute to the federal career of Dr. Snow and his contributions to public health.

11:10 **Standing On The Shoulder Of Giants: Donald Leopold**

Eric H. Holbrook
Massachusetts Eye and Ear/Harvard Medical School, Boston, MA, United States

Donald Leopold--our friend, colleague, teacher, and mentor will truly be missed. As experienced by the numerous previous medical students, residents, and fellows that he trained over many years; he was known for his attention to detail in obtaining a patient history, observing responses to clinical management, and recognizing the anatomic structures of the nose and sinuses that either require surgical correction or were best left undisturbed. He was an early proponent in adopting endoscopes for use in sinus surgery and advanced the field with contribution to development of instruments particularly in improving access to the frontal sinuses. He was a staunch advocate for proper instruction of students and clinical colleagues in the assessment of patients with chemosensory disorders rightly preaching the need for smell and taste testing. His work on diagnosis and management of patients with phantosmia brought a better understanding of central and peripheral causes of this debilitating disorder providing hope for many who sought his care. He was steadfast at looking for ways to improve sense of smell in his chronic sinus patients and understood the quality of life impact this loss contributed to the whole of the disease. AChemS and the American Rhinologic Society were his academic homes always determined to attend annual meetings for both. He loved to learn, forever a student, and in result he was an excellent teacher. He was unselfish in donation of his time to the care of his patients and to the teaching of his students. His legacy will live on in the multitudes of residents and fellows that were fortunate enough to have trained under him and the colleagues and friends who were fortunate to know him.

11:35 **In Honor Of Dr. Gordon Murray Shepherd**

Stuart Firestein[1], Charles Greer[2]
[1]Columbia University, [2]Yale University

Stuart Firestein and Charlie Greer will recount some of the early days in the Shepherd lab and its foundations. They will focus on more personal interactions with GMS and his role in establishing the trajectory of their careers as well as those of others. We will also talk about the early beginnings of AChemS and Gordon's role in the early days of the organization and the meeting.

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

12:30 - 1:30 PM	Calusa ABC
Barry Davis NIH 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

1:30 - 3:00 PM	Calusa ABC
Demystifying the BRAIN Initiative® Program: Guidance to Potential NIDCD Applicants	

The National Institute on Deafness and Other Communication Disorders (NIDCD), one of 10 Institutes and Centers at the National Institutes of Health that make up the NIH BRAIN Initiative, is organizing a workshop to share information about the NIH BRAIN initiative program, provide an overview of various funding opportunities, and provide guidance to potential NIDCD applicants. The workshop will feature presentations from the NIH BRAIN initiative Director and NIDCD staff as well as several BRAIN initiative awardees in the chemical senses space. The presentations will be followed by a Q&A session.

Chair(s): Merav Sabri

1:30 **Introduction To The Nih Brain Initiative® Program And Funding Opportunities**
Dr. John Ngai
Director, NIH BRAIN Initiative

1:50 **The Brain Initiative And The Nidcd**
Dr. Merav Sabri
Program Director, NIDCD

1:55 **Brain Funded Grant Example I: Development Of 3D-Fast Optical Interface For Rapid Volumetric Neural Sensing And Modulation (R01)**
Dr. Emily Gibson
University of Colorado Denver

2:10 **Brain Funded Grant Example Ii: Metastable Dynamics In Cortical Circuits (U01)**
Dr. Arianna Maffei
Stony Brook University

2:25 **Brain Funded Grant Example Iii: Cracking The Olfactory Code (U19)**
Dr. Thomas Bozza
Northwestern University

2:40 **Q&A**

Presenters and NIH staff will answer questions from session attendees.

3:00 **Adjournment**

3:00 - 4:00 PM	Estero Foyer
Diversity Networking Reception (Invite Only)	

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

3:30 - 5:00 PM	Great Egret
Journal Club: From Pasquale Graziadei's Classic Work on Neurogenesis To Degeneration and Regeneration of the Olfactory System in COVID 19	

Organized by the AChemS History Committee: Gary Beauchamp, Jessica Brann, Claire Murphy, Susan Travers, Don Wilson

Chair(s): Jessica Brann

3:30 **Brief Introduction To Achems Journal Club**
Jessica Brann
Firmenich

3:35 **Informal Introduction To The Graziadei Laboratory And Principal Scientific Contributions From Pasquale Graziadei**
Richard M. Costanzo
Virginia Commonwealth University

3:55 **The Classic Paper: Ppc Graziadei And Ga Monti Graziadei: Neurogenesis And Plasticity Of The Olfactory Sensory Neurons**
Jack Finlay
Student from the laboratory of Bradley Goldstein, Duke University

4:10 **To The Present: Degeneration And Regeneration Of The Olfactory System In Covid-19**
Leslie M. Kay
University of Chicago

4:40 **Reminiscences And Comments From The Audience**

5:00 - 5:45 PM	Calusa ABC
AChemS 2023 Codefest Presentations	

Participants from the AChemS 2023 Codefest will present their findings from the National Health and Nutrition Examination Survey (NHANES).

5:00 - 6:00 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 - 7:30 PM	Calusa EFGH
Data Blitz	

Chair(s): Yanina Pepino

7:00 **Neural Mechanisms Of Oxygen Sensing In A Human-Infective Nematode**

Breanna Walsh[1, 2, 3], Elissa A. Hallem[1, 4]

[1]Department of Microbiology, Immunology, and Molecular Genetics, University of California Los Angeles, Los Angeles, CA, United States, [2]Molecular Biology Interdepartmental PhD Program, University of California Los Angeles, Los Angeles, CA, United States, [3]UCLA-Caltech Medical Scientist Training Program, Los Angeles, CA, United States, [4]Molecular Biology Institute, University of California Los Angeles, Los Angeles, CA, United States

Skin-penetrating parasitic nematodes – like insect disease vectors – employ neuronally sensed cues to target human hosts. Sensory stimuli, such as temperature and odorants, aid host-seeking behaviors in *Strongyloides stercoralis*, a skin-penetrating nematode that infects an estimated 610 million people. In its pursuit and subsequent infection of human hosts, *S. stercoralis* encounters a wide range of oxygen (O_2) concentrations, spanning from atmospheric levels (~21%) at the soil surface to near-anaerobic levels in the host intestine. In these contexts, O_2 likely serves as a key sensory cue for environmental navigation and coordinated development; yet, the molecular and neural bases of O_2 sensation remain uncharacterized in *S. stercoralis* and all other parasitic nematodes. We found that *S. stercoralis* infective larvae exhibit robust changes in locomotion upon exposure to acute shifts in O_2 concentration, which is the first evidence of an O_2-evoked behavior in a parasitic nematode. We then identified a subset of putative gas-sensing neurons by anatomic and molecular homology to the O_2-sensing neurons in the free-living model nematode *C. elegans*. Chemogenetic silencing of these neurons resulted in dampened O_2-evoked motile behaviors in *S. stercoralis*. We also identified four candidate soluble guanylate cyclases (sGCs) in *S. stercoralis*, each of which bear protein-level homology to *C. elegans* O_2-sensing sGCs. We posit that these sGCs are molecular O_2 sensors. Using transcriptional reporters, we found that three of the *S. stercoralis* sGCs are expressed in putative O_2-sensing neurons. We are now testing the requirement for these sGCs during host seeking and characterizing their functional properties. Our results will illuminate how gas chemosensation shapes parasite behavior and pathogenesis.

7:04 **Experience-Dependent Plasticity Of Gustatory Insular Cortex Circuits And Taste Preferences**

Hillary C Schiff[1], Joshua F Kogan[1,2,3] , Maria Isaac[1,2], Lindsey A Czarnecki[1], Alfredo Fontanini[1,2], Arianna Maffei[1,2]

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

Experience-dependent refinement of cortical circuits during the postnatal period contributes to the development of our sensory systems, ultimately supporting complex functions like perception and cognition. In human infants, early life taste experience has lasting effects on taste preferences, likely resulting in an appreciation of foods from one’s youth and cultural heritage. These taste preferences influence consumption of nourishing food and

avoidance of dangerous substances. Although evidence suggests that early experience may modify preference, it is not known whether gustatory cortical regions show plasticity in critical periods or remain plastic throughout life. Using a brief access test, we determined that exposure to a variety of tastes in weanling mice (early exposure "EE") persistently enhanced sucrose preference when compared to naïve mice exposed only to water and chow. The same exposure at 8 weeks of age, young adults, did not affect sucrose preference. The change in sucrose preference depended on the presence of nutrients in the exposure tastants and an intact olfactory epithelium. EE modulated neural function, resulting in a relative increase in inhibition in gustatory cortex (GC) and sharpened representation of sucrose concentration. In line with increased inhibition, we observed increased inhibitory synaptic transmission in GC following EE and accelerated association of parvalbumin (PV^+) neurons with perineuronal nets (PNNs). 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 and GC function due to modified inhibitory control of GC.

7:08 **Human Olfactory Navigation Recruits Grid-Like Representations In Entorhinal And Piriform Cortices**

Clara U. Raithel[1,2], Alexander J. Miller[2], Russell A. Epstein[1], Thorsten Kahnt[3,4], Jay A. Gottfried[1,2,4]

[1]Department of Psychology, University of Pennsylvania, Philadelphia, PA, United States, [2]Department of Neurology, University of Pennsylvania, Philadelphia, PA, United States, [3]National Institute on Drug Abuse, Intramural Research Program, Baltimore, MD, United States, [4]Department of Neurology, Northwestern University, Chicago, IL, United States

Olfactory navigation is observed extensively across the animal kingdom. Humans, however, have rarely been considered in this context. In our experiment, we used a combination of olfactometry techniques, Virtual Reality (VR) software applications and neuroimaging methods to investigate whether humans can navigate an olfactory landscape by learning about the spatial relationships among discrete odor cues and integrating this knowledge into a spatial map. Our data show that, over the course of the experiment, participants improved their performance on the odor navigation task, i.e., took more direct paths toward the target, and completed more trials within a given time period. This suggests that humans can successfully navigate a complex odorous environment, reinforcing the notion of olfactory navigation in humans. Functional Magnetic Resonance Imaging (fMRI) data collected during olfactory navigation revealed the presence of grid-like representations in entorhinal and piriform cortices that were attuned to the same grid orientation. This result implies the existence of a functional grid network relying on olfactory cues to guide spatial navigation. In next steps, we plan to examine the interactions between olfactory landmarks and other sensory (e.g., visual) inputs as well as reward to gain a better understanding of spatial navigation at both the neural and behavioral levels.

7:12 **Determining The Roles Of C-Kit And PdgfrA In Sweet Taste Receptor Cell Homeostasis**

Christina M Piarowski[1,2], Elaine T. Lam[3], Peter J. Dempsey[4], Linda A. Barlow[1,2]

[1]Department of Cell and Developmental Biology, University of Colorado Anschutz Medical Campus, Aurora, CO, United States, [2]Rocky Mountain Taste and Smell Center, University of Colorado Anschutz Medical Campus, Aurora, CO, United States, [3]Department of Medical Oncology, School of Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO, United States, [4]Department of Pediatrics - Developmental Biology, University of Colorado Anschutz Medical Campus, Aurora, CO, United States

The sense of taste is mediated by taste buds on the tongue which each house 50-100 continuously renewing, short-lived taste receptor cells (TRCs) comprising type I cells (glial-like), type II cells (sweet-, bitter- or umami-sensitive), and type III cells (sour-sensitive). Since TRCs continuously turn over, pharmacological agents that impede homeostatic pathways can lead to taste dysfunction, or dysgeusia. Dysgeusia is a common symptom associated with oral chemotherapy drugs called tyrosine kinase inhibitors (TKIs), which inhibit receptor tyrosine kinases (RTKs). Our single-cell RNA sequencing data shows that many RTKs inhibited by TKIs are expressed in different populations of progenitors, precursors, or differentiated TRCs. To evaluate if inhibition of these RTKs could hinder taste homeostasis, we treated lingual organoids with three TKIs that commonly cause dysgeusia (axitinib, cabozantinib and sunitinib). While progenitor cell proliferation was not affected by TKI treatment, TRC homeostasis was altered. Specifically, expression of sweet-cell marker Tas1r2 decreased in response to all three drugs. Axitinib has the fewest targets of the TKIs tested, only inhibiting the taste RTKs c-Kit, PDGFRα and RET. Through additional organoid experiments we have determined RET inhibition is not responsible for the Tas1r2 phenotype, leaving c-Kit and PDGFRα as the primary candidates in contributing to sweet-cell biology. Furthermore, immunohistochemistry and HCR *in-situ* hybridization show both candidates are most highly expressed in sweet-cells. We are currently using more specific inhibitors to determine whether c-Kit, PDGFRα, or both, regulate either sweet-cell differentiation or expression of Tas1r2. We are also treating mice with axitinib to determine if sweet-cell homeostasis is impeded *in-vivo*.

7:16 **Correlative Intravital And Histological Imaging On Intact Taste Buds**

Sungho Lee[1,2], MinJae Kim[1,2], Gha Yeon Park[1,2], Jubeen Yoon[3,4], Junsuk Lee[3,4], Chang Ho Sohn[3,4], Myunghwan Choi[1,2]

[1]School of Biological Sciences, Seoul National University, Seoul, *, South Korea, [2]The Institute of Molecular Biology and Genetics, Seoul National University , Seoul, *, South Korea, [3]Center for NanoMedicine, Institute for Basic Science, Seoul, *, South Korea, [4]Department of Nano Biomedical Engineering, Yonsei University, Seoul, *, South Korea

Understanding the physiology of taste cells requires multifaceted cellular information from gene regulation to functional responses. A variety of experimental approaches for obtaining each biological information is available, such as in situ hybridization for gene transcription and microfluidics-integrated intravital microscopy (μTongue) for functional responses. However, the acquisition of genetic and functional information correlatively at a single-cell level has yet to be realized for taste cells, hampering a comprehensive understanding of the causal interaction between gene and function. Here, we report a novel data acquisition pipeline providing correlated information on tastant-evoked functional responses of taste cells *in vivo* and their transcriptional regulation. In this pipeline, *in vivo* functional data is firstly acquired from several taste buds using μTongue and then the vicinities of the taste buds of interest are marked by using near-infrared branding. Using the branding as a landmark, the same taste buds are re-identified in a sliced tissue and processed for in situ hybridization. As a proof-of-principle, we performed *in vivo* imaging of sour-responsive cells in fungiform taste buds, and correlatively performed in situ hybridization targeting OTOP1, resulting in single-cell-level correspondence. Our proposed pipeline is broadly compatible with recent spatial transcriptomics and proteomics approaches for generating large-scale correlative datasets.

7:20 **Development Of A Nonverbal Odor Assessment Map**

Robert Pellegrino[1], Joshua Nsubuga[1], Matthew Andres[1], Jennifer / E. Margolis[1], Joel / D. Mainland[1,2]

[1]Monell Chemical Senses Center, Philadelphia, PA, United States, [2]University of Pennsylvania, Philadelphia, PA, United States

A key problem in quantifying odors is the lack of a rapid tool for individuals to report odor percept. In this study, we develop an odor display device to represent odors spanning a large perceptual gamut to aid subjects in quantifying odor space. First, using Rate-all-that-apply profiling for over 600 odors, we created a relational 2-dimensional odor map (organized map) such that nearby stimuli are perceptually similar and distant stimuli are perceptually different. Second, we provided subjects with a touch display that is coupled to an olfactometer that generates the odor corresponding to a given location on the map. We demonstrate that individuals are better able to match a target odor to the corresponding location on the map when using an organized odor map rather than a map that randomly assigns a location to an odor (shuffled map). Individuals were able to find an odor more accurately ($F = 2.81$, $p < 0.005$) when using an organized odor map rather than a shuffled map. This novel odor display device allows subjects to nonverbally assess odor quality, which may be useful for describing alterations such as parosmia in clinical settings.

7:24 **Endocrine And Sensory Integration In The Caudal Nucleus Of The Solitary Tract Modulates Na^+ Intake In Dehydrated Mice**

Caitlin M. Baumer-Harrison[1], Khalid Elsaafien[1], Sagar Patel[1], Karen A. Scott[1], Guillaume de Lartigue[2], Eric G. Krause[1], Annette D. de Kloet[1]

[1]University of Florida, Gainesville, FL, United States, [2]The Monell Center, Philadelphia, PA, United States

Angiotensin-II (AngII) plays a pivotal role in regulating blood pressure (BP), water (H_2O), and sodium (Na^+) homeostasis, by way of acting on angiotensin type 1a receptors (AT1aR) throughout the body. Of particular relevance here, AT1aR are expressed on afferents that transmit cardiovascular or gustatory sensory information to the nucleus of the solitary tract (NTS). We found that optical excitation of these afferents in the caudal NTS mimics perception of increased vascular stretch and induces frequency-dependent alterations in BP and Fos immunoreactivity in brain regions involved in fluid balance, gustatory, and cardiovascular function. Here, we hypothesized that these $AT1aR^+$ afferents are also sufficient and necessary to modulate H_2O and Na^+ intake during alterations in vascular stretch. Mice expressing excitatory or inhibitory opsins in $AT1aR^+$ cells were implanted with fiber optics targeting the NTS. H_2O and NaCl intakes were measured using two-bottle preference tests in the presence and absence of optical stimulation under euhydration and dehydration. Optical excitation (using a frequency found to be sub-threshold for reducing BP) significantly attenuated, while optical inhibition significantly increased Na^+ intake under dehydrated conditions. Optical stimulation did not impact intakes of AT1aR-Cre control mice. Next, $AT1aR^{Flox}$ mice and AAVs (retro serotype) that express of Cre and/or tdTomato were used to probe the function of AT1aR on neurons that innervate the aortic arch. Preliminary results indicate that deletion of AT1aR(s) from aortic afferents alters fluid consumption in the euhydrated condition, with KO mice having a greater preference for NaCl. Collectively, these results imply that NTS $AT1aR^+$ afferents are both necessary and sufficient to modulate Na^+ intake relative to BP status.

101 **Sugar Exposure Selectively Increases Nucleus Of The Solitary Tract Responses To Glucose In C57Bl/6J Mice**

David Pittman[1], Tatiyana Adkins[1], DeHaven Dickerson[1], Lindsey Schier[2]

[1]Wofford College, Spartanburg, SC, United States, [2]University of Southern California, Los Angeles, CA, United States

Schier et al. previously showed that a sugar exposure paradigm selectively increased licking to glucose relative to fructose in mice. Furthermore, the sugar-exposed mice had significantly higher levels of the glucose-sensing intermediary, glucokinase, in taste buds than naïve control mice. Therefore, it was proposed that upregulation of peripheral gluco-specific transduction mechanisms selectively increase neural and behavioral taste responses to glucose. In this study, we assessed taste responses in the nucleus of the solitary tract (NST) of sugar-exposed and naïve mice. Male C57BL/6J mice were given 1 of 3 concentrations of fructose and glucose as their daily fluid source three times in a counterbalanced design for total of 18 days of sugar exposure (1 sugar solution per day). The naïve group received deionized water as their daily fluid source. Integrated (0.01 s RMS) multi-unit taste responses from the NST were recorded for 20-s trials of 3 concentrations of fructose and glucose, NaCl, citric acid, quinine-HCl, MSG and NH4Cl. Responses were normalized to 500 mM NH4Cl that bracketed the taste stimulation battery. There was no significant difference between the first NH4Cl responses and the last NH4Cl responses indicating stable NST responsiveness. There was a significant effect of concentration for each tastant. The sugar-exposed mice showed significantly higher neural activity in the NST for glucose but not for fructose or any of the other non-sweet taste stimuli. As suggested by previous findings, it appears that sugar-exposure bolsters gluco-specific signals transmitted to the central gustatory system, which could underlie the corresponding increase in glucose appetite.

103 **Oral, Peri-Taste Digestion Contributes To The Hedonic Appeal Of Sugar In Mice**

Aracely Simental-Ramos[1], Ahyun Jung[1], Sandrine Chometton[2], Lindsey Schier[1,2]

[1]Neuroscience Graduate Program, University of Southern California, Los Angeles, CA, United States,

[2]Department of Biological Sciences, University of Southern California, Los Angeles, CA, United States

Cephalic sensory and digestive processing of sugars directly affect downstream events. We previously showed that dietary experience with simple sugars (SugExp), glucose and fructose reinforces taste-driven responsivity to glucose, and this is mediated by a glucokinase-linked sensor in the taste bud cells. SugExp also boosts licking for maltose, a glucose disaccharide, especially in mice that lack a critical intermediary in sweet taste transduction, TRPM5. However, maltose does not directly engage with glucokinase in its bound form. Therefore, we tested two related hypotheses. We assessed whether lingual glucokinase is required to express a relative preference for maltose in SugExp mice. We then tested whether lingual maltose glucoamylase (MGAM), a digestive enzyme that is expressed in taste bud cells and is thought to generate free glucose for sweet receptors and glucosensors, contributes to the behavioral attraction to maltose. We found that knockdown of glucokinase in the major taste fields with a short hairpin RNA (shRNA) reversed the acquired preference for maltose in SugExp, sweet-sensitive mice. SugExp, sweet-sensitive, C57BL6/J mice also expressed more MGAM in the circumvallate taste buds than their naive counterparts. Moreover, there was an inverse relationship between *Tas1r3* and *MGAM* expression in the circumvallate taste buds among sweet-sensitive and sub-sensitive mice, whereby those with less *Tas1r3* had greater *MGAM*. Finally, shRNA-mediated silencing of MGAM in the major taste fields significantly reduced licking avidity for maltose in naïve mice. Taken together, these results provide the first evidence that enzymatic processing of sugars at the very first site of nutrient detection contributes to hedonic appeal, perhaps especially when sweet taste transduction is deficient.

105 **Sensing Taste-Evoked Gaba Release From Taste Buds Using Cellular Biosensors**

Yuryanni A. Rodriguez[1], Gennady Dvoryanchikov [1], Aiyana Ward[2], Stephen D. Roper[1,2], Nirupa Chaudhari[1,2]

[1]Dept. of Physiology and Biophysics, Univ. of Miami Miller School of Medicine, Miami, FL, United States,

[2]Program in Biomedical Sciences, Univ. of Miami Miller School of Medicine, Miami, FL, United States

Taste buds synthesize and accumulate GABA in glial-like Type I and sour-sensing Type III cells. Acid taste elicits GABA release from Type III cells (Huang et al 2011). Whether Type I cells also release GABA is not yet known. Bitter, sweet, and umami tastes stimulate Type II cells to secrete ATP which in turn, activates afferent nerve fibers and Type I cells (Rodriguez et al 2021). We asked if Type I cells then release GABA, e.g. as a gliotransmitter. To detect GABA release we employed CHO cells expressing $GABA_B$ receptors and a chimeric G-protein, Gαqo5. GABA elicits a robust $\Delta[Ca^{2+}]_i$ in these cells that can be imaged with the Ca^{2+} indicator, Cal-590AM. "GABA biosensors" reliably responded to GABA (threshold ≈100 nM; EC_{50} ≈1000nM) and these responses were blocked by 10μM CGP55845, a $GABA_B$ antagonist. To eliminate contamination of GABA biosensor responses with responses from taste-evoked ATP release (because CHO cells express endogenous P2Y receptors), we desensitized biosensors by bathing them in 500μM ATP. To test whether bitter taste-stimulation elicited GABA secretion, we deposited taste buds expressing GCaMP3 in Type I cells onto a monolayer of GABA biosensors and perfused the recording chamber with a bitter tastant, 5μM cycloheximide (CHX). We used 10μM ATP to verify that purinoceptors on GABA biosensors were desensitized. Preliminary data indicate that

GABA biosensors directly below or within 20μm of taste buds responded to CHX stimulation, consistent with taste-evoked GABA release from Type I cells. Further, because Type I cells expressed GCaMP3, we recorded concurrent taste-evoked responses in Type I cells and underlying GABA. Our preliminary results suggest that Type I cells release GABA in a taste-evoked manner. Pharmacological and statistical elaboration of these findings are ongoing.

107 **Don Tucker Finalist: Amino Acid Bitterness: Suppression By Sodium Salts And Individual Differences.**

Caroline P. Harmon[1], Paul A.S. Breslin[1,2], Osama M. Ahmed[3]

[1]Rutgers University, New Brunswick, NJ, United States, [2]Monell Chemical Senses Center, Philadelphia, PA, United States, [3]University of Washington, Seattle, WA, United States

Amino acids are the basic unit of protein and play important roles in muscle growth, cell signaling, and health. Free amino acids are in both natural foods and in health supplements. Products with free amino acids can stimulate “off-tastes” that interfere with consumption and medical compliance. The primary off-taste generated by free amino acids is bitterness. In this study, we aimed to characterize the taste profiles of 19 amino acids, to identify which amino acids generate bitter taste, to suppress their bitterness with sodium salts (NaCl, NaGluconate, NaPropionate, NaSalicylate, and Na Adenosine Monophosphate), and to identify clusters among amino acids based on individual differences in subjects’ bitterness perception. Subjects rated bitterness of amino acids on a labeled magnitude scale. We identified isoleucine, leucine, methionine, phenylalanine, tryptophan, and arginine as the amino acids that elicited bitterness as their main taste quality. The bitter taste of all these amino acids was successfully reduced to varying extents by the sodium salts tested, with the exception of arginine ($p<0.05$). Correlation matrix analysis, Principal Component Analysis, and cluster analysis linked these amino acids and their metabolites into at three to four distinct clusters based on the subjects' individual sensitivities to bitterness. In the future, combinations of the bitter blockers tested may be used to minimize amino acid aversiveness. Minimizing the bitter taste of free amino acids found in muscle growth aids for the elderly, oral rehydration and diarrhea products, and infant hydrolysate formulae can increase compliance to these interventions and improve patient health.

109 **P2Y2 Receptors Are Involved In Taste Signaling Mediated By Type I Taste Receptor Cells**

Catherine Anderson[1,3], Sarah Power[1,3], Mei Li[2,3], Eric D Larson[1,2,3], Sue C Kinnamon[1,3]

[1]Department of Otolaryngology - Head and Neck Surgery, University of Colorado Anschutz Medical Campus, Aurora, CO, United States, [2]Department of Cell and Developmental Biology, University of Colorado Anschutz Medical Campus, Aurora, CO, United States, [3]The Rocky Mountain Taste and Smell Center, University of Colorado Anschutz Medical Campus, Aurora, CO, United States

In taste buds, Type I cells are the most abundant cell type and are considered to have a support, glial-like function. Recent studies challenged this dogma suggesting Type I cells have additional sensory and signaling functions. We and others previously identified Type I cells using GAD65-driven cre. However, we reported expression of GAD65cre in subsets of Type II and III cells, confounding interpretations made using this system. Here, we use a tamoxifen-inducible GAD65cre line more specific to Type I cells. Nerve recording in GAD65cre/Channelrhodopsin mice and histology in GAD65cre/nuclear targeted Tomato mice were dosed daily with tamoxifen for 3,4,5, and 10 days. Chorda tympani responses to light appeared with 4 days of tamoxifen. Histology experiments revealed the number of Tomato+ nuclei increased with successive tamoxifen dosing, but so did the number of Tomato+ nuclei of Type II and III cells. Further physiology experiments were pursued with 4 days of tamoxifen to maximize expression in Type I cells (<3% of Tomato+ nuclei in Type II cells, none in Type III cells). Previous studies showed isolated Type I cells respond to ATP with an increase in intracellular calcium, potentially mediated by P2Y receptors. We tested whether P2Y2 receptors mediated this response. GAD65cre/Tomato+ cells responded to 10 μM ATP and the response was attenuated with pre-treatment of AR-C118925, a selective P2Y2 antagonist. Further, a calcium response was observed when cells were exposed to diquafosol, a selective P2Y2 agonist. However, this was not observed in all GAD65cre/Tomato+ cells, indicating the presence of subpopulations of Type I cells. Further work will examine other functional purinergic receptors in Type I cells and the overall role of P2Y responses in taste signaling. Supported by R01 DC017679 to SCK

111 **Tracing The Terminals And Post-Synaptic Targets Of Penk+ (T3) Gustatory Afferent Neurons**

Gennady Dvoryanchikov[1], Kathleen Depina[1], Andoni Asencor[1], Pantelis Tsoulfas[2], Nirupa Chaudhari[1,3]

[1]Department of Physiology & Biophysics, University of Miami Miller School of Medicine, Miami, FL, United States, [2]Miami Project to Cure Paralysis, University of Miami Miller School of Medicine, Miami, FL, United States, [3]Department of Otolaryngology, University of Miami Miller School of Medicine, Miami, FL, United States

Fungiform and palate taste buds are innervated by geniculate ganglion (GG) neurons, whose projections terminate in the rostral-most region of the nucleus of the solitary tract (NST). GG neurons can be grouped transcriptionally, but it is not known if the neuron types terminate in discrete regions of the NST. Proenkephalin (Penk) marks neurons that respond to sour stimuli (T3 group). Here, we aimed first, to trace the central projections of Penk+ neurons and second, to visualize their NST targets. For the first goal, we examined *Penk;;*tdTomato mice and confirmed immunohistochemically that the correct GG neurons were tdTom+. In cryosections of hindbrain, we defined T3 central projections as aggregates of fibers that (i) enter the hindbrain at ≈ -5.24mm from bregma, (ii) are immunoreactive for P2X2 (a gustatory marker) and (iii) are tdTom+. The terminal arbor of such T3 neurons was in a subregion of P2X2+ neurons, but analysis was confounded by Penk+ hindbrain-resident neurons. Thus, we i.v. injected AAV-PHP.S-*flex*.tdTom to restrict reporter to peripheral

neurons and saw that T3 neuron terminals were concentrated in the ventro-lateral portion of the gustatory NST. For the second goal, we employed WGA/mCherry, a fusion protein that is transported trans-synaptically (Tsai et al, 2022). We constructed AAV-PHP.S-*flex*.WGA/mCherry, and injected it i.v. to limit mCherry to peripheral neurons and their post-synaptic targets. In the NST, mCherry accumulated in a subset of P2X2+ terminals in the same ventro-lateral region as above. Further, a limited number of neuronal cell bodies in this same area displayed mCherry+ puncta. Few mCherry+ neurons were found outside the NST in these sections. Analyses to molecularly identify these putative post-synaptic targets of Penk+ ganglion neurons are in progress.

113 **Investigating The Neural Signals Driving The Consummatory Response In Rats**

Natasha Baas-Thomas, Donald Katz
Brandeis University, Waltham, MA, United States

The gustatory system is an ideal model with which to study the neural circuit processes guiding discriminative, ethologically-relevant behavior. When a taste stimulus reaches the tongue, the gustatory system has one basic goal - to determine whether that stimulus should be consumed or expelled from the mouth. Rats (and many other mammals) produce discriminative orofacial movements (taste reactivity, or TR) reflecting the reaching of a consummatory decision: lateral tongue movements (LTM) for ingestion and gapes for rejection. In response to an aversive tastant, the onset of gapes can be determined *via* electromyography (EMG) of the jaw opener (anterior digastric) muscle. Previous work has demonstrated that gustatory cortex (GC) is integrally involved in reaching a consummatory decision. Taste responses in GC ensembles progress through three firing-rate "epochs", the last of which is decision-related. Furthermore, the relationship between the transition to decision-related firing and the initiation of gapes has proven causal. However, GC is not in and of itself a motor structure, instead only signaling the onset of responding - a central pattern generator localized to the medullary reticular formation (RF) directly drives TR. We propose that a modulatory signal from GC guides the selection and initiation of these behaviors in RF. Using optogenetics, I briefly (0.5s) inhibited GC→RF axons in active rats after taste delivery, while simultaneously monitoring digastric EMG and GC neural activity. Preliminary results suggest that inhibition delays the onset and decreases the likelihood of stereotyped gapes, but only if it preceeded the decision-related epoch of GC activity. These findings enrich our understanding of how GC sensory information is transformed into an appropriate motor response.

115 **In Vivo Characterization Of Olfactory Amygdala Anatomical Projections In Human**

Qiaohan Yang[1], Shiloh Echevarria-Cooper[1], Thorsten Kahnt[2], Christina Zelano[1]
[1]Department of neurology, Feinberg school of medicine, Northwestern University, Chicago, IL, United States,
[2]2. National Institute on Drug Abuse Intramural Research Program, Baltimore, MD, United States

The human olfactory bulb projects to multiple cortical regions in parallel and each is thought to serve distinct functions in olfactory sensory processing. Three subregions of the human amygdala (referred to here as the olfactory amygdala) receive direct bulb input and are therefore considered part of the primary olfactory cortices. These include the medial nucleus, the cortical nucleus, and the periamygdala cortex. The functions of these subregions in human olfactory processing are unknown. Only a handful of human olfaction studies have focused on the amygdala, none of which considered the three amygdala subregions independently. Thus, we lack even a rudimentary understanding of their structural and olfactory functional properties. Here we use diffusion-weighted imaging (DWI) to study the projections of the human olfactory amygdala in vivo. We collected data from 26 healthy adult participants using a multi-shot echo planar imaging (MS-EPI) diffusion-weighted acquisition. Preliminary analyses in some participants found streamline connections from the lateral olfactory tract to the olfactory amygdala subregions, and we found downstream connections from the olfactory amygdala into multiple limbic regions. We plan to further characterize the projections from the olfactory bulb to the olfactory amygdala subregions in all subjects and to examine differences in the downstream anatomical connections from these three regions to the rest of the brain.

117 **Circuitry And Function Of The Anterior Olfactory Nucleus To Nucleus Of Lateral Olfactory Tract Pathway**

Janardhan P Bhattarai, Yingqi Wang, Winqin 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 anterior olfactory nucleus and tenia tecta (AON for simplicity), which then connects to the downstream structures that are crucial for odor-guided behaviors. The functions and anatomical connections of the AON to its downstream targets are not well established. To gain genetic access to the AON neurons, we performed a differential gene expression search in the Allen Brain Atlas and identified the neuromedin B receptor (NMBR) gene as a molecular marker for the AON neurons. Using the CRISPR-Cas9 gene-editing approach, we generated an NMBR-Cre knock-in mouse line. Anatomical tracing from the AON neurons revealed specific reciprocal connections with the nucleus of lateral olfactory tract (NLOT) of the cortical pallial amygdala. Whole-cell patch clamp recordings combined with optogenetic activation confirmed that AON neurons make monosynaptic connections onto NLOT neurons. Furthermore, in vivo fiber photometry revealed odor and/or sniff induced Ca^{2+} signals in the AON neuron axonal terminals in the NLOT as well as in NLOT neurons (specifically targeted in the SLA-Cre mouse line) 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. Consistently, chemogenic inhibition of NLOT projecting AON neurons also disrupted olfactory guided behaviors. Taken together, these results indicate that the NLOT and AONàNLOT pathway play a critical role in olfactory-guided behaviors.

119 **Organization Of Midbrain Dopaminergic Input To The Tubular Striatum**

Anamaria Cotelo[1], Natalie L. Johnson[1], Minghong Ma[2], Daniel W. Wesson[1]

[1]University of Florida, Gainesville, FL, United States, [2]University of Pennsylvania, Philadelphia, PA, United States

Dopamine (DA) is a potent neuromodulator with widespread effects on sensory processing. The tubular striatum (TuS, also known as the olfactory tubercle) receives dense DAergic input from the ventral tegmental area (VTA). This VTA→TuS DAergic pathway mediates odor preference and other naturalistic reward processes. Additionally, phasic DA release in the TuS influences odor valence, and ongoing work in our lab aims to uncover the role of DA release into the TuS on sniffing behavior. To better understand where VTA DAergic neurons innervate the TuS, we injected a cre-dependent anterograde AAV encoding synaptophysin tagged with mCherry into the VTA of DAT-Cre male and female mice. This allows for visualization of fluorescent puncta, indicative of synaptic terminals, in regions that receive midbrain DA input. We quantified fluorescent puncta throughout the anterior to posterior span of the TuS along with neighboring striatal structures. Our preliminary results indicate a topographical arrangement of DAT neuron input to the TuS, with a great portion of synaptic input arriving in the anteromedial TuS. To confirm that VTA neurons synapse onto TuS neurons, we next injected an anterograde transneuronal AAV encoding Cre into the VTA of Ai9 (tdTomato Cre reporter) mice. We observed robust tdTomato expression in first order downstream targets of the VTA, including in TuS neurons. Together, these results inform the neuroanatomical organization of VTA→TuS circuitry and provide a foundation for future studies investigating causal manipulations of DA's effects in the TuS.

121 **Intracranial Recordings From The Human Olfactory Cortex In Response To Odors**

Coralie Mignot[1], Susanne Menzel[1], Dino Podlesek[2], Georg Leonhardt[2], Moustafa Bensafi[3], Thomas Hummel[1]

[1]TU Dresden, Smell and Taste Clinic, Department of Otorhinolaryngology, Dresden, *, Germany, [2]TU Dresden, Neurosurgery of University Hospital Carl Gustav Carus, Dresden, *, Germany, [3]Lyon Neuroscience Research Center, CNRS – INSERM – University Claude Bernard of Lyon, CH Le Vinatier, Lyon, *, France

Little is known about local field potentials elicited by odors in humans, especially the way the limbic system processes odor pleasantness. Here, we describe the single case of a patient (man, 39 years old) with pharmaco-resistant epilepsy monitored via stereotactically implanted depth electrodes (stereoelectroencephalography, SEEG) while passively receiving odors with different hedonic valences (peach or fish). SEEG was recorded from 96 contacts in the right hemisphere, located amongst others in structures belonging to the primary olfactory cortex. Odors were delivered 20 times and for 3s each using a computer-controlled olfactometer in precise timing and constant airflow. Respiration was monitored to align the stimulus onset on the following inspiration. Using time-frequency analysis, oscillations of different frequency bands were described. From the closest contact to the piriform cortex in the amygdala, a specific olfactory pattern was observed in the form of early theta/beta oscillations linked to the inspiration phase, followed by beta and gamma oscillations, as described in the literature. Unlike the pleasant peach odor, the unpleasant fish odor produced an early and sustained reinforcement of the delta band. For both odors, contrary to active sniff-induced olfactory processing seen in the literature, the gamma oscillations seemed to last less for passive smelling, with periodic repetition of the olfactory pattern. The parahippocampal gyrus showed differential oscillations between fish and peach. Taken together, these results provide first insights into differential oscillations patterns in the human primary olfactory cortex for pleasant and unpleasant odors.

123 **Don Tucker Finalist: Neural Mechanisms Of Oxygen Sensing In A Human-Infective Nematode**

Breanna Walsh[1, 2, 3], Elissa A. Hallem[1, 4]

[1]Department of Microbiology, Immunology, and Molecular Genetics, University of California Los Angeles, Los Angeles, CA, United States, [2]Molecular Biology Interdepartmental PhD Program, University of California Los Angeles, Los Angeles, CA, United States, [3]UCLA-Caltech Medical Scientist Training Program, Los Angeles, CA, United States, [4]Molecular Biology Institute, University of California Los Angeles, Los Angeles, CA, United States

Skin-penetrating parasitic nematodes – like insect disease vectors – employ neuronally sensed cues to target human hosts. Sensory stimuli, such as temperature and odorants, aid host-seeking behaviors in *Strongyloides stercoralis*, a skin-penetrating nematode that infects an estimated 610 million people. In its pursuit and subsequent infection of human hosts, *S. stercoralis* encounters a wide range of oxygen (O_2) concentrations, spanning from atmospheric levels (~21%) at the soil surface to near-anaerobic levels in the host intestine. In these contexts, O_2 likely serves as a key sensory cue for environmental navigation and coordinated development; yet, the molecular and neural bases of O_2 sensation remain uncharacterized in *S. stercoralis* and all other parasitic nematodes. We found that *S. stercoralis* infective larvae exhibit robust changes in locomotion upon exposure to acute shifts in O_2 concentration, which is the first evidence of an O_2-evoked behavior in a parasitic nematode. We then identified a subset of putative gas-sensing neurons by anatomic and molecular homology to the O_2-sensing neurons in the free-living model nematode *C. elegans*. Chemogenetic silencing of these neurons resulted in dampened O_2-evoked motile behaviors in *S. stercoralis*. We also identified four candidate soluble guanylate cyclases (sGCs) in *S. stercoralis*, each of which bear protein-level homology to *C. elegans* O_2-sensing sGCs. We posit that these sGCs are molecular O_2 sensors. Using transcriptional reporters, we found that three of the *S. stercoralis* sGCs are expressed in putative O_2-sensing neurons. We are now testing the requirement for

these sGCs during host seeking and characterizing their functional properties. Our results will illuminate how gas chemosensation shapes parasite behavior and pathogenesis.

125 **Chronic Consumption Of Sugar Attenuates Appetitive Licking For Sweeteners In Mice**

John I Glendinning, Niki Williams
Barnard College, Columbia University, New York, NY, United States

Humans and rodents are attracted to the flavor of sugar solutions. What remains unclear is whether prolonged exposure to sugar solutions alters this attraction. Resolving this issue may help explain why modern humans exhibit high such daily intakes of sugar. We provided C57BL/6 mice with two 28-day exposures to a control (chow + water) or experimental (chow, water + an 11 or 34% sugar solution) diet. We used brief-access lick tests to assess flavor-mediated attraction to sucrose and sucralose before, between and after the exposure periods. We operationally defined attraction as high oral acceptability (lick rate during 5-s trials) and high motivation for the sweeteners (number of trials initiated across the lick test). Exposure to the control diet had no impact on lick rates or number of trials initiated for the sweeteners; both measures remained high across the exposure periods. In contrast, exposure to 11% sucrose reduced number of trials initiated, but did not alter lick rates. Exposure to 34% sucrose reduced lick rates and number of trials initiated. In Experiment 2, we exposed mice to 11 or 34% concentrations of glucose or high-fructose syrups. Exposure to 11 and 34% glucose syrups reduced lick rates and number of trials initiated. Exposure to high-fructose syrups reduced lick rates and number of trials initiated less reliably. Despite the attenuated attraction to the sweeteners, the mice increased daily intake of most sugar solutions across the exposure periods. We conclude that (a) prolonged exposure to the sugar solutions reduced attraction to the sweetener solutions, (b) the extent of this effect depended on the concentration and type of sugar, and (c) the reduced attraction to the sweeteners could not explain the increase in daily intake of the sugar solutions across the exposure periods.

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

Hillary C Schiff[1], Joshua F Kogan[1,2,3] , Maria Isaac[1,2], Lindsey A Czarnecki[1], Alfredo Fontanini[1,2], Arianna Maffei[1,2]

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

Experience-dependent refinement of cortical circuits during the postnatal period contributes to the development of our sensory systems, ultimately supporting complex functions like perception and cognition. In human infants, early life taste experience has lasting effects on taste preferences, likely resulting in an appreciation of foods from one's youth and cultural heritage. These taste preferences influence consumption of nourishing food and avoidance of dangerous substances. Although evidence suggests that early experience may modify preference, it is not known whether gustatory cortical regions show plasticity in critical periods or remain plastic throughout life. Using a brief access test, we determined that exposure to a variety of tastes in weanling mice (early exposure "EE") persistently enhanced sucrose preference when compared to naïve mice exposed only to water and chow. The same exposure at 8 weeks of age, young adults, did not affect sucrose preference. The change in sucrose preference depended on the presence of nutrients in the exposure tastants and an intact olfactory epithelium. EE modulated neural function, resulting in a relative increase in inhibition in gustatory cortex (GC) and sharpened representation of sucrose concentration. In line with increased inhibition, we observed increased inhibitory synaptic transmission in GC following EE and accelerated association of parvalbumin (PV^+) neurons with perineuronal nets (PNNs). 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 and GC function due to modified inhibitory control of GC.

129 **Examining Taste Preference, Taste Sensitivity, And Appetite Hormones Following Ad Libitum Consumption Of Low-Carb And Low-Fat Diets: A Randomized Controlled Pilot Study**

Rosario Jaime-Lara[1,2,3], Alexis Franks[2], Brianna Brooks[2], Khushbu Agarwal[1,2], Monica Atkinson[1,2], Nafisa Nawal[1,2], Meaghan Steck[1,2], Amber Courville[4], Juen Guo[4], Shanna Yang[5], Valerie Darcey[4], Stephanie Chung[4], Ciaran Forde[6], Kevin Hall[4], Paule Joseph[1,2]

[1]National Institute of Alcohol Abuse and Alcoholism, Bethesda, MD, United States, [2]National Institute of Nursing Research, Bethesda, MD, United States, [3]University of California Los Angeles, Lose Angeles, CA, United States, [4]National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, United States, [5]National Institutes of Health Clinical Center, Bethesda, MD, United States, [6]Wageningen University and Research, Wageningen, *, Netherlands

As obesity rates continue to escalate, many dietary interventions have become popular to promote weight loss. Two common diets often utilized in the treatment/management of obesity are the low-carbohydrate (LC) and low-fat (LF) diets. While both diets can promote weight loss, the compared effectiveness of each intervention has been a subject of debate. Although taste is known to influence food selection and intake, less is known regarding the role that taste preference and sensitivity play in eating behavior following LC and LF diets. This study sought to: (1)compare sweet and salty taste detection thresholds and preferences following a two-week ad libitum LF diet compared to a LC diet, (2)determine whether sweet and salty taste detection thresholds and preferences were associated with gut hormones (Leptin, ghrelin, PYY, and GLP-1) within each diet arm. We

analyzed and compared taste preference and detection during an *ad libitum*, randomized, controlled inpatient feeding study (n=18) comparing LC (10%carbohydrate, 75%fat and 15%protein) and LF (75%carbohydrate, 10%fat and 15%protein) diets. Taste measures and hormone levels were measured at the end of each diet period. There were no significant differences in taste measures between the LC and LF diets. However, we did observe a negative association between salt preference and leptin levels during both LC (r_s=0.59;p<0.01) and LF (r_s=-0.47;p<0.05) diet arms. Salt preference was also negatively correlated with GLP-1 after the LF diet (r_s=-0.61;p<0.01). This suggests that while taste measures may not differ between diet arms, hormones associated with satiety and appetite may impact taste parameters following both LC and LF diets. The effect of these hormones on taste may be a potential mechanism underlying the observed weight loss following both diets.

131 **Achems Undergrad Finalist: Glucose But Not Sucralose Detection Thresholds Vary As A Function Of Hormone Status Throughout The Menstrual Cycle**

Sarah M. Sywanycz[1], Emily C. Hanselman[1], Elizabeth Kaye B. Leonardo[1], Paul A.S Breslin[1,2]
[1]Rutgers University, New Brunswick, NJ, United States, [2]Monell Chemical Senses Center, Philadelphia , PA, United States

In vitro studies showed that estrogen (17-β-estradiol) is a positive modifier for the ATP-sensitive potassium channel (K_{ATP}) in pancreatic beta islet cells. The K_{ATP} channel also plays a functional role in the oral metabolic signaling pathway in taste cells for glucose detection and the anticipatory insulin responses in mice. This same pathway has also been identified in human taste cells and is involved in glucose detection. Hypothesis: We hypothesize that oral glucose detection thresholds in humans will decrease as a function of increasing 17-β-estradiol (ovulation). Methods: Oral glucose detection thresholds in eumenorrheic humans (n=10) were collected during menstruation and ovulation using a modified staircase method and compared to non-ovulating humans (n= 6). Ovulation was verified using fertility LH and E3G urine test strips from Modern Fertility and Mira. Sucralose detection thresholds were collected as a negative taste control, since ATP is not generated by this non-metabolizable sweetener. Results: Oral glucose thresholds were slightly elevated during the ovulatory phase versus the menstrual phase. Sucralose detection thresholds did not change. Conclusion: Subjects were unexpectedly less sensitive to glucose during ovulation compared to menstruation. But we will continue to explore the role, if any, the metabolic signaling pathway plays in the found effect. Future direction will continue to determine the relationship between glucose detection as a function of 17-β-estradiol cyclicity. Individual participants vary in hormone levels throughout the menstrual cycle. We will consider the magnitude of change between high and low estrogen, as well as the slopes of individual estrogen functions during the menstrual cycle.

133 **Cross-Country Comparison Of Sweet Preferences In Online Reviews Of Food Products**

Evan Guerra, Valentina Parma
Monell Chemical Senses Center, Philadelphia, PA, United States

Sweet taste preference is an important determinant in greater intake of high-calorie sugary foods, which predisposes to increased risk of obesity and related metabolic disorders. Some diets are richer in sugars than others, affecting sweet preferences. Using Natural Language Processing methods, online food product reviews can be harnessed to evaluate regional dietary exposure to sweetness and the associated sweetness preference profile. We designed a study to examine the differences in sweetness level, liking, and ingredients in Amazon food products across five countries (China, France, Mexico, Turkey and USA). We scraped from country-specific amazon domains all products included in the Amazon Fine Foods Dataset during the period June 23, 2000 to November 3, 2022. Preliminary analyses evaluated a total of 554,046 reviews (2% China, 7% France, 16% Mexico, <1% Turkey and 75% USA) on a total of 74386 products. Reviews for each product were classified according to phrases included in the reviews (i.e., the phrase“too sweet” is classified as oversweet whereas “not too sweet” is classified as not oversweet), and star ratings and ingredient list were extracted and matched to each review. As hypothesized, preliminary analyses reveal regional trends for oversweetness. For instance, France was ranked as having the most products with oversweet reviews and the USA was ranked as the least products with oversweet reviews, suggesting a lower sweet preference in France than in the USA. Relationships between reported sweetness, liking and ingredient are used to characterize country-specific sweetness profiles and date is used to assess the influence of the pandemic on such profiles. The present work offers insights on quantifying in a highly-replicable manner regional dietary exposure to sweetness.

135 **Replication Of An Effect Of Gnat3 Polymorphisms For Sucrose Solutions But Not Foods**

Alissa A. Nolden[1], Emma L. Feeney[2], John E. Hayes[3,4]
[1]Department of Food Science, University of Massachusetts, Amherst, MA, United States, [2]Institute of Food and Health, University of College Dublin, Dublin, *, Ireland, [3]Department of Food Science, The Pennsylvania State University , University Park, PA, United States, [4]Sensory Evaluation Center, The Pennsylvania State University , University Park, PA, United States

Genetic variability in taste receptors is known to explain individual differences in perception, with potential implications for dietary intake. Here, we re-visit a putative relationship between sweetness perception and genetic variability in *GNAT3*, a gene involved in the pathway for sweet, bitter, and sour tastes. *Present work builds on prior studies reporting a significant association between sucrose sensitivity* and *GNAT3* SNP rs7792845 (Fushan *et al*., 2010 and Eriksson *et al*., 2019). Conversely, a GWA study using 0.35M sucrose failed to confirm *reported associations with* sucrose intensity or liking (Hwang *et al*. 2019). Here, we test putative associations in a lab-based cohort using a wider range of concentrations. Specifically, 153 American participants of European ancestry (mean age 25.4 y, 6.6 SD) rated the intensity of 7 sucrose solutions (0.065, 0.125, 0.25, 0.5,

1.0, 2.0, and 2.5M) on a general Labeled Magnitude Scale; liking data were also collected. We performed 2-way repeated measures ANOVAs across concentration and genotype to test the relationship between intensity and liking ratings and the *rs7792845 variant in GNAT3*. Sweetness intensity and liking for sucrose were each associated with *GNAT3 (P = 0.0124 and 0.0136, respectively), but we observed no association between GNAT3 variation and reported liking of sweet foods or beverages*. While our findings confirm earlier reports that *GNAT3* variants may be associated with liking and intensity of sucrose, we are reminded of RM Pangborn's warning over 3 decades ago that using model systems to generalize to liking and intake of foods "can be an exercise in futility." Additional work is needed to determine if this variant might be able to explain differences in ingestive behavior for real foods and beverages in other cohorts using other methods.

137 **Oral Glucose Sensing In Cephalic Phase Insulin Release**

Alexa J Pullicin, Juyun Lim
Oregon State University, Corvallis, OR, United States

Cephalic phase insulin release (CPIR) is a rapid secretion of insulin following sensory stimulation. Of the numerous sensory inputs that are likely involved in eliciting CPIR, gustatory stimulation, specifically, appears to be particularly important for inducing the response. Most CPIR research targeting gustation has focused on the role of carbohydrates—principally glucose-based saccharides—in eliciting CPIR, and recent work in mice points to oral sensing of free glucose itself as being critical. Notably, more than one mechanism may be involved in sensing glucose and glucose-based saccharides in the oral cavity: glucose transporters, which detect free glucose; the T1R2+T1R3 receptor, which detects glucose as well as other sugars and leads to perceptible sweet taste; and an undefined mechanism that detects certain glucose oligomers. This study was conducted to understand which of these oral carbohydrate sensing mechanisms contribute to CPIR in humans. Four stimuli were designed to target one or more oral carbohydrate sensing mechanism present in the oral cavity. Fasted participants (N=17) each attended four sessions where blood samples were drawn before and after modified sham-feeding with one of the stimuli. Plasma c-peptide, insulin, and glucose concentrations were then analyzed. We found that a 1 M glucose solution elicited CPIR in the group (p <0.05), but that it did not stimulate the response when lactisole—a sweet taste inhibitor—was added. In addition, we found that 22% w/v maltodextrin stimulated CPIR (p <0.05), but that it did not in the presence of lactisole and acarbose—an α-amylase inhibitor. These findings suggest that glucose is important to elicit CPIR in humans, but that sweet taste may also play a role.

139 **Sweet Taste Perception During An Oral Glucose Tolerance Test (Ogtt) Is Associated With Changes In Insulin Secretion And Insulin Clearance In Females With Obesity**

Mariel Molina[1], Clara Salame[2], Blair Rowitz[2,3], M. Yanina Pepino[1,2,3]

[1]Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, Urbana, IL, United States, [2]Division of Nutritional Sciences, University of Illinois at Urbana-Champaign, Urbana, IL, United States, [3]Carle Illinois College of Medicine, Urbana, IL, United States

The overall goals of this study are to test the hypotheses that sweet taste helps regulate postprandial glucose metabolism, and that habitual low-calorie sweetener (LCS) consumption alters these sweet-signaling regulation. To this aim, using a randomized crossover design, 18 habitual (7 males and 11 females) and 14 non-habitual (8 males and 6 females) LCS consumers, all with obesity and none with diabetes, were assessed using 75 g oral glucose tolerance test (OGTT) under three conditions: 1) a control OGTT, 2) an OGTT mixed with lactisole, a broad sweet taste receptor antagonist, and 3) tasting and expectorating sucralose 10 min before an OGTT mixed with lactisole. Indices of β-cell function (insulinogenic index), insulin sensitivity (S_I), and insulin clearance rates were estimated by mathematical models that use plasma glucose, insulin, and C-peptide measurements. We found that inhibiting sweetness had no effect on glucose concentrations in either sex, but in females, it increased insulin concentration, and this effect was ameliorated when tasting sucralose before the OGTT+lactisole (P=0.01). There was a trend for this effect to take place in non-habitual LCS consumers solely (P=0.06). Similarly, only in non-habitual LCS consumers, inhibiting sweetness increased insulin secretion (insulinogenic index) when compared to the control condition and the perception of sweetness before the glucose load increased the overall insulin clearance rate when compared to the OGTT+lactisole (consumption x condition; both P<0.05). There were no significant differences in S_I between LCS groups or within conditions. These data suggest that in obesity, sweet taste plays a role in postprandial glucose responses in females, and that habitual consumption of LCS might disrupt these responses.

141 **Evaluation Of The Validity Of Temporal Sensory Evaluation Methods Carried Out By Consumers On Controlled Stimuli Delivered By A Gustometer.**

Noëlle Béno[1,2], Léna Nicolle[1,2], Michel Visalli[1,2]

[1]Centre des Sciences du Goût et de l'Alimentation, L'Institut Agro, CNRS, INRAE, Université Bourgogne Franche-Comté, Dijon, *, France, [2]CNRS, INRAE, PROBE research infrastructure, ChemoSens facility, Dijon, *, France

Temporal sensory evaluation is increasingly used with consumers. However, very few studies have investigated the repeatability and resolution of the methods used. Thus, this study aimed to fill in the gap by comparing the conclusions drawn from data collected using three temporal sensory methods performed with consumers on time model solutions delivered using a gustometer. One hundred and fifty consumers were recruited. After familiarization and a recognition task, they were divided into 3 panels, each using a different method to describe their temporal perception: Temporal Dominance of Sensations (TDS), Temporal Check-All-That-Apply (TCATA), and Attack-Evolution-Finish (AEF). Fourteen solutions of varying complexity were evaluated, including four evaluated twice. These solutions delivered by the gustometer varied in composition (including 3

to 5 sapid or aromatic compounds: acid, salty, sweet, lemon, and basil), in intensity levels (weak, medium, and strong), and in delivering order. The temporal resolution and the repeatability of the 3 sensory evaluation methods decreased with the complexity of the stimuli. While the sensory perception concurred with the composition of the solutions for simple stimuli, some unexpected results were observed when interactions between compounds occurred. Surprisingly, at the panel level, the conclusions were very similar to TDS and TCATA although they were supposed to measure different concepts (dominance vs. applicability). As expected, TDS and TCATA were superior to AEF in their capacity to highlight temporal changes during the tasting, but as a static method, AEF performed better to detect the presence of the compounds in more complex solutions. These results show that taking into account the temporal dimension in sensory perception is very challenging.

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Endocrine And Sensory Integration In The Caudal Nucleus Of The Solitary Tract Modulates Na^+ Intake In Dehydrated Mice

Caitlin M. Baumer-Harrison[1], Khalid Elsaafien[1], Sagar Patel[1], Karen A. Scott[1], Guillaume de Lartigue[2], Eric G. Krause[1], Annette D. de Kloet[1]

[1]University of Florida, Gainesville, FL, United States, [2]The Monell Center, Philadelphia, PA, United States

Angiotensin-II (AngII) plays a pivotal role in regulating blood pressure (BP), water (H_2O), and sodium (Na^+) homeostasis, by way of acting on angiotensin type 1a receptors (AT1aR) throughout the body. Of particular relevance here, AT1aR are expressed on afferents that transmit cardiovascular or gustatory sensory information to the nucleus of the solitary tract (NTS). We found that optical excitation of these afferents in the caudal NTS mimics perception of increased vascular stretch and induces frequency-dependent alterations in BP and Fos immunoreactivity in brain regions involved in fluid balance, gustatory, and cardiovascular function. Here, we hypothesized that these $AT1aR^+$ afferents are also sufficient and necessary to modulate H_2O and Na^+ intake during alterations in vascular stretch. Mice expressing excitatory or inhibitory opsins in $AT1aR^+$ cells were implanted with fiber optics targeting the NTS. H_2O and NaCl intakes were measured using two-bottle preference tests in the presence and absence of optical stimulation under euhydration and dehydration. Optical excitation (using a frequency found to be sub-threshold for reducing BP) significantly attenuated, while optical inhibition significantly increased Na^+ intake under dehydrated conditions. Optical stimulation did not impact intakes of AT1aR-Cre control mice. Next, $AT1aR^{Flox}$ mice and AAVs (retro serotype) that express of Cre and/or tdTomato were used to probe the function of AT1aR on neurons that innervate the aortic arch. Preliminary results indicate that deletion of AT1aR(s) from aortic afferents alters fluid consumption in the euhydrated condition, with KO mice having a greater preference for NaCl. Collectively, these results imply that NTS $AT1aR^+$ afferents are both necessary and sufficient to modulate Na^+ intake relative to BP status.

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Management Of Chemosensory, Trigeminal And Salivary Dysfunction In Long-Covid Patients

Preet Bano Singh, Åsmund Rogn, Janicke Liaaen Jensen
Institute of Clinical Dentistry, University of Oslo, Oslo, *, Norway

Sudden smell loss is an early symptom of COVID-19, and 10%-20% of patients develop persisting smell disorders. Oral disturbances like loss of taste function, burning sensation, and oral dryness are less studied in Long-COVID patients. The aim of this prospective study was to (i) investigate the prevalence of chemosensory, trigeminal, and salivary dysfunction in Long-COVID patients, and (ii) examine recovery over time, with and without treatment. Long-COVID patients (≥2 months since COVID-19 infection) were recruited among patients referred to Faculty of Dentistry for treatment of persisting chemosensory, trigeminal and salivary disorders. Patients' smell, taste, trigeminal, and salivary status were determined at first consultation day to establish a baseline. Sixty-two patients (41 women, mean age 39.7±12.4 yr) were treated for their symptoms. As a positive control, chemosensory, trigeminal, and salivary status were established for 25 patients (20 women, mean age 42.7±12.0 yr) who had not undergone any treatment since Covid-19 infection (≥9 months since COVID-19 infection). Prevalence of parosmia reduced from 80% to 17.9% and anosmia from 38.7% to 7.7% after treatment. Among untreated patients, parosmia was observed in 88.0% and anosmia in 48.0%. Reduction was found in number of patients complaining of dysgeusia (43,5% to 5,1%), dysesthesia (14,5% to 5,1%), and dry mouth (17,7% to 10,3%), among treated patients. Dysgeusia, dysesthesia, and dry mouth were more prevalent in untreated than treated patients, ≥1yr since COVID-19 infection. These results suggest that Long-COVID patients' chemosensory, trigeminal and salivary functions improved after treatment. Among untreated patients, olfactory and salivary dysfunction were highly prevalent and gustatory and trigeminal dysfunction were less frequent.

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Blockade Of Taste By The P2X2/3 Antagonist Af353: The Path To Increasing Medical Compliance By Briefly And Selectively Blocking Taste

Linda J. Flammer[1], Paul A.S. Breslin[1,2], Natasha Rivers[1], Michael Tordoff[1], Carol M. Christensen[1], Peihua Jiang[1]

[1]Monell Chemical Senses Center, Philadelphia, PA, United States, [2]Department of Nutritional Sciences, Rutgers University, New Brunswick, NJ, United States

Many people, especially children, older adults, and the very ill have difficulty swallowing pills. Therefore, they rely on liquid formulations of life-saving medicines, which are often strongly bitter tasting and aversive. Strong bitter taste can cause failure to comply with medical prescriptions, which threatens the patients' lives. Previous attempts to block bitter taste have focused on inhibiting T2R receptors in the mouth and on cognitive masking by

adding sugar and flavorings. Another potential site of action is downstream where taste receptor cells communicate with primary afferent neurons by releasing adenosine triphosphate (ATP) into the cleft between them. Activation of the taste nerves by ATP is through ionotropic purinoceptors, which are ligand-gated ion channels composed of P2X2 and/or P2X3 subunits. Previous work in mice showed taste blockade when the P2X2 and/or P2X3 receptors were blocked with a purinergic antagonist, Afferent Pharmaceutical 353 (AF-353) [Vandenbeuch, et al. 2015]. We hypothesized that blocking the activation of the P2X2/P2X3 subunits in humans with AF-353 would result in attenuation of bitter taste, as well as sweet, sour, salty, and savory tastes, but not other upper airway sensations such as astringency, irritation, and aroma. We found that rinsing with the P2X2/P2X3 antagonist, AF-353, reduced all taste sensations within five minutes in every human subject tested. Across all subjects, bitterness ratings were reduced from "strong" to nearly "barely detectable." For several subjects there was complete bitter blockade. Interestingly, the degree of blockade for each of the taste qualities varied, with sweetness showing the least and saltiness showing the most. On average, taste recovery began at 10 minutes post-treatment with full recovery occurring in approximately 90 minutes. As predicted, ratings of non-taste oral sensations were unaffected. We believe that short term, reversible blockade of taste purinergic signaling is a means to increase medical compliance in patients who need bitter medicines but cannot swallow pills. In the future, we would like to understand the relationship between concentration of antagonist and time of oral exposure on the degree of bitterness blockade for a variety of life-saving active pharmaceutical ingredients.

151 **New Feasible Test For Clinical Assessment Of Taste And Oral Somatosensory Function &Ndash; The &Lsquo;Seven-Itt&Rsquo;**

Mariano Mastinu[1], Michał Pieniak[1,2], Anne Wolf[1], Tomer Green[3], Antje Hähner[1], Masha Y Niv[3], Thomas Hummel[1]

[1]Smell and Taste Clinic, Department of Otorhinolaryngology, Technical University of Dresden, Dresden, *, Germany, [2]Institute of Psychology, University of Wroclaw, Wroclaw, *, Poland, [3]Institute of Biochemistry, Food Science and Nutrition, The Hebrew University of Jerusalem, Jerusalem, *, Israel

Taste dysfunctions may occur, for example, after viral infection, surgery, medications, or with age. In clinical practice, it is important to assess patients' taste function with rapidity and reliability. Moreover, tactile sensations in the tongue (burning, astringency) are also difficult to evaluate. This study aimed to develop a test that assesses human gustatory sensitivity together with somatosensory functions of astringency and spiciness. A total of 154 healthy subjects from Germany and Israel, and 51 patients with chemosensory dysfunction rated their gustatory sensitivity. They underwent a whole-mouth identification test of 12 filter-paper strips impregnated with low and high concentrations of sweet, sour, salty, bitter (sucrose, citric acid, NaCl, quinine), astringency (tannin), and spiciness (capsaicin). The percentage of correct identifications for high-concentrated sweet and sour, and for low-concentrated salty, bitter and spicy was lower in patients as compared with healthy participants ($\chi^2 \geq 3.94$; $p \leq 0.047$). Interestingly, a lower identification in patients for both astringent concentrations was found ($\chi^2 \geq 10.7$; $p < 0.001$). Based on the results, we proposed the 7-items Taste Test (Seven-iTT) to assess chemo/somatosensory function, with a cut-off of 6 out of 7 (sensitivity: 84.3%, specificity: 51.0%, Cohen's $\varkappa = 0.25$). Mann-Whitney analysis showed that the test score discriminated patients from healthy controls ($p < 0.001$), and showed gender differences among healthy controls ($p < 0.001$). This quantitative test seems to be suitable for routine clinical screening of gustatory and trigeminal function. The present results also provide new evidence on the mutual interaction between the taste and oral somatosensory function.

153 **Determining The Roles Of C-Kit And PdgfrA In Sweet Taste Receptor Cell Homeostasis**

Christina M Piarowski[1,2], Elaine T. Lam[3], Peter J. Dempsey[4], Linda A. Barlow[1,2]

[1]Department of Cell and Developmental Biology, University of Colorado Anschutz Medical Campus, Aurora, CO, United States, [2]Rocky Mountain Taste and Smell Center, University of Colorado Anschutz Medical Campus, Aurora, CO, United States, [3]Department of Medical Oncology, School of Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO, United States, [4]Department of Pediatrics - Developmental Biology, University of Colorado Anschutz Medical Campus, Aurora, CO, United States

The sense of taste is mediated by taste buds on the tongue which each house 50-100 continuously renewing, short-lived taste receptor cells (TRCs) comprising type I cells (glial-like), type II cells (sweet-, bitter- or umami-sensitive), and type III cells (sour-sensitive). Since TRCs continuously turn over, pharmacological agents that impede homeostatic pathways can lead to taste dysfunction, or dysgeusia. Dysgeusia is a common symptom associated with oral chemotherapy drugs called tyrosine kinase inhibitors (TKIs), which inhibit receptor tyrosine kinases (RTKs). Our single-cell RNA sequencing data shows that many RTKs inhibited by TKIs are expressed in different populations of progenitors, precursors, or differentiated TRCs. To evaluate if inhibition of these RTKs could hinder taste homeostasis, we treated lingual organoids with three TKIs that commonly cause dysgeusia (axitinib, cabozantinib and sunitinib). While progenitor cell proliferation was not affected by TKI treatment, TRC homeostasis was altered. Specifically, expression of sweet-cell marker Tas1r2 decreased in response to all three drugs. Axitinib has the fewest targets of the TKIs tested, only inhibiting the taste RTKs c-Kit, PDGFRα and RET. Through additional organoid experiments we have determined RET inhibition is not responsible for the Tas1r2 phenotype, leaving c-Kit and PDGFRα as the primary candidates in contributing to sweet-cell biology. Furthermore, immunohistochemistry and HCR *in-situ* hybridization show both candidates are most highly expressed in sweet-cells. We are currently using more specific inhibitors to determine whether c-Kit, PDGFRα, or both, regulate either sweet-cell differentiation or expression of Tas1r2. We are also treating mice with axitinib to determine if sweet-cell homeostasis is impeded *in-vivo*.

155 **Neuronal Activity And Connectivity Changes That Underlie Memory Acquisition, Consolidation, And Extinction**

Neta Dagan[1], Anan Moran[1,2]

[1]School of Neurobiology, Biochemistry & Biophysics, The George S. Wise Faculty of Life Science, Tel-Aviv University, Tel Aviv, *, Israel, [2]Sagol School of Neuroscience, Tel-Aviv University, Tel Aviv, *, Israel

Memory formation is not an instantaneous event, but rather a dynamic process that progressively evolves across different brain regions. In conditioned taste aversion (CTA) learning, a classical conditioning paradigm in which an association is formed between a palatable taste and malaise, molecular studies suggested the existence of 2 distinct memory phases in the gustatory cortex (GC): an early acquisition phase (2-3 hours following training), followed by a memory consolidation phase about 3 hours later. Recently we showed that distinct neuronal activity changes in the GC occur in relation to these phases: the population response to the conditioned taste changes continuously, its overall magnitude only increases during the acquisition and consolidation phases, and the known quickening of the ensemble-state dynamics appears only after consolidation. These results suggest the existence of rules that govern neuronal network reconfiguration and neuronal taste coding changes that underlie the evolvement of CTA memory, and optionally even during its extinction. To reveal these rules we implanted rats with Neuropixels probes in the GC and neighboring brain regions, and record the continuous activity of hundreds of neurons simultaneously during the acquisition, consolidation, and extinction of CTA. We employed pairwise neuronal cross-correlation-based techniques to characterize the connectivity map between the recorded neurons, and regular single-neuron analyses to portray the coding information of each neuron. Comparing changes in the response patterns of neurons across the learning, as well as the changes in connectivity maps reveal the rules by which memories evolve.

157 **The Presence Of Salivary Proteins Alters Post-Oral Feedback**

Verenice Ascencio Gutierrez[1], Laura E. Martin[3], Samantha L. Brooker[1], Kimberly F. James[1], Ann-Marie Torregrossa[1,2]

[1]Department of Psychology, University at Buffalo, Buffalo, NY, United States, [2]Center for Ingestive Behavior Research, University at Buffalo, Buffalo, NY, United States, [3]Department of Food Science and Technology,Oregon State University, Corvallis, OR, United States

We have demonstrated that a subset of salivary proteins (SPs) upregulate in response to a diet containing the bitter stimulus quinine resulting in decreased bitter taste perception and taste nerve signaling in response to quinine. “Bitter taste” receptors in the oral cavity also line the gut, so we asked: do SPs alter gut feedback? We used 2 paradigms to explore the role of SPs in the gut. In both the animal is given a test solution directly into the gut while licking to a neutral solution (Kool-Aid). When a bitter solution is infused into the gut, animals decrease on-going intake of the neutral solution (within-session suppression) and learn to avoid the neutral solution that was paired with gastric bitter receptor activation (conditioned avoidance). To ask if SPs could modify these behaviors, male Long Evans rats implanted with gastric catheters were trained to lick a bottle of Kool-Aid while simultaneously receiving a gastric infusion. Donor saliva was collected from a separate group of rats treated with isoproterenol and combined into a homogenous sample for gastric delivery of SPs. In the within-session paradigm, there was no difference in total licks to Kool-Aid when animals received either water or SPs alone in the gut ($p=0.70$). Licking was suppressed (compared to water control) when rats were infused with quinine ($p<0.001$); however, when infused with quinine+SPs, licking increased to levels equivalent to control group ($p=0.88$). In the conditioning paradigm, rats showed significant conditioned avoidance of the quinine-paired flavor compared to the water-paired flavor ($p=0.01$). This avoidance was rescued by SPs; rats do not show a conditioned avoidance to the quinine+SPs-paired flavor compared to artificial saliva-paired flavor ($p=0.14$). These data suggest SPs alter post-oral feedback.

159 **A Novel Approach To Investigating Anticipatory Cortical Responses To Taste Associated Cues**

Emma A. Barash, Daniel Å. Svedberg, Adam Weissman, 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 (e.g., the color of a fruit) with a post-consumption outcome (e.g., eating green, unripe fruit made me sick) to guide approach-avoidance decisions. While cue-driven-association research is common, little research focuses on the decision-making which follow food-cues and lead to food consumption/avoidance. To address this gap, we have developed a novel version of a classic go/no-go task, wherein a rat must trigger a cue and then decide whether to retrieve a reward. This framework allows us to probe the anticipation of food advertised by cues in a multisensory setting designed to separate behaviors elicited by cues from those related to consumption. The task pairs audio-visual cues with palatable (sucrose) and aversive (quinine) taste stimuli. We tested whether rats successfully learn cue-taste associations by determining if they differentially respond to cues corresponding to more preferred tastes. Here, we show rats tend to approach only palatable stimuli advertised by the cue, suggesting that cue-taste association was learned. Furthermore, the occasional responses to cues advertising aversive taste were long-latency compared to those advertising palatable taste, suggesting uncertainty about a cue’s meaning may elicit more careful consideration in making the decision. 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.

161 **Cortical Population Dynamics Underlying Learned And Unlearned Aversive Behavior**

Christina Mazzio, Jian-You Lin, Hannah Germaine, Donald B. Katz
Brandeis University, Waltham, MA, United States

The ability to rapidly detect, process, and respond to potentially harmful tastes is important for survival. The most basic of these responses is an easily observable, stereotyped oral 'gape' that serves to push the taste out of the mouth. While gapes can occur in a decerebrated rat, gustatory cortex (GC) is important for the occurrence and timing of gaping *in situ*: neuronal ensemble activity in GC progresses through a series of coherent firing-rate transitions in the 0.2-1.5s following taste delivery, such that firing encodes first taste identity and then hedonic value of the taste (i.e. 'palatability'); the onset of this palatability-related firing directly precedes, and drives, gaping to naturally aversive quinine. Rats also learn to gape to naturally appetitive tastes, which become aversive once paired with gastric malaise in a form of learning called conditioned taste aversion (CTA). While appearing similar in form, evidence suggests that different neural mechanisms may underlie innate and learned gapes. Here, we tested whether GC palatability activity is differentially related to unlearned gaping to quinine and learned gaping to saccharin in rats following CTA. Rats received passive deliveries of 0.1% saccharin via pre-implanted IOCs, and 30min later, were injected with 0.6M LiCl. Twenty-four hrs after this CTA training, the rats were given aliquots of saccharin and novel quinine. During both CTA training and testing, GC activity (via 64-channels electrodes) and mouth movements (via electromyography of the anterior digastric muscle) were simultaneously recorded. Preliminary results show that learned gapes have an earlier onset than unlearned gapes, suggesting that we will also see differences between GC palatability firing preceding unlearned and learned gaping in this ongoing study.

163 **Activation Of Hippocampal Pv Interneurons At Distinct Phases Of Theta Oscillations Stimulates Changes In Phase Amplitude Coupling**

Daniel Ramirez-Gordillo[1,2], Jocelyn Contreras[3], Diego Restrepo[2,4]
[1]Department of Neurosurgery, University of Colorado Anschutz Medical Campus School of Medicine, Aurora, CO, United States, [2]Department of Cell and Developmental Biology, University of Colorado Anschutz Medical Campus School of Medicine, Aurora, CO, United States, [3]Biology department, New Mexico State University , Las Cruces, NM, United States, [4]4Neuroscience Program, University of Colorado Anschutz Medical Campus, Aurora, CO, United States

The balance between excitatory and inhibitory signals in the brain is essential for learning and memory. Interneurons regulate inhibitory and excitatory signals. In particular, parvalbumin (PV) interneurons are fast spiking interneurons that contribute to the occurrence of gamma (65-95 Hz) bursts of neuronal activity at specific phases of theta (6-14 Hz) oscillations (phase-amplitude coupling, or PAC). We asked whether PV interneuron-modulated theta-referenced PAC is involved in learning to discriminate odorants in a go-no go associative learning task where the animal responds to the rewarded odorant by licking on a spout to obtain a water reward. The mice received bilateral optetrode implants targeting hippocampal dorsal CA1. PV interneurons were stimulated at specific phases of the theta local field potential (LFP) using closed loop optogenetics in both hippocampi in PV-Cre mice infected with AAV virus expressing channelrhodopsin 2. We found a significant difference in the strength of PAC when the stimulation occurred at peak or trough of theta. Furthermore, when we surveyed theta phase-referenced power, we found a significant difference between stimulation at peak vs. trough. These results suggest that dorsal CA1 is involved in encoding information in the olfactory go-no go task.

165 **Olfactory Coding In Hippocampal Area Ca2**

Sami I. Hassan, Shivani Bigler, Steven A. Siegelbaum
Columbia University, New York City, NY, United States

Although the hippocampus is crucial for social memory, how social sensory information is incorporated with contextual information to form episodic social memories remains unknown. Here we investigate the mechanisms for social sensory information processing by performing high-resolution calcium imaging from hippocampal neurons in awake head-fixed mice exposed to social and non-social odors. We focus on the CA2 region because of its importance for social memory. We describe for the first time specific and robust social odors representations of individual conspecifics by a subpopulation of CA2 neurons. The representations are flexibly modulated during associative social odor-reward learning, and CA2 activity is important for this learning. Finally, we find that odor representations in CA2 neural activity space contain higher order structure that allows for generalization along categories of reward and social relevance. Thus, our study provides the first evidence that hippocampus encodes and distinguishes complex social odors and their reward associations, providing a likely substrate for social recognition memory.

167 **The Precision Of Olfactory Information Guides Odor Identification And Learning In Humans**

Sam H. Lyons, Paola A. Alicea-Román, Jay A. Gottfried
University of Pennsylvania, Philadelphia, PA, United States

The natural odors we encounter every day are complex, multi-component mixtures that shift dynamically through space and time, face interference from other stimuli, and appear across a wide variety of contexts. The noise that arises from these complex sources of variability poses a significant challenge to the olfactory system, which must reliably identify odors to support robust decision-making and behavior. However, we know very little about if or how the olfactory system might account for such noise during odor identification and learning. We hypothesize that olfactory noise is encoded in a manner consistent with Bayesian inference in which sensory information is weighted according to its precision (inverse uncertainty). Precise information is amplified and

used to update beliefs about odor identity, whereas imprecise information is minimized or ignored. To test this hypothesis, we developed a probabilistic olfactory learning paradigm in which participants were asked to identify the dominant odor in odor mixtures comprised of β-pinene (pine) and isoamyl acetate (banana). Critically, we manipulated the precision of the dominant odor by varying the ratios of the odor mixtures along a continuum from 100% pine to 100% banana. We then used variational Bayesian methods to model each participant's trial-to-trial olfactory uncertainty and belief updating. Consistent with our hypothesis, we discovered that participants encoded information about the precision of the dominant odor and used this information to proportionately update their beliefs about its identity. Overall, these results suggest a precision-weighting mechanism by which odor identification and learning can remain robust in the face of noise and additionally point to a more general Bayesian theory of olfactory inference.

169 **The Impact Of Tubular Striatum Projecting *Drd1* And *Drd2* Positive Amygdala Neurons On Odor Valence**

Sarah E. Sniffen[1], Sang Eun Ryu[1], Graylin M. Skates[1], Milayna M. Kokoska[1], Ellyse Thomas[1], Natalie L. Johnson[1], Andy Chavez[1], Daisy Valle[1], Amanda M. Dossat[1], Minghong Ma[2], Daniel W. Wesson[1]

[1]Depts of Pharmacology & Therapeutics, Neuroscience, & Center for Smell and Taste, Univ of Florida, Gainesville, FL, United States, [2]Dept of Neuroscience, Univ of Pennsylvania, Philadelphia, PA, United States

Odors can be potent triggers of emotional and behavioral responses. The amygdala is well known for its role in both influencing olfactory behavior and for its necessity in establishing learned emotional responses. However, the mechanisms whereby the amygdala influences odor-evoked affective responses remain elusive. Our lab is currently identifying and characterizing tubular striatum (TuS; also known as olfactory tubercle) projecting *drd1* and d*rd2* positive basolateral amygdala (BLA) neurons and have found that these neural pathways are active during odor-fear learning. Here, we used a chemogenetic approach to express DREADDs in both *drd1* and *drd2* positive BLA to TuS pathways by using either D1- or D2-Cre mice, respectively. The influence of these cell types was examined in either a spontaneous or learned assay for odor hedonics by locally infusing the DREADD ligand J60 into the BLA, thereby silencing the activity of TuS projecting BLA *drd1* or *drd2* positive neurons. DREADD ligand infusion in nontransgenic and control AAV injected D1- and D2-Cre mice did not indicate any off-target effects. Ongoing work is resolving the unique contributions of TuS projecting BLA *drd1* and *drd2* positive neurons to odor preference and learning. Given the expression of D1 and D2 receptors on BLA to TuS neurons, additional work is ongoing to investigate the involvement of dopaminergic transmission on the function of these circuits. Overall, these preliminary results contribute to a model whereby Tus projecting BLA d*rd1* and d*rd2* positive neurons contribute to odor learning and odor valence.

173 **Development Of A Nonverbal Odor Assessment Map**

Robert Pellegrino[1], Joshua Nsubuga[1], Matthew Andres[1], Jennifer / E. Margolis[1], Joel / D. Mainland[1,2]

[1]Monell Chemical Senses Center, Philadelphia, PA, United States, [2]University of Pennsylvania, Philadelphia, PA, United States

A key problem in quantifying odors is the lack of a rapid tool for individuals to report odor percept. In this study, we develop an odor display device to represent odors spanning a large perceptual gamut to aid subjects in quantifying odor space. First, using Rate-all-that-apply profiling for over 600 odors, we created a relational 2-dimensional odor map (organized map) such that nearby stimuli are perceptually similar and distant stimuli are perceptually different. Second, we provided subjects with a touch display that is coupled to an olfactometer that generates the odor corresponding to a given location on the map. We demonstrate that individuals are better able to match a target odor to the corresponding location on the map when using an organized odor map rather than a map that randomly assigns a location to an odor (shuffled map). Individuals were able to find an odor more accurately ($F = 2.81$, $p < 0.005$) when using an organized odor map rather than a shuffled map. This novel odor display device allows subjects to nonverbally assess odor quality, which may be useful for describing alterations such as parosmia in clinical settings.

175 **Identification Of Tubular Striatum Projecting *Drd1* And *Drd2* Positive Amygdala Neurons And Their Activity During Odor Learning**

Sang Eun Ryu[1], Sarah E. Sniffen[1], Graylin M. Skates[1], Milayna M. Kokoska[1], Ellyse Thomas[1], Andy Chavez[1], Natalie L. Johnson[1], Daisy Valle[1], Amanda M. Dossat[1], Minghong Ma[2], Daniel W. Wesson[1]

[1]Depts of Pharmacology & Therapeutics, Neuroscience, & Center for Smell and Taste, Univ of Florida, Gainesville, FL, United States, [2]Dept of Neuroscience, Univ of Pennsylvania, Philadelphia, PA, United States

Odors are potent triggers of emotional responses. The amygdala is well known for its role in both influencing olfactory behavior and for its necessity in establishing learned emotional responses. However, the mechanisms whereby the amygdala influences odor-evoked affective responses remain elusive. Using a combination of anterograde and retrograde viral tracing strategies, we identified two distinct populations of basolateral amygdala (BLA) projection neurons expressing *drd1* or *drd2* genes specifically in the basal nucleus of the BLA, which innervate the tubular striatum (TuS; also known as the olfactory tubercle). In D1- and D2-Cre mice, we found that both cell types synapse throughout the entire span of the TuS, but three times more *drd1* neurons innervate the TuS than *drd2* neurons. Furthermore, while the BLA comprises both GABAergic and glutamatergic neurons that can project to regions outside of the BLA, we found through both RNAscope and immunohistochemistry that these TuS projecting BLA neurons appear to be solely glutamatergic. Since work by several groups, including ours, has established the TuS as a key region for linking odor information with learned responses, we next investigated the activation of these neurons during odor learning. We used cell-type specific fiber photometry of TuS projecting BLA neurons expressing GCaMP8m and found that TuS projecting BLA neurons

are activated during Pavlovian odor fear learning. The results contribute to a better understanding whereby the brain learns to associate odors with outcomes.

177 **The Role Of Parvalbumin And Somatostatin Interneurons In Assembly Formation In Piriform Cortex During Odor Discrimination Training**

F. Kathryn Friason[1], Changyu Sun[2], Anne-Marie M. Oswald[1,2]

[1]University of Pittsburgh, Pittsburgh, PA, United States, [2]University of Chicago, Chicago, IL, United States

In the piriform cortex, odors are represented by distributed ensembles of neurons. A long-held hypothesis is that ensemble representations are stabilized by excitatory synaptic plasticity to form odor assemblies whose activity signals the correct odor identity even for degraded or altered stimuli. The role of inhibitory plasticity in this process has yet to be elucidated. We have previously found that following odor discrimination training, excitatory and inhibitory synapses are strengthened within ensembles of neurons co-activated by the rewarded stimulus. Moreover, enhanced inhibition scales with enhanced excitation. However, excitation remains weak within the unrewarded ensemble. In this study, we used Fos^{ERT} mice to fluorescently label active pyramidal neuron ensembles in an odor discrimination paradigm. We then investigated the strength of inhibitory connections from Parvalbumin or Somatostatin interneurons onto pyramidal neurons within these ensembles *in vitro*. For ensembles that responded to the rewarded odor, inhibition from Parvalbumin interneurons tends to be stronger than inhibition from Somatostatin interneurons. This suggests that Parvalbumin inhibition scales with excitation. Following this, we hypothesize that parvalbumin inhibition will be weaker onto the unrewarded ensemble consistent with weaker excitation. We further hypothesize that somatostatin inhibition will be stronger onto the unrewarded ensemble. This would be consistent with the role of Somatostatin interneurons mediating dendritic inhibition and preventing excitatory enhancement. Ongoing experiments are addressing these hypotheses.

179 **Sleep Stage-Dependent Coherence In Olfactory And Hippocampal Oscillations**

Rui He[1,2], Abigail E. Stuart[2], Leslie M. Kay[1,2]

[1]Department of Psychology, University of Chicago, Chicago, IL, United States, [2]Institute for Mind and Biology, University of Chicago, Chicago, IL, United States

Sleep staging is often used to characterize post-learning consolidation in hippocampal and neocortical activity. Olfactory system sleep analysis has been primarily confined to anesthetized pseudo-sleep, which may not be equivalent to physiological sleep. To understand the system-wide neural coordination and memory consolidation of olfactory learning during sleep, we recorded local field potentials from ipsilateral olfactory bulb (OB), piriform cortex, olfactory tubercle, hippocampus, and nasal respiration from 8 freely-behaving rats (4F) during 90-minute sessions before and after odor discrimination learning. Sleep stages were identified using the OB gamma (60-100Hz) power and hippocampal theta (5-10Hz) to delta (1.5-4Hz) ratio and verified with video coding. Respiration frequency is strongly coherent with OB oscillations during sleep and alternates between low-frequency breathing during slow wave sleep (2-4Hz) and waking-like fast-frequency sniffing during rapid-eye-movement sleep (REM; 6-8Hz). Fast sniffing entrains olfactory and hippocampal oscillations during waking and REM and engages the same network seen in olfactory learning in these rats. Comparisons between the network pre- and post-learning allow us to track memory consolidation reflected in neural connectivity.

181 **The Relationship Between Taste Perception And Affect**

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

[1]Brown University, Providence, RI, United States, [2]Le Moyne College, Syracuse, NY, United States, [3]Le Moyne College, Syracuse, NY, United States, [4]Le Moyne College, Syracuse, NY, United States

Past literature on taste perception has reported that tasting a sweet food or drink increases agreeableness (Meier et al., 2012) and that tasting bitter substances increases hostility (Eskine et al., 2011; Saigioglu & Greietmeier, 2014). However, our recent work (Herz, Cunningham, & White, 2022) did not find any support for the effects of taste on self-rated affect. As this result was unexpected, we are now conducting a detailed study to explore and expand research in this area. In our current pre-registered study, participants are given a cover story to disguise the true experimental purpose and asked to report both their hunger level and when they last ate. Participants then taste a substance randomly selected from the six experimental tastants (2 bitter, 2 sweet and 2 neutral) and complete both the agreeableness and hostility questionnaires that have been previously examined in past research (counterbalanced across participants). Participants then rate all 6 tastants for pleasantness, familiarity, intensity, sweetness, sourness, bitterness, saltiness, and liking on VAS scales. Finally, participants' taster status is assessed using the gLMS and 6-n-propylthiouracil. Data collection is ongoing and to date we have only examined main taste effects on a subset of participants. Current results from 46 participants suggest a replication of our prior null finding between sweet and bitter taste perception and self-rated agreeableness and hostility, and challenge the literature reporting effects of basic taste perception on affect.

183 **Time Frequency Analysis To Characterize Odor-Induced Taste Enhancement (Oite) In People With Normal-Weight Or Living With Obesity**

Shirley X. L. Lim, Christopher Aveline, Charlotte Sinding
Centre des Sciences du Goût et de l'Alimentation, CNRS, INRAE, Institut Agro, Université de Bourgogne Franche-Comté, F-21000 Dijon, France, Dijon, *, France

Flavour perception is the integration of taste and odour which can produce a phenomenon known as odour-

induced taste effect (OITE). Chemosensory event-related potential (CERP) has been employed to understand the underlying brain mechanism of OITE. However, CERP often exhibit low signal-to-noise ratio (SNR) caused by significant amount of trial-to-trial temporal jitter due to methodological challenges of chemosensory stimulation. Moreover, for in-mouth flavour stimulation, aroma (the odour component of flavour) may not be time-locked to the onset of the flavour stimulation. Therefore, here we proposed to probe the brains' responses of OITE using time-frequency analysis (TF) approach because it can measure activity that are both phase-locked and non-phase-locked. In this EEG experiment, 33 normal weight and 28 obese participants evaluated the intensity of three solutions, i.e., odour (vanillin), taste (sucrose) and flavour (vanillin + sucrose). All solutions are dissolved in water. Behavioural results showed significant OITE effect within NW and OB but this effect is not significantly different between groups. For participants living with obesity, preliminary EEG results showed event-related synchronization (ERS) activity in the delta band (1Hz-4Hz) for taste, odour and flavour, across all electrodes. Importantly, we found that ERS (delta band) for flavour begins at 200ms after stimulus onset but for OITE, event-related desynchronization (ERD) activity begins at 400ms (Cz electrode). This preliminary result suggests that although the processing of flavour information starts earlier, the OITE induced by the integration between taste and odour is a late brain processing phenomenon. The EEG data for OB group is currently still under analysis.

185 **Event-Related Potential Analysis Of The Brain Integration Of Odor And Taste In Normal-Weight And People Living With Obesity**

Christopher Aveline[1], Maud Blouri[1], Anaïs Guilbert[1], Damien Gabriel[2,3], Thierry Thomas-Danguin[1], Charlotte Sinding[1]

[1]Centre des Sciences du Goût et de l'Alimentation, INRAE, Université Bourgogne Franche-Comté, CNRS, Institut Agro Dijon, F-21000, Dijon, *, France, [2]Laboratoire de Recherches Intégratives en Neurosciences et Psychologie Cognitive (LINC), Université de Franche-Comté, Besançon, *, France, [3]Hôpital Universitaire CHRU, Besançon, *, France

An odor can acquire a taste property, following the brain integration of odor and taste, that results in a taste enhancement. We aimed to highlight the brain chronometry of this phenomenon called Odor-Induced Taste Enhancement (OITE) in people living with normal weight (NW) or obesity (OB). We recorded the brain activity of 33 NW and 28 OB, while they were stimulated in-mouth. An apple juice (Aj) or a salty water (Wsa) bases were declining in three conditions: base with sucrose or NaCl (*taste*), base with vanillin or bacon odorant (*odor*) and base with tastant and odorants (*flavor*). Event-Related Potential (ERP) was used to measure the brain chronometry of the OITE, while source localization revealed the brain areas involved in OITE processing. We hypothesized that OITE would occur in the late brain processing of odor and taste (P3 peak) and would involve higher processing brain areas. Furthermore, the P3 peak latency would be increased in both groups and amplitude would be increased in OB only. Concerning the source localization, we hypothesized that high level areas would be activated to integrate *odor* and *taste*. Our results showed a delayed P3 peak as a correlate of OITE in both populations. Moreover, OB presented higher P3 amplitude than NW participants. The source localization analysis showed an activation of the amygdala, the parahippocampus and the anterior cingulate gyrus only in OB. Moreover, we found that the anterior insula and the frontal operculum was activated during the late processing of flavor for both group indicating possibly a feedback loop that may have increased the activity of the gustatory cortex and allowed OITE. These results are in line with the classical view of odor/taste integration.

187 **The Psychological Value Of The Senses Smell And Taste**

Jonas Y Junge[1], Rachel Herz[2], Martha Bajec[3], Michelle Niedziela[3], Valentina Parma[1]

[1]Monell Chemical Senses Center, Philadelphia, PA, United States, [2]Brown University, Providence, RI, United States, [3]HCD Research, Flemington, NJ, United States

In Western tradition, smell is typically perceived as the least important of the senses. This belief persists even if there is little actual research on the psychological significance of smell and taste. Herz & Bajec (2022) studied how US college students and older adults perceived the value of smell as compared to hearing and sight and to items representing digital (e.g., phone), material (e.g., money), personal (e.g., pet) and physical commodities (e.g., hair) of varying social and emotional meaningfulness. Here we preregistered a replication and extension of that study to assess not only the perceived value of smell but also the perceived value of taste against other senses and commodities. To improve generalizability of observations, we included 1138 participants (age range 18-85 years old, 50.3% women) from 22 countries across 6 continents. By using generalized linear mixed models (GLMM) with binomial distribution and logit link functions, we confirmed that respondents preferred losing their sense of smell and taste over hearing and vision, with smell and taste being equivalent. This hierarchy is not representative of all countries (e.g., Thailand) where the value of vision is reported to be lower than hearing and taste. Overall, participants value all senses, including smell and taste, as more important than other body parts (i.e., little left toe). The relative value of smell and taste was not influenced by the age of the respondents, though age influenced the importance of tech-related commodities (greater in <28 years old vs. 70-85 years old). Overall, women and men similarly valued smell and taste. These results confirm that, despite widespread coverage of the chemical senses during the COVID-19 pandemic, the value of smell and taste remains underappreciated across the globe.

189 **Human Olfactory Navigation Recruits Grid-Like Representations In Entorhinal And Piriform Cortices**

Clara U. Raithel[1,2], Alexander J. Miller[2], Russell A. Epstein[1], Thorsten Kahnt[3,4], Jay A. Gottfried[1,2,4]

[1]Department of Psychology, University of Pennsylvania, Philadelphia, PA, United States, [2]Department of

Neurology, University of Pennsylvania, Philadelphia, PA, United States, [3]National Institute on Drug Abuse, Intramural Research Program, Baltimore, MD, United States, [4]Department of Neurology, Northwestern University, Chicago, IL, United States

Olfactory navigation is observed extensively across the animal kingdom. Humans, however, have rarely been considered in this context. In our experiment, we used a combination of olfactometry techniques, Virtual Reality (VR) software applications and neuroimaging methods to investigate whether humans can navigate an olfactory landscape by learning about the spatial relationships among discrete odor cues and integrating this knowledge into a spatial map. Our data show that, over the course of the experiment, participants improved their performance on the odor navigation task, i.e., took more direct paths toward the target, and completed more trials within a given time period. This suggests that humans can successfully navigate a complex odorous environment, reinforcing the notion of olfactory navigation in humans. Functional Magnetic Resonance Imaging (fMRI) data collected during olfactory navigation revealed the presence of grid-like representations in entorhinal and piriform cortices that were attuned to the same grid orientation. This result implies the existence of a functional grid network relying on olfactory cues to guide spatial navigation. In next steps, we plan to examine the interactions between olfactory landmarks and other sensory (e.g., visual) inputs as well as reward to gain a better understanding of spatial navigation at both the neural and behavioral levels.

191 **Olfaction And Verbal Memory In Aging: A Meta-Analysis**

Benoit Jobin[1,2,3], Frederique Roy-Cote[4], Benjamin Boller[1,3], Johannes Frasnelli[2,5]
[1]Université du Québec à Trois-Rivières, Department of Psychology, Trois-Rivières, QC, Canada, [2]Research Centre of the Hôpital du Sacré-Cœur de Montréal, Montréal, QC, Canada, [3]Research Centre of the Institut universitaire de gériatrie de Montréal, Montréal, QC, Canada, [4]Université du Québec à Montréal, Department of Psychology, Montréal, QC, Canada, [5]Université du Québec à Trois-Rivières, Department of Anatomy, Trois-Rivières, QC, Canada

Olfaction and memory are intimately associated. Both functions involve limbic and orbitofrontal areas; both decline in aging and even more so in neurodegenerative diseases such as Alzheimer's disease. Several studies have investigated the association between performance in verbal memory tasks and olfactory identification and/or detection threshold scores. We summarize these studies and verify the association between two distinct olfactory tasks, namely olfactory identification and olfactory detection threshold with episodic and semantic memory performance in cognitively unimpaired older adults. We searched for published studies in the following databases: PsychNet, PubMed, and Academic Search Complete (Ebsco). We included articles according to the following criteria: 1) inclusion of cognitively unimpaired older adults; 2) assessment of verbal memory (episodic or semantic); 3) assessment of olfactory identification or detection threshold. Olfactory identification was associated with episodic memory (small effect size: $r = 0.19$, 95% CI [0.13, 0.25]; $k = 22$) and semantic memory (small effect size: $r = 0.16$, 95% CI [0.09, 0.22]; $k = 23$). Both effect sizes were significantly heterogeneous. Similarly, olfactory detection threshold was associated with both episodic memory (small to medium effect size: $r = 0.25$, 95% CI [0.01, 0.45]; $k = 5$) and semantic memory (small effect size: $r = 0.17$, 95% CI [0.04, 0.29]; $k = 7$). Both effect sizes were significantly homogenous. Age was not a significant moderator in any meta-analysis. In cognitively unimpaired older adults, olfactory identification and detection threshold functions are both similarly slightly related to verbal memory performance. In contrast with previous reports, olfactory detection threshold is related to cognitive performance in older adults.

193 **Odor Stimuli Are Separable By Ratings Of Identity, Pleasantness, And Edibility**

Sarah Cormiea, Gulce Dikecligil, Jay Gottfried
University of Pennsylvania, Philadelphia, PA, United States

Odors are not static stimuli; they unfold over time. Previous research in human olfaction has sought to understand the salient perceptual features that differentiate odors as well as the time-course over which such features arise in behavior and brain activity. Here, we devised an odor rating task to measure how people perceive and identify odors. Resultant odor ratings provide a framework to decipher neural responses to odors in a time-resolved manner. On each trial, participants were presented with one of eight real-world odors (e.g., cheese, dirt, cake, shampoo) and asked to evaluate it. Odors were rated on one of three feature dimensions, which were presented randomly on a trial-by-trial basis: (i) pleasantness, (ii) edibility, or (iii) identity. Behavioral results reveal that participants reliably differentiate between pleasant and unpleasant odors as well as between edible and inedible odors. When asked to endorse the true label of an odor versus an incorrect foil label, participants overwhelmingly chose the true label. Past research has shown that odor identity is decodable from intracranial EEG activity recorded in piriform cortex. Participants in the present study performed behavioral tasks while their brain activity was being simultaneously recorded via surgically implanted electrodes, which were placed as part of treatment plan for intractable epilepsy. We will take advantage of the high temporal resolution of intracranial EEG to investigate how odor-related brain activity evolves during perception, memory, and evaluation of odor stimuli. Behavioral results have already shown that odor features such as valence, edibility, and identity are separable from stimulus ratings alone. Future analyses will reveal when such relationships between stimuli arise and how they unfold over time.

195 **Breathing As An Underlying Rhythm Of Human Sleep-Related Cortical Slow Waves And Spindles**

Justin Morgenthaler[1], Andrew Sheriff[1], Christopher Cyr[1], Guangyu Zhou[1], Navid Shadlou[1], Julia Jamka[1], Joshua Rosenow[2], Stephan Schuele[1], Gregory Lane[1], Christina Zelano[1]
[1]Department of Neurology, Northwestern University Feinberg School of Medicine, Chicago, IL, United States,

[2]Department of Neurosurgery, Northwestern University Feinberg School of Medicine, Chicago, IL, United States

During sleep, brain rhythms play an active role in organizing and strengthening our memories. Cortical slow waves and sleep spindles are two prominent brain rhythms that facilitate memory consolidation during sleep. A large body of literature has shown that sleep spindles are coupled to cortical slow waves and up/down states, which are coupled to hippocampal ripples associated with memory replay. Together, these nested rhythms—up/down states, slow waves, spindles and ripples—coordinate a complex interplay between the cortex, hippocampus and thalamus to organize memory consolidation during sleep. At the same time, a growing body of literature has shown that nasal breathing drives neural oscillations in the olfactory epithelium, olfactory bulb, piriform cortex, and other limbic and neocortical areas. Respiratory behavior has also been shown to impact both memory performance and sleep. Here we explore the relationship between breathing rhythms, sleep stages, up/down states, and spindles to test several hypotheses. First, we hypothesize that respiratory waveforms differ across sleep stages including N1, N2, N3 and REM. Second, we hypothesize that slow waves shift in phase to align with breathing during N2 and N3 sleep. Third, we hypothesize that during N2 and N3, respiration-aligned slow waves preferentially include spindle activity over non-aligned slow waves. Taken together, we will test the overarching idea that respiration is an organizational rhythm underlying nested slow waves, up/down states and spindles during sleep, which are critical for memory consolidation. These findings may shed light on the importance and impact of breathing on memory consolidation during sleep in humans.

197 **The Cognition Of Olfaction - Characterizing Structural Mri Correlates Of Odor Identification**

Mahmoud Omidbeigi, Michael Chodakiewitz, Saeed Ghodsi, Ausaf Bari
University of California Los Angeles, Los Angeles, CA, United States

The ability to identify odors has been reported to be closely related to various domains of general cognitive function in healthy subjects. However, the brain structures and pathways contributing to odor identification (OI), as well as their relationships with cognition in healthy subjects, are not fully understood. Here, we aim to characterize the neuroanatomical correlates of OI test performance in healthy individuals. Diffusion and structural MRI scans were obtained from 586 healthy subjects sampled from the Human Connectome database. Surface-based morphometry analysis was performed for two morphometric measures: gyrification and cortical thickness. Using probabilistic whole-brain tractography, a network-based statistics approach was used to identify subnetworks with significant correlation with OI scores. We found that the shape and thickness of piriform cortex and right medial orbitofrontal cortex—including the subcallosal region, gyrus rectus, and olfactory sulcus—were correlated with OI scores. Connectivity within two subnetworks in the left hemisphere was significantly associated with OI performance ($P<.05$): The first subnetwork included the subcallosal region, amygdala, thalamus, and superior temporal sulcus, and second included the calcarine sulcus, superior occipital gyrus, parieto-occipital sulcus, and lingual sulcus. Our results also indicate that OI performance is associated with better semantic knowledge ($P<.001$). In conclusion, we found large-scale cortical and subcortical networks associated with OI performance. Some of these were in the secondary olfactory cortex, whereas others were in areas not traditionally associated with olfactory function. Our findings suggests that in addition to olfaction, the OI test measures cognitive performance subserved by a distributed brain network.

199 **Olfactory Oddball Detection: The Role Of Left Temporoparietal Junction**

Prasanna Karunanayaka[1], Rommy Elyan[1], Biyar Ahmed[1], Jian-li Wang Wang[1], Ran Pang[3], Sangam Kanekar[1], William Jens[2], Deepak Kalra[2], Paul Eslinger[2], Qing Yang[3]
[1]Department of Radiology, Penn State University College of Medicine, Hershey, PA, United States, [2]Department of Neurology, Penn State University College of Medicine, Hershey, PA, United States, [3]Department of Neurosurgery, Penn State University College of , Hershey, PA, United States

Introduction: The neurobiological basis of odor-identification remains unknown. We hypothesized that match and mismatch tasks in the olfactory domain could be used to isolate areas of odor-identification. When we combined functional magnetic resonance imaging (fMRI) with an olfactory oddball detection task, we found strong left temporoparietal junction (TPJ) activation. This region is heavily involved in match/mismatch activity. *Methods*: Eighteen subjects (mean age=28.5; 10 F) took part in the study. Subjects completed two pseudorandomized and counterbalanced runs of the task on a Siemens 3T MRI system. The tasks required subjects to press a button with their right index finger to identify the oddball: coffee, from a list of distractors. A T2*-weighted EPI sequence was used for fMRI image acquisition. A T1-weighted MPRAGE image was acquired for anatomical overlay. *Results*: A group general linear model in SPM 12 detected activity in olfactory structures for all odorants. When coffee was contrasted with distractors, the left TPJ was significantly activated. A generalized psychophysiological interaction (gPPI) analysis of the left TPJ revealed higher connectivity for oddball, with the left middle and frontal gyri, insula, frontal superior medial gyrus, cuneus, and frontal inferior gyrus lateralized to the left hemisphere; the precuneus, and calcarine fissure lateralized to the right. *Conclusion*: The novel finding of our study is the activity in left TPJ during olfactory oddball detection. This finding is consistent with the hypothesis that the left TPJ encodes matches between expected and actual sensory, motor, or cognitive stimuli. Our paradigm adds critical information to a seldom studied modality: olfaction; it may also provide a basis by which a neurocognitive model for odor-identification can be developed.

8:00 - 10:00 PM	Calusa EFGH
Polak Award Lectures	

Chair(s): Emily Liman and Robert Datta

8:00 **Characterization Of Gastrin-Releasing Peptide In Neurons Of The Gustatory Cortex**

Lindsey A Czarnecki[1], John Chen[1,2], Olivia K Swanson[1,2], Siddarth Swaminathan[1], Arianna Maffei[1,2], Alfredo Fontanini[1,2]

[1]Stony Brook University Deparmtent of Neurobiology & Behavior, SUNY Stony Brook, Stony Brook, NY, United States, [2]Graduate Program in Neuroscience, SUNY Stony Brook, Stony Brook, NY, United States

Gastrin-releasing peptide (GRP) has been tied to satiety both by peripheral and central action. Central administration of GRP reduces food intake and extends intermeal intervals in animal models. However, the neural mechanisms responsible for meal regulation are not understood. Here, we show that GRP is expressed in ~5% of neurons in GC. It is most prominently expressed in more superficial neurons where the density of GRP positive cells reaches ~15%. Whole cell patch clamp recordings show intrinsic properties consistent with these cells being excitatory neurons. Using anterograde viral tracing, we observe a dense projection of GRP positive GC neurons to the basolateral amygdala. Consistent with previous reports, bath application of GRP in acute slices induces an increase in inhibitory events. To explore the coding properties of GRP-expressing GC neurons, we imaged their activity in mice licking for multiple tastants. Preliminary data suggest that a larger proportion of GRP-expressing neurons are modulated by Ensure consumption compared to neurons not expressing GRP. We then used Ensure to further explore the putative role of GC GRP neurons in satiety. We hypothesized that GC neurons may, at least in part, underlie the pharmacological effects of GRP on food intake. To test this hypothesis, we expressed channelrhodopsin-2 in GRP-expressing cells in GC. Food restricted animals were trained to make instrumental licks to a spout in order to receive an 8uL drop of Ensure. On test days, animals experienced a laser burst stimulation paradigm in alternating blocks of trials. Preliminary data show that animals make fewer instrumental responses and consume less Ensure on laser stimulation days. Altogether, these results demonstrate the existence of GRP-expressing neurons in GC, show their propensity to respond to Ensure and their potential role in mediating a reduction of food intake.

8:20 **Piriform Cortex Takes Sides: Temporally-Segregated Odor Representations From Ipsilateral And Contralateral Nostrils Within A Single Sniff**

Naz Dikecligil[1], Andrew I Yang[2], Nisha Sanghani[1], Kathryn A Davis[1], Jay A Gottfried[1]

[1]Department of Neurology, University of Pennsylvania, Philadelphia, PA, United States, [2]Department of Neurosurgery, University of Pennsylvania , Philadelphia, PA, United States

Olfactory epithelia in the left and right nostrils, isolated from one another by the nasal septum, each receive a snapshot of the sensory world upon inhalation. While olfactory information arising from these two channels ultimately results in a unified odor percept, studies across animals and humans have shown that internostril differences can nonetheless inform and shape behavior. These findings suggest that the olfactory system can both integrate and segregate odor information arising from the two nostrils. However, relatively little is known about how information from the two nostrils is integrated and differentiated in the human olfactory system. Specifically, if piriform cortex (PC), a region critical for encoding odor identity information, favors integration or segregation of odor information across the two nostrils, remains poorly understood. To address this question, we recorded intracranial EEG signals directly from PC while human subjects participated in an odor identification task where odors were delivered to the left, right, or both nostrils via a computer controlled olfactometer. We analyzed the time-course of odor-identity coding using machine learning approaches, and found that odor inputs from the ipsilateral nostril are encoded hundreds of miliseconds faster than odor inputs from the contralateral nostril. This temporal staggering across the nostrils gave rise to two non-overlapping epochs of odor coding within a single sniff when odors were sampled through both nostrils. Thus, our findings reveal that PC maintains distinct representations from each nostril by temporally segregating odor information, highlighting an olfactory coding scheme that can parse odor information across nostrils within a single sniff.

8:40 **Sensorimotor Prediction Errors In The Mouse Olfactory Cortex**

Priyanka Gupta[1], Marie Dussauze[1,2], Uri Livneh[1], Dinu F Albeanu[1,2]

[1]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, United States, [2]Cold Spring Harbor School of Biological Sciences, Cold Spring Harbor, NY, United States

During behavior, sensation and action are in closed-loop. Through experience, the brain may learn the reciprocal relationship between sensory inputs and movements to build internal models that predict the sensory consequences of upcoming actions (sensorimotor predictions). While odor sampling is tightly coupled to motor actions, the effect of movements on odor representations has rarely been analyzed. We hypothesize that, in closed-loop olfaction, mice predict the sensory consequences of their actions (next most probable odor input). Movement related predictions of expected odor input get compared with current odor input within olfactory cortex to represent olfacto-motor prediction errors. To test this, we developed a behavioral task where head-fixed mice are trained to steer the left-right location of an odor source by controlling a lever with their forepaws. In this manner, we link movement to well-defined sensory expectations (odor location) and subsequently violate the learnt expectations via online sensory feedback perturbations in expert animals. Strikingly, *mice readily counter brief sensorimotor perturbations, by making precise corrective movements that provide a read-out of their*

individually learnt sensorimotor predictions. Importantly, cortical odor representations are strongly re-shaped by olfacto-motor expectations. Transient perturbations often trigger responses that are stronger than those evoked by any other task variable. Our results suggest that olfactory cortex computes sensorimotor prediction errors by integrating sensory information with movement-related predictions, presumably relayed via top-down feedback. Using cell-type analysis and activity manipulations, we are currently identifying the circuit elements that facilitate the comparison of olfactory inputs with predictions.

9:00 **Neural And Algorithmic Bases Of Odor Guided Trail Following In Mice**

Siddharth Jayakumar[1,2], William Tong[1,2], Gautam Reddy[1,2,3], Alexander Mathis[1,2,4], Venkatesh N. Murthy[1,2]
[1]MCB, Harvard University, Cambridge, MA, United States, [2]CBS,Harvard University, Cambridge, MA, United States, [3]NTT Research Inc, Sunnyvale, CA, United States, [4]École Polytechnique Fédérale de Lausanne, Lausanne, *, Switzerland

Animals actively sense the environment to acquire features of interest. An everyday example of active sensing behavior is our use of saccadic eye movements for scene recognition. Many behaviors in rodents are guided by odor cues, and active modulation of sniffing is likely to play an important role. For example, during trail following, an animal actively modulates sniffing and uses features beyond odor concentrations to successfully follow and reach a destination. The strategies adopted by rodents to execute such flexible yet precise odor-guided behavior are poorly understood. Here, we use an “infinite” paper treadmill with dynamic odor trails that challenge mice to continuously navigate with high precision. By combining high speed videography with markerless tracking we estimate mouse and trail positions with high accuracy. We corroborated previous findings that occluding a nostril biases trail following. Although mice predominantly cast while tracking, they also use other strategies for trail following such as biased searches based on previous encounters with the trail. To address the neural basis of trail-following, we chose to study the AON as it has privileged access to information from both nostrils. Targeted unilateral chemogenetic perturbation in AON activity leads to lateralized deficits in tracking. We also recapitulated trail following behavior in a neural-network-based reinforcement learning (RL) framework, where an agent is tasked with finding a reward at the end of a stochastically meandering odor trail. Further examination of how agent behavior is affected by virtual perturbations will provide insights into mechanisms that drive robust navigation. In summary, we aim to contrast mouse behavior and RL-agent based simulations to unveil strategies relevant to trail following.

9:20 **A Map Of Ionotropic Receptors In The Mosquito Olfactory Appendage**

Joshua Raji, Christopher Potter
Johns Hopkins University, Baltimore, MD, United States

The mosquito antenna is the main olfactory appendage for detecting volatile chemical cues from the environment. A map of Ionotropic Receptors (IRs) expression and the functional contributions of odor-specific tuning IRs are largely elusive in the malaria mosquito. Whole mount fluorescence in situ hybridization of IRs expressed in the antennae revealed that the antenna might be divisible into proximal and distal functional domains. The number of IR-positive cells were stereotyped within each antennal segment and sexually dimorphic. Highly expressed odor-tuning IRs exhibit distinct co-localization patterns with the IR coreceptors Ir8a, Ir25a, and Ir76b that might predict their functional properties. IR neuron populations are not changed after blood meal or in aged adults. CRISPR genetic knock-in captured IR41c endogenous expression pattern which faithfully recapitulates IR41c probe-labeled cells. Neurogenetic labeling revealed IR41c neurons innervate a distinct ventral-posterior glomerulus in the antennal lobe. In vivo functional imaging of IR41c neurons indicated both odor-induced activation and inhibition in response to select amine compounds. Targeted mutagenesis of IR41c did not abolish behavioral responses to the amine compounds. Our study provides a comprehensive map of IR-expressing neurons in the main olfactory appendage of mosquitoes. These findings reveal previously unknown organizing principles of IR-expressing neurons in malaria mosquito which might underlie their functional contribution to the detection of behaviorally relevant odors.

9:40 **Genetic Ablation Of Lgr5^{+} Taste Progenitor Cells Reveals A Novel Mechanism Of Taste Bud Regeneration Following Injury.**

Sushan Zhang[1, 2], Jennnifer.K Scott[1, 2], Eric.D Larson[1, 2, 3], Linda.A Barlow[1, 2]
[1]Department of Cell & Developmental Biology, University of Colorado Anschutz Medical Campus, Aurora, CO, United States, [2]The Rocky Mountain Taste and Smell Center, University of Colorado Anschutz Medical Campus, Aurora, CO, United States, [3]Department of Otolaryngology – Head and Neck Surgery, University of Colorado Anschutz Medical Campus, Aurora, CO, United States

The circumvallate taste papilla (CVP) of rodents contains hundreds of taste buds, each containing ~60 type I, II and III taste receptor cells (TRCs). All TRCs are renewed steadily from adult stem cells, which express LGR5 and/or GLI1 (10.1002/stem.1338; 10.1016/j.ydbio.2013.07.022). To test the requirement for LGR5^{+} progenitors in taste homeostasis, *Lgr5$^{DTR\text{-}mGFP}$* mice were treated with Diphtheria Toxin (DT) to kill LGR5^{+} cells, and CVP taste epithelium assessed at progressive times. At 24hr post-DT, LGR5-GFP^{+} cells were successfully ablated from CVP epithelium, but small numbers of LGR5-GFP^{+} cells were already evident at 48hr. Taste buds were also ablated at 24hr, while sparse cytokeratin (KRT8) $^{+}$ immature TRCs were detected at 48hr. LGR5-GFP^{+} cells and small KRT8^{+} TRC clusters that also expressed type I, II and III TRC markers rapidly increased over the first week, with gradual recovery up to control metrics at 2 weeks post-LGR5^{+} cell ablation. As LGR5-GFP^{+} and

KRT8^{+} taste cells reappeared coincidentally and rapidly, we posited GLI1^{+} cells were responsible for this rapid regeneration process. To test this, *Gli1*^{CreER};*Rosa*^{tdTomato};*Lgr5*$^{DTR\text{-}mGFP}$ mice were given tamoxifen to lineage trace GLI1^{+} cells, and then treated with DT to ablate LGR5^{+} progenitors. At 24hr, GLI1-Tomato^{+} cells were abundant in regenerating CVP epithelium indicating GLI1-derived cells were activated following LGR5^{+} cell ablation. At 72hr, many LGR5-GFP^{+} cells and KRT8^{+} TRCs were GLI1-Tomato^{+} indicating GLI1-derived cells rapidly regenerated both populations. In sum, ablation of LGR5^{+} cells triggers a rapid and novel regenerative response in taste epithelium. We are now comparing transcriptomes of LGR5^{+} cell-ablated CVP to controls to identify candidate genetic regulators of this new regeneration process in taste epithelium.

Friday, April 21, 2023

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

8:00 - 11:00 AM	Estero Ballroom
Poster Session III	

200 **Histone Inheritance Patterns In Mammalian Olfactory Horizontal Basal Cells**

Binbin Ma[1], Jonathan Yao[1], Gabriel Manske[2], Saher S Hammoud[2], Xin Chen[1], Haiqing Zhao[1]

[1]Johns Hopkins University, Baltimore, MD, United States, [2]University of Michigan, Ann Arbor, MI, United States

A long-standing question in stem cell research is how distinct cell fates are defined during one round of division. Histones, a major carrier of epigenetic information, play essential roles in regulating gene expression and cell fates. Previous studies have shown asymmetric histone inheritance in male germline stem cells and intestinal stem cells *in Drosophila*. It is still unclear whether asymmetric histone inheritance occurs in mammalian stem cells. To address this question, we investigate horizontal basal cell (HBC) divisions in the mouse olfactory epithelium (OE). We first screened the time window of HBCs activation during methimazole-induced OE regeneration and found that HBCs acquire the highest proliferation rates at 48 h post injury. In this injury-regeneration paradigm, ~60% of mitotic HBCs display asymmetric distribution of a stem cell marker, p63. Notably, the two resulting daughter cell nuclei seem to have asynchronized cell cycle progression during the mitotic exit. We further found asymmetric H4 nucleosome density in asymmetrically dividing HBCs but not in symmetrically dividing HBCs, indicating the cellular specificity of the asymmetric histone inheritance. The asymmetric histone density in asymmetrically dividing daughters may indicate the differential level of chromatin accessibility during HBCs fate transitions. Moreover, histone H2A-H2B exhibited symmetric distribution patterns in both symmetrically and asymmetrically dividing HBCs, further suggesting the molecular specificity of the asymmetric histone inheritance. Overall, these results support that asymmetric histone inheritance in stem cells is a conserved phenomenon across different species and tissue contexts. These on-going studies help to enhance our understanding of how stem cells retain their epigenetic memory.

202 **Differential Expression Of Surface Adhesion Molecules Defines Neuronal Cellular Identity**

Nikki M. Dolphin[1], Paolo E. Forni[1]

[1]Department of Biological Sciences, University at Albany, Albany, NY, United States, [2]The RNA Institute, University at Albany, Albany, NY, United States

The accessory olfactory system (AOS) in rodents is comprised of the vomeronasal organ (VNO) and the accessory olfactory bulb (AOB). The vomeronasal epithelium (VNE) is composed of two main types of vomeronasal neurons (VNs) expressing vomeronasal receptors (VR) of either V1R or V2R gene families. Basal VNs express V2R receptors and project to the posterior AOB (pAOB), while apical VNs express V1R receptors and project to the anterior AOB. The VNs' axon terminals form synapses with the dendrites of second-order neurons in the AOB, forming neuropil-rich structures called glomeruli. Adhesion molecules play key roles in cell adhesion, axon growth, pathfinding and fasciculation, synapse formation, and stabilization. Protocadherin's (Pcdh), a family of adhesion molecules have already been extensively studied in the main olfactory system. However, whether the Pcdh's have a role in the AOS has yet to be studied. Analyzing single-cell RNA sequencing of VNs, we found that distinct types and subtypes of VNs express unique combinatorial adhesion molecule profiles. Seven non-clustered Pcdh were found to be expressed at highly variable levels across neurons expressing distinct V1R receptors. Notably, Pcdh7 was found to be selectively expressed in V1R neurons while the V2R-expressing neurons were found to express higher levels of Pcdh9. Analysis of Pcdh7 knockout mice revealed ectopic projection and glomeruli formation of the apical VNs to the pAOB. Our data also suggest that several Pcdh are locally translated in the axon terminals of VSNs as they form glomeruli. We propose that local combinatorial expression of Pcdh is crucial for defining the spatial and specificity of targeting of apical VSNs to the AOB.

204 **Elucidating The Role Of Gli3 In Olfactory Ensheathing Cell Development Using Single-Cell Rna-Sequencing**

Ed Zandro M. Taroc[1,2], Paolo E. Forni[1,2]

[1]Biological Sciences, University at Albany, Albany, NY, United States, [2]The RNA Institute, University at Albany, Albany, NY, United States

Olfactory Ensheathing Cells (OECs) are the peripheral glial cells of the olfactory system (OS). The OECs have

been proposed to play several developmental functions in the OS, such as guiding newly formed axons to the olfactory bulb (OB), fasciculation of the axons into bundles, de-fasiculating the axons for fine targeting once arriving proximal to the OBs and assisting in the migration of the hypothalamic Gonadotropin Releasing Hormone-1 (GnRH-1) neurons. The OECs are derived from the neural crest (NC) and share several molecular features with other NC-derived peripheral glial cells known as Schwann Cells. $Sox10^{NULL}$ mouse mutants do not have OECs, in these mice, loss of olfactory neurons, as well as disorganization of the olfactory axons, has been previously reported. These data suggest that the OECs are important for the OS's development, maintenance, and repair. A limited number of studies have focused on the development of the OECs. We previously demonstrated that a loss of the transcription factor Gli3 compromise OEC development. However, why Gli3 loss of function compromises the development of the OECs remains an open question. To investigate this, we generated and analyzed single-cell RNA Sequencing data from nasal cells of control and Gli3 null mutant mice. Our data highlighted divergent developmental trajectories between the OECs of control and Gli3 mutants. Our initial data offer a key framework to further investigate the crosstalk between the developing olfactory system and the induction of olfactory ensheathing cells starting from neural crest-derived progenitors in the nasal area.

206 **Examining The Olfactory Receptor Fates Of Individual Olfactory Sensory Neuron Progenitors In Mice Using Polyloxexpress Barcoding And Single-Cell Rna Sequencing**

Karlin E. Rufenacht, Kawsar Hossain, Caitlin C. Winkler, Stephen W. Santoro
University of Colorado Anschutz Medical Campus, Aurora, CO, United States

Neurogenesis occurs throughout life in mammalian olfactory epithelia (OE). In mice, each differentiating olfactory sensory neuron (OSN) precursor selects 1 out of ~1200 possible olfactory receptor (OR) genes to express, which determines the subtype of the mature OSN. Recently, our lab found that stimulation can increase the number of OSNs of specific subtypes via subtype-specific acceleration of neurogenesis. These findings conflict with the current model of OSN neurogenesis, according to which the relative birth rates of distinct subtypes are constant due to the stochastic process of OR choice in post-mitotic immature OSNs. Although OR choice is assumed to occur in post-mitotic immature OSNs based on OR expression onset, whether OR choice can precede expression and occur in mitotic progenitors is unknown. If so, it might be possible for stimulation to promote the selective proliferation of progenitors to accelerate the neurogenesis of specific OSN subtypes. In this study, we tested the hypothesis that some mitotic OSN progenitors are inclined toward specific subtype fates, which predicts that OSNs arising from a single progenitor should tend to express the same OR. In support of this, Cre reporter-based lineage tracing combined with RNA-FISH revealed some groups of OSNs that appear to belong to the same lineage and express the same OR. To further test this, we used PolyloxExpress genetic barcoding, in which progenitors are labeled with one of >1 M possible barcodes, and scRNA-seq, to examine the OSN subtype composition of individual lineages in mouse OE. We found that the proportion of OSN subtypes with replicated Polylox barcodes was significantly higher than what would be expected to occur by chance, supporting the hypothesis that some progenitors are inclined toward specific OSN subtype fates.

208 **The Endoplasmic Reticulum Protein Canopy1 Is Necessary For The Homeostasis Of The V2R-Expressing Vomeronasal Sensory Neurons**

Nicholas A. Mathias[1,2], Paolo E. Forni[1,2]

[1]Biological Sciences, University at Albany, Albany, NY, United States, [2]The RNA Institute, University at Albany, Albany, NY, United States

The process of neuronal differentiation and establishment of cellular identity results from a complex interaction between extrinsic signals, combinatorial expression of transcription factors, protein synthesis, and chromatin modifications. Neural progenitors in the murine vomeronasal organ yield two main types of vomeronasal sensory neurons (VSN) that differ in their molecular features, connectivity, and function. Apical VSNs reside towards the lumen, express Gαi2 G-Protein subunits, and the V1R family of odorant receptors, whereas Basal VSNs reside adjacent to the basal lamina, express Gαo G-Protein subunits, and the V2R family of receptors. Previously our lab has shown basal VSN dependence on bone morphogenetic protein (Bmp) to mature and express dendritic knobs facilitating chemosensory activation and subsequent long-term neuron survival. scRNA sequencing data identified selective Canopy 1 (Cnpy1) expression in basal VSNs. Cnpy1 is a protein found in the endoplasmic reticulum with a putative role in regulating Fibroblast growth factor receptor 1 (Fgfr1) processing and membrane trafficking. Previous work in zebrafish has shown that Cnpy1 loss of function compromises Fgf signaling as well as the expression of downstream cadherins, t-box proteins, which affect transcriptional dynamics, and Engrailed 2, a marker found to be associated with autism. Preliminary data from Cnpy1 knockout mice indicate that the basal VSNs fail to survive into adulthood with the exception of those closest to the basal lamina. This is a phenotype that overlaps with several mutants in which V2R signaling is lost such as, TRPC2 KOs, Ga KOs, Gg8 KOs, Tfap2e KOs and Smad4 KOs. Our data suggest a key role for Cnpy1, and likely Fgfr1 signaling, in the development, signaling, and homeostasis of the V2R expressing VSNs.

210 **Trace Amine Associated Receptors (Taars) Response To Amines Are Largely Affected By Sequence Variants.**

Jody Pacalon[1], Christine Belloir[2], Sébastien Fiorucci[1], Loïc Briand[2], Jérémie Topin[1]

[1]Université Côte d'Azur (UCA), UMR7272, Faculté des sciences Parc Valrose, 28 avenue Valrose, 06000, Nice, *, France, [2]Centre des Sciences du Goût et de l'Alimentation, CNRS, INRAE, Institut Agro, Université Bourgogne Franche-Comté, F-21000, Dijon, *, France

The Trace Amine Associated Receptors (TAARs) are a family of chemosensory receptors that recognize volatile amines. These receptors are few in number and highly conserved, compared to regular olfactory receptors.

Polymorphisms in the TAAR family can have a drastic impact on our perception of amine compounds. Our study combine numerical simulations with in vitro experiments to reveal the activation mechanisms of the human TAAR5 receptor. The study focused on the hTAAR5-S95P polymorphism, which is found at high frequency in Nordic countries. This mutation affects the perception of trimethylamine (TMA), making individuals less able to perceive the smell of rotten fish caused by this molecule. The study's 3D model captures the inability of the hTAAR5-S95P variant to be activated by TMA in vitro, as well as the activation of the receptor by different agonists. Long-scale molecular dynamics simulations were used to study the system bound to ligands with different efficacies, providing insight into the features of a prototypical active state of G protein-coupled receptors. The study has identified two specific features of the TAAR family that are responsible for the altered perception of TMA in individuals with this polymorphism.

214 **Testis Expressed 15 Is Required For Diverse Olfactory Receptor Choice**

Nusrath Yusuf[1], David H Brann[2], Sandeep Robert Datta[2], Kevin Monahan[1]

[1]Department of Molecular Biology and Biochemistry, Rutgers University, Piscataway, NJ, United States, [2]Department of Neurobiology, Harvard Medical School, Boston, MA, United States

Mammals have evolved hundreds of G-protein coupled olfactory receptors (ORs) that bind and signal the presence of environmental odorants. ORs are expressed by olfactory sensory neurons (OSNs), with each OSN expressing only one allele of one OR gene. As a result, expression of the vast genetically encoded set of OR genes requires a diverse population of OSNs expressing different OR genes. Here, we show that testis expressed 15 (Tex15), a protein that silences transposable elements in the male germline, is required for diverse OR gene expression by OSNs. We find that Tex15 is transiently expressed by immediate neuronal progenitors in the olfactory epithelium, coincident with the onset of OR gene expression, and that Tex15 protein localizes to the nucleus of these cells. Tex15 knockout (Tex15 KO) mice exhibit a dramatic alteration in the expression of OR genes. In these mice, most OR genes are no longer detectable, whereas a subset of ORs exhibit dramatically increased expression, with 50% of OSNs expressing one of six OR genes. Importantly, Hi-C analysis shows that these upregulated OR genes form interchromosomal contacts with OR enhancers, and that their expression requires Ldb1, suggesting that loss of Tex15 alters OR gene choice but not the interchromosomal enhancer hub mechanism that drives OR gene expression. Finally, using single-cell RNA-seq we find that the OR genes that predominate in Tex15 KO mice are the same as the first OR genes to be transcriptionally activated in wild-type OSN progenitors. We propose that Tex15 is required to downregulate these first-activated OR genes, thereby preventing their early expression from skewing subsequent OR gene choice.

216 **Characterization Of Enhancer Motifs And Transcription Factors That Drive Trace Amine-Associated Receptor Gene Choice**

Madison Ratkowski[1], Rya Muller[1], Kyungho Seong[1], David Brann[2], Tatsuya Tsukahara[2], Sandeep R Datta[2], Thomas Bozza[1]

[1]Northwestern University, Evanston, IL, United States, [2]Harvard Medical School, Boston, MA, United States

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. 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. Most of what is known about olfactory receptor gene choice has come from studying the ORs. 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. We previously identified and characterized two enhancers in the TAAR gene cluster that are necessary and sufficient for TAAR gene choice. Unlike typical OR enhancers, we find that the TAAR enhancers share common, evolutionarily conserved motifs known to be important in OR gene choice, as well as multiple shared motifs that are specific to the TAAR enhancers. These shared motifs contain multiple predicted binding motifs for the transcription factor Tbr1. Moreover, single-cell sequencing reveals that Tbr1 is significantly upregulated in TAAR-expressing OSNs in comparison to OR-expressing cells. We are currently characterizing the function of Tbr1 in the olfactory epithelium and the effects of mutating Tbr1 binding sites in the TAAR enhancers. Our data suggest that the mechanism of singular TAAR expression not only shares some similarities with that of the canonical ORs, but also is dictated by a unique combination of previously uncharacterized factors.

218 **Computational Fluid Dynamic Modeling Of The Effect Of Dupilumab In The Management Of Surgical-Resistant Crswnp With Anosmia**

Zachary T Root, Thomas J Lepley, Zhenxing Wu, Bradley A Otto, Kathleen Kelly, Kai Zhao
The Ohio State University, Columbus, OH, United States

Background: Chronic Rhinosinusitis with Nasal Polyposis (CRSwNP) is among the most common causes of olfactory loss, potentially mediated by inflammatory sensorineural loss or by conductive obstruction to the olfactory receptor sites. Dupilumab is a monoclonal antibody treatment for CRSwNP that often results in olfactory improvement, yet the mechanism by which dupilumab improves olfaction remains unclear. Objectives: To use Computational Fluid Dynamic (CFD) modeling to gain a better understanding of the mechanism by which dupilumab alleviates olfactory loss in patients with CRSwNP. Methods: A three-dimensional CFD model was constructed based on computerized tomography scans of one CRSwNP patient who did not symptomatically improve with surgery but did with dupilumab (SNOT-22 score: 84 at baseline; 74 at 7 months post-surgery; 24 at 11 months post-dupilumab). Airflow velocity and pattern changes throughout the nasal airway were compared after sinus surgery and after dupilumab administration. Results: CFD modeling demonstrated significant improvement in patency of the olfactory cleft, marked by increased airflow distribution (from 0.5% to 26.4%, or 51.8-fold increase), when comparing the post-dupilumab to the post-operative state. Interestingly, there was

minimal change in nasal resistance between the two states (<1%). The improvement in olfactory airflow corresponded to significant improvement in smell identification score (16 at post-surgery to 35 post-dupilumab). Conclusion: In addition to the potential reversal of inflammatory sensorineural loss, our report suggests that dupilumab may improve olfaction in patients with CRSwNP by alleviating regional olfactory-specific obstruction and increasing olfactory-relevant airflow.

220 **Vasopressin Receptor 1A, Oxytocin Receptor, And Oxytocin Knockout Mice Display Normal Perceptual Abilities Toward Non-Social Odorants.**

Chloe E. Johnson, Elizabeth A. D. Hammock, Adam K. Dewan
Florida State University, Tallahassee, FL, United States

Genetic knockouts of the vasopressin receptor 1a (A*vpr1a*), oxytocin receptor (*Oxtr*), or oxytocin (*Oxt*) gene in mice have helped cement the causal relationship between these neuropeptide systems and various social behaviors (e.g., social investigation, recognition, and communication, as well as territoriality and aggression). In mice, these social behaviors depend upon the olfactory system. Thus, it is critical to assess the olfactory capabilities of these knockout models to accurately interpret the observed differences in social behavior. Prior studies utilizing these transgenic mice have sought to test for baseline deficits in olfactory processing; predominantly through use of odor habituation/dishabituation tasks, buried food tests, or investigation assays using non-social odorants. While informative, these assays rely on the animal's intrinsic motivation and locomotor behavior to measure olfactory capabilities and thus, have yielded mixed results. Instead, psychophysical analyses using head-fixed operant conditioning procedures and flow-dilution olfactometry are ideally suited to precisely quantify olfactory perception. In the present study, we used these methods to assess the main olfactory capabilities of adult male and female *Avpr1a, Oxtr,* and *Oxt* transgenic mice to volatile non-social odorants. Our results indicate that homozygous and heterozygous knockout mice of all three strains have the same odor learning, sensitivity, and discrimination ability as their wild-type littermates to non-social odors (p>0.05, two-way ANOVA with multiple comparisons). These data strongly support the hypothesis that the observed social deficits of these global knockout mice are not due to a ubiquitous deficit of their main olfactory system.

222 **The Impact Of Subjective Sense Of Smell Prior To Olfactory Dysfunction On Quality Of Life**

Emily A Garvey[1], Bita Naimi[1], Stephanie Hunter[2], Alexander Duffy[1], Pamela Silberman[3], Katie Boateng[3], Suz Schrandt[5], Paule V. Joseph[6], Claire Murphy[4], Jenifer Trachtman[2], Pamela H. Dalton [2], Nany E. Rawson[2], Gurston Nyquist[1]

[1]Thomas Jefferson University Hospital, Philadelphia, PA, United States, [2]Monell Chemical Senses Center, Philadelphia, PA, United States, [3]Smell and Taste Association of North America, Philadelphia, PA, United States, [4]Department of Psychology, San Diego State University, San Diego, CA, United States, [5]ExPPect, LLC, Arlington , VA, United States, [6]National Institute of Alcohol Abuse and Alcoholism, Section of Sensory Science and Metabolism, Bethesda, MD, United States

Introduction: Studies have demonstrated that olfactory disturbance (OD) can negatively impact quality of life (QOL). However, those who perceive their olfaction as greatly reduced may suffer greater QOL deficits after loss of olfactory function. We aimed to determine whether subjective perception of smell function prior to smell loss affects QOL across etiologies. Methods: Data were collected in April 2022 using an online questionnaire made available throughout the United States for those with taste and/or smell disorders. Individuals who rated their sense of smell as "excellent" before their OD onset were compared to those who rated their sense of smell as "extremely good," "extremely bad," "somewhat bad," "neither good nor bad," and "somewhat good," which were combined into one group. Binary and logistic regression was used to determine the effects of subjective olfactory function on QOL. Results: A total of 5,032 participants were included. 67% of participants reported having an excellent sense of smell prior to OD, of which 77% were female. Patients with a prior excellent sense of smell were more likely to report greater difficulty in relationships (B=0.241, p<0.001), worse mental health (B= 0.483, p<0.001), and decreased happiness (B=0.590, p<0.001). However, those with a prior excellent sense of smell were less likely to seek mental health treatment after OD onset (B= -1.129, p<0.001). Conclusion: Individuals with perceived "excellent sense of smell" prior to OD onset experienced a greater impact on mental health and relationships however, were less likely to seek treatment for mental health. Providers should consider inquiring about a patient's subjective sense of smell prior to OD to provide insights on how to best provide support.

224 **Four Odorants For Olfactory Training Is Enough**

Nicole Power Guerra, Emely Kruschwitz, Thomas Hummel
Smell & Taste Clinic, Department of Otorhinolaryngology, TU Dresden, Dresden, *, Germany

Background: Olfactory training (OT) is the most valid method to treat olfactory disorders of different etiologies, which affects ~20% of humans. Nevertheless, there is an ongoing debate about the most effective OT regimen. Aim of the study: We aimed to compare OT with 7 items (rose, lemon, eucalyptus, cloves, cinnamon, mint, apple) to OT with 4 items (rose, lemon, eucalyptus, gloves) over 3 months. Methods: Participants were 60 normosmic individuals receiving no OT, 4-item-OT, or 7-item-OT, and 40 gender and aged matched patients with olfactory dysfunction receiving 4-item-OT or 7-item-OT. Before and after the OT we assessed phenyl ethyl alcohol odor thresholds, odor discrimination, and odor identification (TDI score). In addition, thresholds were obtained for (R)-(-)-carvone, β-damascenone, and salicyclic acid benzylester. We also measured the degree of phantosmia and parosmia, cognitive function (MOCA score), and ratings of olfactory function. Results: OT was

accompanied by an increase of the TDI score independently of the number of odors used for OT in both patient groups (*p*

226 **The Patient Voice Matters: Outcomes From A Patient Engagement Award From The Patient-Centered Outcomes Research Institute (Pcori).**

Nancy E. Rawson[1], Katie Boateng[2], Suz Schrandt[3], Claire Murphy[4], Gurston Nyquist[5], Jenifer Trachtman[1], Stephanie R. Hunter[1], Pamela Silberman[2], Paule V. Joseph[6], Bita Naimi[5], Emily Garvey[5], Pamela H. Dalton[1]
[1]Monell Chemical Senses Center, Philadelphia, PA, United States, [2]The Smell and Taste Association of North America, Philadelphia, PA, United States, [3]ExPPect, LLC, USA, Arlington, VA, United States, [4]San Diego State University, San Diego, CA, United States, [5]Thomas Jefferson University, Arlington, VA, United States, [6]National Institute of Alcohol Abuse and Alcoholism, Section of Sensory Science and Metabolism & National Institute of Nursing Research, Bethesda, MD, United States

With COVID-19, there is a greater awareness and appreciation that patients with taste and smell disorders possess vital information and experiences that can help enhance care, lead researchers and clinicians to develop future preventive measures and treatments, and improve overall quality of life. However, information was limited regarding the experience of individuals suffering from these disorders in the United States (US), and clinical outcomes research has been hindered by systems which do not facilitate collaboration among patients, researchers, and clinicians. To understand the needs and experiences of these patients, the Monell Chemical Senses Center, the Smell and Taste Association of North America and Jefferson Health, conducted a survey of people who have experienced smell and/or taste dysfunction, as well as their caregivers and family. The response to this survey exceeded our goals several-fold with nearly 6,000 responses representing every US State within two weeks. This remarkable response speaks to the urgent need for more patient-centered efforts to address the needs of those experiencing taste/smell dysfunction. However, the vast majority of respondents were white (88%) and female (73%), indicating a need for future efforts to improve outreach to more diverse populations. We also conducted a series of listening sessions (n=6) with identified subgroups to gain deeper insight into the challenges highlighted by the respondents. Insights from these efforts were used to develop a relationship map and identify priorities to shape future work. Results are also being integrated into a white paper highlighting gaps in resources, research, training and education that must be addressed to better serve this growing patient population and integrate their voice throughout the research endeavor.

228 **A Confectionary-Based Tool For Prospective Screening For Covid-19 Related Chemosensory Losses In At-Risk Population**

Kym Man[1], Zhenxing Wu[2], Susan Travers[3], Christopher Simons[1], Kai Zhao[2]
[1]Department of Food Science and Technology, The Ohio State University, Columbus, OH, United States, [2]Department of Otolaryngology, Head&Neck surgery, 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 tools are needed to identify suspected COVID-19 cases. Sudden chemosensory loss is a cardinal symptom of COVID-19. Using 7 different fruit-flavored but visually identical hard candies, we developed an easy-to-use, prospective screening tool to monitor smell and taste function in an at-risk population (no COVID-19 infection in the prior 3 months). With a 2.5 month supply, every day, each subject was asked to randomly unwrap a candy and assess smell and taste function via a customized smartphone app. First, orthonasal identification (ID) and perceived aroma intensity were evaluated, followed by retronasal flavor intensity and ID after placing the candy in the mouth. The app also recorded daily temperature, exposure, symptoms, and COVID testing results. To date, a total of 14219 daily entries have been collected from 456 subjects (53.6% fully vaccinated), 325 of whom completed at least 10 daily testing sessions (Median=43 days). Of those, 14 tested positive for COVID-19 (11 fully vaccinated) that span from 10/18/2021 to 7/2/2022, with 9 on or after 12/29/2021 (likely Omicron). We found that the retronasal odor ID score started to decline as early as 1 day after testing positive and lasted ~8 days (GLM, $p<0.05$); however, only 3 of the 14 positives self-reported smell or taste losses. Odor and taste intensity ratings did not differ between subjects who tested positive for COVID-19 vs. those who did not in any date range. Temperature was only significantly different on the day of testing positive. Our results suggest olfactory loss using the candy test is among the early indicators of COVID-19, is a better objective predictor of infection than temperature, and has potential utility as a public health screening tool.

230 **Impaired Olfactory Function In Parkinson&Rsquo;S Disease Patients Detected By Ultra-High Field Functional Mri**

Yu Luo[1,2], Xinyuan Miao[1,2], Adrian G. Paez[1,2], Alex Y. Pantelyat[3], Liana I. Rosenthal[3], Vidyulata Kamath[4], Jun Hua*[1,2]
[1]Neurosection, Division of MRI Research, Russell H. Morgan Department of Radiology and Radiological Science, Johns Hopkins University School of Medicine, Baltimore, MD, United States, [2]F.M. Kirby Research Center for Functional Brain Imaging, Kennedy Krieger Institute, Baltimore, MD, United States, [3]Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD, United States, [4]Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, MD, United States

Parkinson’s disease (PD) is a neurodegenerative disorder characterized by widespread impairments in motor,

cognitive and behavioral functions. Olfactory deficits in PD are prevalent and reportedly present in up to 80-90% of sporadic PD cases. Such deficits can precede the onset of motor symptoms, and may be one of the earliest symptoms in PD. Postmortem pathological studies have demonstrated that Lewy bodies appear in the olfactory bulb (Braak Stage 1) before spreading to lower brainstem nuclei and the amygdala (Braak stages 2–3), and eventually substantia nigra and other midbrain regions (Braak stage 4: clinical disease stage), when motor functions become clearly affected. Olfactory loss has been linked to α-synuclein pathology, a major pathological hallmark of PD. Therefore, non-invasive and repeatable in vivo measures associated with olfactory deficits may be excellent candidates as quantitative in vivo biomarkers in early PD for tracking disease progression and evaluating treatment outcomes. In this study, high resolution functional MRI scans were performed in early PD patients and matched controls to investigate functional abnormalities in the primary and secondary olfactory regions. The experiments were conducted on ultra-high magnetic field (7.0 Tesla or 7T) human MRI scanner to enhance the sensitivity. Significantly impaired functional activities during olfactory stimulation in the olfactory regions in PD patients compared to control were found, which showed strong correlations with behavioral olfactory measures in the same subjects.

232 **Possible Effects Of Phytochemicals With Bioactive Properties On Chemosensory Dysfunction**

Sachiko Koyama[1], Vonnie Shields[2], Thomas Heinbockel[3], Poonam Adhikari[4], Rafieh Alizadeh[5], Angela Bassoli[6], Surabhi Bhutani[7], Orietta Calcinoni[8], Jingguo Chen[9], GCCR Phytochemical Project Group , Antonella Di Pizio[10], Daniel Strub[11], Rumi Ueha[12], Vera Voznessenskaya[13], Paule Joseph[14]

[1]Indiana University, Indianapolis, IN, United States, [2]Towson University, Towson, MD, United States, [3]Howard University, Washington, D.C., DC, United States, [4]Indian Institute of Technology Ropar, Ropar, *, India, [5]Iran University of Medical Sciences, Tehran, *, United States, [6]University of Milan, Milan, *, Italy, [7]San Diego State University, San Diego, CA, United States, [8]ENT, Phoniatrician, Voice and Music Professionals' Care Team, Milan, *, Italy, [9]Second Affiliated Hospital of Xi'an Jiaotong University (Xibei Hospital), Xi'an, *, China, [10]Leibniz Institute for Food Systems Biology at the Technical University of Munich, Freising, *, Germany, [11]Wroclaw University of Science and Technology, Wroclaw, *, Poland, [12]The University of Tokyo, Tokyo, *, Japan, [13]Severtsov Institute of Ecology & Evolution, Moscow, *, Russia, [14]NIAAA NIH, Bethesda, MD, United States

Following the outbreak of SARS-CoV-2, many COVID-19 patients have shown chemosensory dysfunction, which is deemed to be a better early sign of COVID-19 than other symptoms, like coughs and fever. In case of COVID-19, many of the patients who have mild to moderate level of symptoms are experiencing long-hauling symptoms. One such symptom is long-term chemosensory dysfunction. Many people tried various methods to help accelerate the recovery from chemosensory dysfunction, the PASC, and the stress caused by the pandemic itself. One of the most popular approaches is the so-called at home remedy. Many reports on daily foods, drinks, essential oils, and their ingredients suggest these remedies work. Other reports and follow-up studies, however, claim that they did not work. In these cases, one of the problems of these home remedies could lie in their self-test modalities and accuracy about the preparation, the composition, the quantities, and control conditions. Although there are many phytochemicals that are reported to have anti-inflammatory and anti-viral effects, the lack of the aforementioned factors makes their efficacy unclear. In this project, we have been conducting a survey to elucidate the impact of some phytochemicals with respect to whether they improved or worsened the PASC symptoms. We focused on anosmia and ageusia, but we also asked about the influence on other PASC symptoms. The survey was conducted in 10 different languages and asking the participants the foods and beverages that they take, and the symptoms/recovery. Currently we have over 1400 participants. In this report, we will summarize our findings so far and discuss the possible use of phytochemicals with bioactive properties on recovery from chemosensory dysfunction. This project is one of the GCCR (Global Consortium for Chemosensory Research) projects, ID: NDS005.

234 **Differential Impact Of Olfactory Disorders On Quality Of Life**

Pamela Dalton[1], Gurston Nyquist[2], Claire Murphy[3], Nancy Rawson[1], Paule Joseph[5], Katie Boateng[4], Pamela Silberman[4], Stephanie Hunter[1]

[1]Monell Chemical Senses Center, Philadelphia, PA, United States, [2]Thomas Jefferson University , Philadelphia, PA, United States, [3]San Diego State University, San Diego, CA, United States, [4]Smell and Taste Association of North America, Philadelphia, PA, United States, [5]National Institute of Alcohol Abuse and Alcoholism, Bethesda, MD, United States

The COVID-19 pandemic has increased the prevalence of smell and taste loss throughout the population. Patients often report that their quality of life (QOL) is negatively impacted by long-term smell loss. However, some demographic characteristics may differentially predispose individuals to experience QOL issues. Here, we assessed whether QOLwas impacted based on age, gender, COVID-19 etiology, and type of olfactory disorder. Data were collected between April 6, 2022 through April 28, 2022 using an online questionnaire made available to people throughout the United States with taste and/or smell disorders. A total of 5,352 participants (11% 18-24 years old, 30% 25-39 years old, 38% 40-60 years old, 21% 60 years old or older; 73% female; 88% white) completed the questionnaire. Ingestive behavior-related quality of life (nutrition, appetite, eating habits, food choices, cooking) were more affected in females and those with parosmia compared to males ($p<0.001$) and those with anosmia or hyposmia ($p<0.002$), respectively. Social and relationship - related quality of life (relationships, sex, and hygiene) were less affected in those 60 years of age and older compared to those 18-60 years old ($p<0.02$). Mental health was affected more in females compared to males ($p=0.008$) and less affected in

those 60 or older compared to those 18-60 years old ($p<0.05$), while pleasure and joy was affected more in females and those with parosmia compared to males ($p=0.01$) and those with anosmia and hyposmia ($p<0.01$), respectively. Safety was affected more in those with anosmia compared to those with parosmia ($p=0.01$). Thus, quality of life is differentially impacted by the type of olfactory disorder as well as patient age and gender.

236 **Taste Detection Of Maltooligosaccharides With Varying Degrees Of Polymerization**

Laura E Martin[1], Toren S Andrewson[1,2], Michael H Penner[1], Juyun Lim[1]

[1]Department of Food Science and Technology, Oregon State University, Corvallis, OR, United States, [2]Kaiser Permanente Bernard J. Tyson School of Medicine, Pasadena, CA, United States

We and others have previously demonstrated that humans can taste maltooligosaccharides (MOS) but not maltopolysaccharides (MPS). This taste detection of MOS is independent of the canonical sweet taste receptor, but the identity of the putative oligosaccharide receptor is unknown, making it unclear how the receptor interacts with MOS. The objective of this study was to investigate the effect of chain length on MOS detectability and taste sensitivity. To do this, we first prepared three food-grade MOS samples with narrow degree of polymerization (DP) ranges using flash chromatography: low (DP 4-6), medium (DP 7-12), and high (14-21) DP samples. We then asked subjects to discriminate MOS stimuli from blanks after the stimuli were swabbed on the tip of tongue, using a triangle test procedure. All stimuli were presented at 75mM, with acarbose, an α-glucosidase inhibitor, to prevent oral hydrolysis of MOS. Subjects were able to detect all three MOS classes. In a second experiment, we explored whether the detection of these samples differed at a range of concentrations (18-56 mM), using the same procedure as in experiment 1. Detection rates of medium- and high-DP MOS varied in a concentration-dependent manner (p <0.05). In contrast, low-DP MOS showed a consistent detection rate across the concentrations tested. These results demonstrate that humans can taste MOS stimuli of all chain lengths, and that relative taste detection does not decrease as MOS with increases in chain length.

238 **Taste Perception Of Pullulan Derived Oligosaccharides Containing A-1,6 Glycosidic Linkages**

Shashwat Damani, Michael H. Penner, Juyun Lim
Oregon State University, Corvallis, OR, United States

It has been shown that humans can taste linear maltooligosaccharides linked exclusively via α-1,4 glycosidic bonds. However, dietary oligosaccharides may contain α-1,6 glycosidic linkages in addition to the α-1,4 bonds. Yet, the role of α-1,6 glycosidic linkages on taste detection is currently unknown. To investigate the impact of α-1,6 glycosidic linkages on taste perception of oligosaccharides, three target stimuli were derived from pullulan, a polysaccharide consisting of maltotriose units linked via α-1,6 glycosidic bonds, following enzyme hydrolysis. The resulting products were food-grade oligosaccharides with degree of polymerization (DP) of 3, 6, and 9 containing 0, 1, and 2 α-1,6 glycosidic linkage(s). When target stimuli were presented, subjects (N=28) could discriminate all targets from blanks at a significant degree (p <0.05). In the presence of lactisole (a known sweet taste inhibitor), subjects were unable to detect DP 3 (p >0.05); yet they were able to detect DP 6 and 9 (p <0.05), although the level of detection dropped significantly (p <0.05). In a follow-up qualitative study, subjects (N=6) grouped the target stimuli and glucose into two categories (glucose/DP 3 vs. DP 6/DP 9) and characterized them as mostly "sweet" with having different sweetness intensities. With lactisole, the same individuals described glucose and DP 3 as "tasting like blank" (lactisole water) and found it challenging to describe the DP 6 and 9 stimuli due to their subtle characteristics. The findings of this study suggest that the taste perception of pullulan-derived oligosaccharides (DP 6 and 9) containing α-1,6 glycosidic linkage primarily depends on the sweet taste receptor although they may possess other sensory attributes that made it detectable when the sweet taste receptor was blocked.

240 **Effects Of Pre-Exposure To Sweeteners On Sensitivity To Increments In Sweeter Concentration**

Paul M Wise[1], Anilet Tharp[1], Natasha Rivers[1], May M Cheung[2], Paul AS Breslin[1,3]

[1]Monell Chemical Senses Center, Philadelpha, PA, United States, [2]Brooklyn College, City University of New York, Brooklyn, NY, United States, [3]Rutgers University, New Brunswick, NJ, United States

Taste intensity tends to decrease with continuous or repeated exposure to a fixed stimulus, a phenomenon often called adaptation, sensory fatigue, or desensitization. The terms "fatigue" and "desensitization" imply that the system becomes unresponsive, whereas "adaptation" implies that the system remains responsive but shifts its responsive range. The current experiment tested the hypothesis that sweetness adapts rather than desensitizes. We measured participants' ability to detect increments in the concentration of sweetness-matched glucose, sucralose, and rebaudioside A using a 2-alternative, forced-choice staircase procedure. Increment sensitivity, or just noticeable differences (JNDs) were measured after either holding the same sweetener in the mouth for several minutes (pre-exposure condition) or holding water (control condition). Sweetness intensity was measured during pre-exposure using the general Labeled Magnitude Scale. Effects of pre-exposure on JNDs were assessed using ANOVA models. After glucose pre-exposure, glucose was perceived as less sweet, but JNDs were smaller relative to pre-exposure to water, i.e., greater sensitivity to increments. For the non-nutritive sweeteners, pre-exposure reduced sweetness more profoundly than it did for glucose, consistent with past work. However, unlike glucose, JNDs did not decrease. Thus, for glucose we gain sensitivity to increments in concentration from the pre-exposure level in exchange for reduced sweetness, consistent with adaptation rather than desensitization. This was not true for the non-nutritive sweeteners, which suggests differences in the underlying physiology of dynamic response to sugars versus non-nutritive sweeteners. One possibility is that metabolic pathways in taste cells play a role in dynamic response to sweeteners.

242 **Does Phlorizin Affect Glucose Taste In Humans?**

R. Kyle Palmer, Anna Nechiporenko

Opertech Bio, Inc., Philadelphia, PA 19146, PA, United States

Recent evidence has suggested that glucose, a metabolizable sweet-tasting agonist of the TAS1R2/TAS1R3 receptor, can activate an additional taste signaling pathway through uptake by a sodium-dependent glucose transporter protein (SGLT) in humans. We are conducting an ongoing study of this potential alternative sweet-taste signaling pathway using the TaStation, a rapid throughput, game-like operant taste discrimination assay that enables testing of multiple tastants in replicate trials within single test sessions (Palmer et al, 2021, JPET, 377:133). A forced choice method of constant stimuli (MCS) approach is being used to determine the discriminability of 5 randomly presented concentrations of glucose (20, 40, 60, 80 and 100 mM; 12 trials each per test) from water (36 trials per test). Each of 4 subjects to date has been tested 6 times on this design. Signal detection analysis of the resulting dataset averaged across all 24 tests yielded a *d'* value for discriminability 0.55 for 20 mM glucose, indicating that this concentration was near detection threshold. The test was repeated 6 times with 20 mM NaCl replacing water as a stimulus and as the solvent for all glucose concentrations. Discriminability of glucose was increased when dissolved in 20 mM NaCl; the *d'* value for 20 mM glucose under these conditions increased to 1.01, indicating that the presence of sodium enhanced detection of glucose. The test was repeated 6 times again with 0.2 mM phlorizin added to all glucose solutions and water, but no statistically detected impact of phlorizin on discriminability of any concentration of glucose was observed. Taken together, thus far, the enhancing effect of sodium on discriminability of low glucose concentrations does not appear to involve an SGLT.

244 **Lateralized Dominance Of The Nasal Cycle Is Not Reflected In Olfactory Bulb Volumes And Cerebral Activations**

Akshita Joshi, Divesh Thaploo, Marie Thomas, Thomas Hummel
Smell & Taste Clinic, Department of Otorhinolaryngology, Technical University of Dresden, Dresden, *, Germany

Background- Nasal cycle (NC) is a rhythmic change of lateralized nasal airflow mediated by the autonomous nervous system. Previous studies reported the dependence of NC dominance on handedness and hemispheric cerebral activity. Objective- We aimed to investigate firstly, the possible lateralized effect of NC dominance on olfactory bulb volume: the first cerebral region processing olfactory information, and secondly, the association of NC dominance with the lateralized cerebral dominance in terms of olfactory processing (piriform cortex and orbitofrontal cortex). Methods- Thirty- five subjects (22 women, mean age 26 ± 3 years) participated in the study. NC dominance was ascertained using a portable rhino-flowmeter, the "Nasal Holter" for a duration of 24 h, out of which 22 subjects had right-dominant NC and 13 subjects had left-sided dominance. Structural and functional brain measurements were assessed using a 3T MR scanner (Siemens). Vanillin odor was presented during functional scans using a computer-controlled olfactometer. Results- NC dominance was found to be independent of the lateralization of olfactory bulb volumes. Also, NC dominance was found to be independent of the cerebral activations during odor perception. Conclusion- NC dominance is not associated with lateralized structural or functional differences in the cerebral olfactory system.

246 **Don Tucker Finalist: The Role Of Cortical Feedback And Gabaergic Synapses In Generating Oscillatory Activity In The Neonatal Olfactory Bulb In Vivo**

Zihao Zhang, Timothy Vladimir Dong, Chad Collins, Joost X Maier
Wake Forest School of Medicine, Winston Salem, NC, United States

The olfactory system of neonatal rat pups exhibits highly stable activity patterns: starting at birth (P0) until around postnatal day 15 (P15), odor stimuli evoke 10-20 Hz spindle oscillations that are coherent between the olfactory bulb (OB) and the piriform cortex (PC). Previous work from our lab demonstrated that neonatal spindle oscillations rely on cortical feedback, that targets the OB granule cell layer, and that removing cortical feedback increases OB oscillation frequency. Based on these findings, we hypothesize that the neonatal olfactory system exhibits adult-like circuit motifs, particularly net inhibitory corticobulbar feedback. In the present study, we tested two predictions that follow from this hypothesis: 1) Suppressing cortical feedback increases the excitability of OB mitral cells; and 2) Effects of cortical feedback are mediated via inhibitory GABAergic synapses. In order to test these predictions, we quantified spontaneous and odor-evoked spiking activity of single mitral cells in the OB of awake rat pups (P4-8, n=38) under three conditions: 1) Intact circuit ; 2) Suppressed corticobulbar feedback, accomplished by injecting lidocaine into the lateral olfactory tract or olfactory peduncle; and 3) Suppressed GABAergic function, accomplished through injecting the GABA antagonist bicuculline into the granule cell layer of olfactory bulb. The results confirm our predictions: 1) Suppressing cortical feedback increases mitral cell excitability; and 2) Suppressing GABAergic function increases oscillation frequency in the OB. Taken together, these findings suggest that inhibitory GABAergic synapses mediate a net inhibitory effect of corticobulbar feedback on neonatal odor processing. The inhibitory effect of GABA stands in stark contrast to other neural systems, where GABA is excitatory during the first week of life.

248 **Defining The Role Of Immature Olfactory Sensory Neurons In Olfaction**

Jordan D. Gregory[1,2], Claire E. Cheetham[1]
[1]Dept of Neurobiology, University of Pittsburgh, Pittsburgh, PA, United States, [2]Center for Neuroscience at the University of Pittsburgh, Pittsburgh, PA, United States

To better develop strategies to repair damaged brain areas via stem cell-derived neurons, it is imperative to understand how endogenously generated cells functionally integrate into existing neural circuitry. The mammalian olfactory bulb (OB) is a valuable model to study the functional integration of adult-born neurons in both the healthy and regenerating brain. Adult-born olfactory sensory neurons (OSNs), which are generated throughout a mammal's life, go through immature and mature developmental stages as they wire into the OB. We

have shown recently that immature OSNs provide odor input to the mouse OB, where they form monosynaptic connections with excitatory neurons. Furthermore, immature OSNs exhibited graded responses across a wider odorant concentration range than mature OSNs. Therefore, our hypothesis is that immature and mature OSNs provide distinct, but complementary, odor input to OB neurons. To test this, we employ Gg8-tTA;tetO-hM4Di and OMP-IRES-tTA;tetO-hM4Di transgenic mice to chemogenetically silence either immature or mature OSNs respectively via clozapine N-oxide (CNO) mediated activation of the inhibitory DREADD hM4Di. Validation of these models consisted of co-staining olfactory epithelium sections for OMP and HA-tagged hM4Di to determine expression selectivity; and phospho-S6 staining of OB sections following odor exposure to quantify CNO-mediated silencing. To determine the functional contribution of immature vs. mature OSNs, we will image odor-evoked responses in GCaMP6s-expressing mitral cells via *in vivo* 2-photon microscopy before and after hM4Di-mediated silencing. We will also determine the effect of silencing immature or mature OSNs on odor-guided behavior. Together, these experiments will provide new insight into the contribution of immature OSNs to healthy OB function.

250 **Loss Of The Ciliary Protein, Arl13B, Causes Axonal And Synaptic Defects**

Julien C. Habif[1,2], Jordan C. Moretta[1,2], Warren W. Green[1,2], Kirill Ukhanov[1,2], Chao Xie[1,2], Carlos de Celis[1,2], Lian Zhang[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 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 disruptions in the cilia that emanate from OSNs which possess the necessary machinery for odor detection. However, besides the expected impairments in olfactory ciliation, various ciliopathy murine models have displayed defects in the size and targeting of glomeruli, where OSN axons arborize and form functional synapses. Previously, we showed that the loss of the ciliopathy protein, ARL13B, lead to glomerular deformation and hypo-innervation. However, no study has detailed a mechanism for the axonal defects in ciliopathy mouse models; therefore, we aimed to do so utilizing a conditional murine model in which *Arl13b* is excised under the *OMP* promoter expressed in mature OSNs (*OMP-Arl13b*). We observed that *OMP-Arl13b* glomeruli were smaller and possessed a central region devoid of axonal penetrance, which we term a cavitation. Additionally, there was a loss in overall afferent activity and OSN terminals. Transducing OSNs with a bicistronic construct allowed the analysis of the arborization and pre-synaptic vesicles of individual OSNs. We determined that the loss of *Arl13b* in OSNs resulted in exuberant axonal arborization and an increase in the number of synaptic vesicles compared to WT. Finally, utilizing an odorant receptor reporter line, we assayed glomerular targeting and observed that *OMP-Arl13b* mice targeted additional, ectopic glomeruli. For the first time we show that loss of a ciliopathy protein in OSNs causes exuberant arborization and synaptic defects, providing an additional mechanism for ciliopathy induced olfactory dysfunction beyond the loss of cilia.

252 **The Contribution Of Smell To Olfactory-Hippocampal Gamma Oscillation Changes Observed In Alzheimer&Rsquo;S Disease-Associated Pathology**

Joseph A. Villanueva[1,2], Christy S. Niemeyer[2], Andrew N. Bubak[2], Maria A. Nagel[2,3], Diego Restrepo[1,2,4]

[1]Department of Cell & Developmental Biology, University of Colorado Anschutz Medical Campus, Aurora, CO, United States, [2]Department of Neurology, University of Colorado Anschutz Medical Campus, Aurora, CO, United States, [3]Department of Ophthalmology, University of Colorado Anschutz Medical Campus, Aurora, CO, United States, [4]Neuroscience Graduate Program, University of Colorado Anschutz Medical Campus, Aurora, CO, United States

Alzheimer's disease (AD) is a progressive, neurodegenerative disorder that affects 1 in 9 Americans over the age of 65 and is the 7th leading cause of death. Currently, ~6.5 million Americans over the age of 65 years of age have AD, costing roughly $321 billion in healthcare and long-term care, and unless treatments are developed, by 2060, ~13.8 mil Americans will have AD, costing just under $1 trillion by 2050. Early processes in AD are characterized by loss of sense of smell, amyloid deposition in the olfactory epithelium, and olfactory sensory neuron dysfunction. We performed transcriptomic and proteomic analysis of olfactory bulb (OB) and olfactory tract (OT) tissue from familial AD and controls. We found a signature of viral infection in the OB and inflammation and demyelination in the OT suggesting dysfunctional communication from the OB to the limbic system. Because sniff induced gamma oscillations generated in the olfactory bulb (OB) are directionally coupled to the hippocampus, the loss of smell would result in decreased hippocampal gamma oscillations that has been hypothesized to lead to neurodegeneration and cognitive decline. Moreover, AD associated pathology can be attenuated via entrainment of gamma oscillations in the hippocampus by activation of visual or auditory cortex through recruitment of both neuronal and glial responses. Together these data raise the question whether activation of gamma oscillations in the OB-hippocampus axis attenuates AD associated pathology. My work tests the hypothesis that gamma frequency optogenetic stimulation of OB inputs will reduce microglial activation, phosphorylated tau and β- amyloid deposition in the hippocampus in 5xFAD mice, an animal model of familial Alzheimer's disease.

254 **How Sensitive Are Mri Measures Of Human Olfactory Bulb Volume? A Post-Mortem Approach**

Elbrich M Postma[1,2], Laura E Jonkman[3,4], Paul AM Smeets[1], Evelien Huisman[3,4], Wilma DJ van de Berg[3,4], Sanne Boesveldt[1]

[1]Division of Human Nutrition and Health, Wageningen University, Wageningen, *, Netherlands, [2]ENT

Department, Hospital Gelderse Vallei, Ede, *, Netherlands, [3]AmsterdamUMC, Vrije Universiteit Amsterdam, Department of Anatomy and Neurosciences, section Clinical Neuroanatomy and Biobanking, Amsterdam, *, Netherlands, [4]Amsterdam Neuroscience, program Brain imaging and Neurodegeneration, Amsterdam, *, Netherlands

The olfactory bulb (OB) plays a pivotal role in the olfactory pathway and odor detection. Typically, OB volume is measured based on manual delineation on MRI scans and used to relate to clinical measures (e.g. greater volume is related to better recovery prognosis after smell loss). Accuracy and sensitivity of these MRI-based OB volume measures are however yet unknown. The aim of this study was to compare MRI OB volumes to OB tissue volumes of the same individuals using a post-mortem approach. Using the Normal Aging Brain Collection Amsterdam pipeline, 8 (5F, 57-88 years) in-situ post-mortem 3T MRI scans, including a T_2-weighted sequence to image the OB (voxel size 0.47x0.47x1 mm), were collected to obtain OB volume with manual delineation. After scanning and craniotomy, the right OB was dissected, fixed in 4% PFA and sliced in 40 μm sections. Every 5th section underwent Nissl staining to enable delineation of the OB tissue and generation of morphometric data. OB volumes showed a large variation: MRI volumes ranged between 21.54-63.30 mm^3, whereas tissue volumes ranged between 26.15-61.87 mm^3. The average difference between measurements was 12.7 mm^3 (SD: 9.71; range: 1.15-27.64). According to Bland-Altman plot, mean difference value was -3.03. All results fell between the upper and lower 95% CI. Spearman's rank correlation showed no significant correlation between both measures ($r(6) = 0.24$, $p = .570$). These first analyses indicate that OB MRI-measures reflect tissue volume; however, correlation between measurements was not significant. More cases will be included to determine validity and sensitivity of MRI-based OB volume measurements, and if this is related to other factors (e.g. age and gender). These results contribute to an increased accuracy of future MRI-based OB volume measurements.

256 **Mitral/Tufted Glomeruli Exhibit A Range Of Different Concentration Response Relationships In The Awake Mouse Olfactory Bulb**

Narayan Subramanian[1], Douglas A Storace[1,2,3]

[1]Florida State University, Department of Biological Science, Tallahassee, FL, United States, [2]Florida State University, Program in Neuroscience, Tallahassee, FL, United States, [3]Florida State University, Institute of Molecular Biophysics, Tallahassee, FL, United States

Detecting and recognizing important odor stimuli in natural odor scenes requires that an animal be able to recognize an odor as the same across a large range of concentrations, while also maintaining sensitivity to variations in the concentration gradient of the same odor. However, it remains unclear where these perceptual functions are generated within the brain. Olfactory receptor neurons exhibit a primarily monotonic relationship with changes in odor concentration and have concentration-response functions that are well fit by the Hill equation. Comparisons between glomerular measurements of the olfactory receptor neuron input to the bulb and the apical dendrites of mitral/tufted cells have found that odor representations are more concentration invariant in the bulb output. This suggests an important role in olfactory bulb processing in transforming the neural representations of olfactory stimuli. Here we tested whether the transformation is heterogeneous across the glomerular population. We carried out glomerular measurements from the apical dendrites of mitral/tufted cells in response to odors presented across a ~100-fold concentration range using 2-photon calcium imaging in awake mice. Mitral/tufted glomeruli responded with a heterogeneous mix of concentration-response profiles which were quantified using a monotonicity index. Approximately half of the glomeruli had odor responses that increased monotonically with concentration increases, while the remainder had a range of different nonmonotonic relationships. The results support a model in which higher concentrations can activate stronger modulatory processing within the bulb via broad activation across the olfactory receptor neuron glomerular input.

258 **Evidence That Feedback From Higher Centers Does Not Affect The Olfactory Bulb Concentration Invariance Transformation.**

Yunsook Choi[1,2], Lawrence Cohen[1,2]

[1]Yale University, New Haven, CT, United States, [2]Brain Science, KIST, Seoul, *, Korea

The olfactory bulb is known to carry out computations for the perception of concentration invariance of odor recognition (Storace and Cohen, 2017; Bolding and Franks, 2018), a perception that is essential for life as we know it. The present experiments were carried out to determine the role of the extensive feedback from higher centers (e.g. Shipley and Ennis, 1996) on this input-output transformation. We have measured the concentration dependence of the mitral cell output of the bulb before and after blocking the feedback by lidocaine infusion into the olfactory peduncle (Martin et al., 2006; Mandairon et al., 2014 (following Gray and Skinner, 1986)). In preliminary experiments we found that the concentration dependence of the mitral cell output was unaffected by the lidocaine block of feedback. We conclude that the computations for concentration invariance are likely carried out within the olfactory bulb itself.

260 **Effect Of Odorant Concentration On The Structure Of Glomerular Odor Representations In The Mouse Olfactory Bulb**

Matt Wachowiak[1], Michael Schmuker[2], Shawn Burton[3]

[1]University of Utah School of Medicine, Salt Lake City, UT, United States, [2]University of Hertfordshire, Hertfordshire, *, United Kingdom, [3]Lehigh University, Bethlehem, PA, United States

At the first synaptic level of the olfactory pathway, odorants are represented by combinatorial patterns of

olfactory sensory neuron (OSN) input to glomeruli. We recently generated an atlas of glomerular sensitivities to 185 odorants in the mouse olfactory bulb (Burton et al., doi: 10.7554/eLife.80470); these data established that glomeruli are extremely sensitive to their 'best' odorants, responding at picomolar- to nanomolar concentrations and, in this concentration range, are exceptionally narrowly tuned, resulting in a sparsely structured representation of olfactory chemical 'space' by the OSN population. Since environmental odorant concentrations vary widely, in the present study we asked how glomerular/OSN tuning and odor representations change as odorant concentrations increase. We used GCaMP6 imaging to map glomerular inputs across 59 odorants (a subset of the original 185), with each odorant presented at two concentrations differing by 25-fold. These concentrations were 100 - 1000-fold greater than those used in our earlier study, allowing us to compare odorant representations for the same 59 odorants across a concentration range of ~ 3 orders of magnitude. We found that glomerular tuning broadens substantially at higher odorant concentrations, although response spectra are still well-explained by subsets of shared chemical features as well as by putative odorant receptor class. In addition, the dimensionality of odor representations across the glomerular population decreased as odorant concentrations increased, suggesting that different strategies for decoding odorant identity may be optimal in low- versus high regimes of odorant concentration. These results highlight the importance of establishing relevant ranges of odorant concentrations encountered by an organism in natural settings.

262 **Multidisciplinary Approach To Explore Interactions In Odor Mixture Perception**

Thierry Thomas-Danguin[1], Anne-Marie Le Bon[1], Olivier Taboureau[2], David Jarriault[3], Frédérique Datiche[1], Christopher Aveline[1], Noëlle Beno[1], Florian Koensgen[1], Marylène Rugard[4], Karine Audouze[4], Vanessa Soubeyre[1], Olivier Bouchez[5], Christophe Klopp[5], Elisabeth Guichard[1], Charlotte Sinding[1], Anne Tromelin[1]
[1]Centre des Sciences du Goût et de l'Alimentation, INRAE, CNRS, Institut Agro, Université de Bourgogne Franche-Comté, Dijon, *, France, [2]Université Paris Cité, CNRS, INSERM U1133, Unité de Biologie Fonctionnelle et Adaptative, Paris, *, France, [3]Univ. Bordeaux, INRAE, Bordeaux INP, NutriNeuro, UMR 1286, Bordeaux, *, France, [4]Université Paris Cité, T3S, Inserm UMR S-1124, Paris, *, France, [5]Plateform Genotoul, Castanet-Tolosan, *, France

Odors and Aromas perceived in food and in the environment result from the processing of complex chemical mixtures of volatile compounds that should be efficiently processed by the olfactory system. It is known for decades that this processing generates perceptual interactions, such as masking, synergy, or perceptual blending, which contribute to elaborating a synthetic brain representation of the chemical information. Nevertheless, the perceptual processes underlying these interactions are still poorly known. In this project, we set out a multidisciplinary approach to identify the characteristics of odorants and olfactory receptors (ORs) that could support perceptual interactions. We hypothesized that odorants involved in interactions at the peripheral level should share common structural characteristics to allow the activation of a common set of ORs. First, using an RNA-seq approach in mice, we identified the ORs responding to either single odorants or mixtures exhibiting specific perceptual interactions. Then, we confirmed that the target ORs responded to odorants in an *in vitro* cellular system and that the expected perceptual interactions occurred between the odorants at the olfactory periphery, through EOG recordings on mice's olfactory mucosa, but also through sensory evaluation in humans. Additionally, a computational study revealed common molecular features between the odorants involved in interactions. When combined, all the results highlight that perceptual interactions such as masking could rise from competition between odorants at the OR level, but that other interactions such as perceptual blending most likely originate from more central integrative brain processing. This work was supported by the Agence Nationale de la Recherche (ANR-18-CE21-0006 MULTIMIX).

264 **Olfactory Evidence Accumulation In Mice**

Luis Boero[1], Hao Wu[1], Paul Masset[1], Siddharth Jayakumar[1], Joseph Zak[2], Venkatesh Murthy[1]
[1]Department of Molecular and Cellular Biology - Harvard Unviersity, Cambridge, MA, United States, [2]Department of Biological Sciences - University of Illinois at Chicago, Chicago, IL, United States

Odor cues from distant objects are sparse and highly fluctuating due to turbulent transport. Whether, and which aspects of olfactory stimuli statistics are used by animals when they seek to locate an odorous object are still unknown. To address if animals can integrate and weigh discrete olfactory sensory evidence over time, we developed a new behavioral task in which head-restrained, water-restricted mice make binary decisions under fluctuating odor stimuli over many seconds to obtain a water reward. A custom-built device allowed us to precisely deliver discrete, short pulses of odors at arbitrary Poisson-distributed pulse rates. Trained mice can readily differentiate stochastic odor stimuli with different total numbers of pulses presented over many seconds and reductions in the difference in the total number of pulses between the two conditions had a detrimental effect in performance. Logistic regression of (binary) behavioral outcome against the timing of odor pulses in the breathing cycle revealed that mice weighed sensory information differentially depending on the phase of the breathing cycle in which pulses arrived. We built a decision model assuming a Gaussian-distributed estimation of number of pulses and obtained Maximum Likelihood Estimates (MLE) of model parameters. We found that the variance in the estimation of the total number of pulses per trial scaled linearly with the number of pulses and this dependence was refined by accounting for pulse arrival times in the breathing cycle. Our study indicates that mice can integrate discrete, intermittent olfactory inputs over several seconds to make decisions and that perceptual evidence is weighted by the arrival time of sensory information with respect to breathing cycle.

266 **Odor Coding In The Piriform Cortex Of Awake, Freely Moving Mice**

Ian F. Chapman[1,2], Max L. Fletcher[1]

[1]University of Tennessee Health Science Center, Memphis, TN, United States, [2]Monell Chemical Senses Center, Philadelphia , PA, United States

In rodents, activity in the piriform cortex (PC) has been shown to reliably encode the identity of olfactory information within single sessions of odor delivery. Recent work, however, has leveraged technical advances to record from the same PC neurons and found greater unreliability in PC odor coding over long periods of time. The causes of this phenomenon, termed representational drift, are still being explored across multiple sensory systems, but evidence has suggested a role for animal behavior in the observed unreliability of coding. To explore this possibility in PC, we recorded from conserved populations of neurons using micro-endoscopic calcium imaging in freely-moving and awake mice as they gained passive experience with a panel of 6 chemically diverse odors. We also developed behavioral tracking measurements using DeepLabCut to assess investigation of odor trials over time. We find PC odor responses become less consistent across days of experience, but responses within a single session can still be used to train linear classifiers to accurately predict odor identity. Classifiers trained across days, however, perform significantly worse at odor classification due to inconsistent tuning of response preference over time. As the mice gained experience with the odor panel, they investigated the odors less, particularly towards the end of sessions on the later recording days. This decrease in attention coincided with a decrease in response correlation to the initial day of odor presentations, indicating that behavioral state of an animal plays a role in response inconsistency over time. Future studies will aim to further untangle the relationship between experience and behaviorally driven changes to odor coding in PC.

268 **Altered Olfactory Coding Of Social And Non-Social Cues In A Mouse Model Of Fragile X Syndrome**

Felipe Arancibia, Marcela Navarrete, Marcelo Rojas, Magdalena Sanhueza, Jorge Mpodozis, Alexia Nunez-Parra
Biology Department, Faculty of Science, Universidad de Chile, Santiago, *, Chile

Sensory perception is one of the most fundamental brain functions allowing individuals to properly interact and adapt to a constantly changing environment. Individuals with Fragile X Syndrome (FXS), the most common monogenetic cause of autism spectrum disorder, exhibit atypical sensory perception across sensory modalities including olfaction and social interaction deficyts greatly affecting their life quality. Here, we use a combination of behavioral, anatomical and electrophysiological tools to study the neuronal circuitry in olfactory-guided behaviors of the FXS mouse model (*Fmr1*-KO). We found that these animals exhibit altered olfactory sensitivity, olfactory memory and pheromonal signaling, which is central for social behavior. When perceptual stability, the cognitive process consisting in recalling an odor-objects even though there are some features of the sensory stimulus missing was evaluated, we found that cortical representations are incredibly stable and inflexible in the *Fmr1*-KO. This augmented stability prevented animals for discriminating similar mixtures in contrast with was is found in WT controls. Moreover, this deficiency is accompanied with an excitatory/inhibitory disbalance leading to a hyperexcited cortical network in the *Fmr1*-KO. Altogether, our results suggest that *Fmr1*-KO create inappropriate olfactory representations for social and no-social odors partially relying on a dysfunctional bulbar and cortical processing that could underpin the observed social interaction deficits.

270 **Odor Interaction At Peri-Threshold And Sub-Threshold Levels**

Jianbo Huang, Shuyi Wu, Leto Solla , Terry Acree
Cornell University, Ithaca, NY, United States

Both odor synergy and odor masking have been observed in binary odor mixtures. recently we found that Iso-valeric Acid (IVA) (“stinky feet” odor) could be masked by sub-threshold level of Perfumery Raw Materials (PRMs). However, whether peri-threshold levels of PRMs could be masked by sub-threshold levels of IVA has yet to be explored. In this study, the effects of sub threshold concentrations of Iso-valeric Acid (IVA at 25% threshold concentration) on the recognition probability of *s*-Limonene (“citrus” odor), Neohivernal (“Clean Laundry” odor), Methyl *Iso*-Eugenol (“Woody” odor), and Florhydral (“Floral” odor) were evaluated using Sniff Olfactometry (SO). 8 subjects selected for this study evaluated mixtures of PRMs at threshold and IVA at 25% of threshold concentration to establish the probability of detecting PRMs. Our results demonstrated that sub threshold levels of IVA could effectively mask the perception of PRMS. However, the sub-threshold level of IVA showed individual differences against the PRMs, indicating a potential for synergistic effects for some individuals.

272 ***Unlocking The Combinatorial Code Of Olfaction: A Deep Learning Approach***

Matej Hladis, Maxence Lalis, Sebastien Fiorucci, Jeremie Topin
Institute of Chemistry in Nice, Université Côte d’Azur, Nice, *, France

The olfactory system is capable of detecting and distinguishing between a wide range of odors using a combinatorial coding scheme, in which different odors are represented by the activity patterns of multiple olfactory receptors (ORs). However, determining the combinatorial code for a specific molecule can be costly and time-consuming, requiring the completion of numerous laboratory experiments. Recently, machine learning has emerged as a tool that can overcome this limitation and fill the gaps in the combinatorial codes. In this study, we introduce a deep learning model that utilizes a novel architecture combining protein language and graph neural networks to predict the combinatorial code of olfaction. This model is interpretable and able to identify molecule-residue correlations and structural characteristics that contribute to the activation of ORs. Our results reveal a consistent combinatorial coding for a large number of odor families. By analyzing the predicted combinatorial codes, we discovered several insights, including ORs specific for certain smell families and previously unknown pairs of enantiomers with distinct combinatorial codes. Additionally, this model allows us to estimate the broadness of ORs by predicting the activation by more than 6000 odorants, providing the first broadness estimate based on the full recognition spectra. The broadness is in full agreement with the well-known broad receptors, and we identify several new, undertested broad ORs. Finally, we extend the definition of

broadness to odorant molecules and highlight the importance of odorant broadness for the diversity of *in vitro* screenings.

274 **Chemodb: A Database Of Olfactory Receptor-Odorant Pairs For Understanding The Molecular Mechanisms Of Olfaction**

Maxence Lalis, Matej Hladis, Sébastien Fiorucci, Jérémie Topin
University Côte d'Azur, Nice, *, France

The sense of smell, or olfaction, is triggered when specific odorants bind to olfactory receptors (ORs) in the olfactory system and activate signaling pathways, leading to the perception of distinct odors. To gain a deeper understanding of the molecular mechanisms involved in olfaction, we have developed a database of OR-odorant pairs, drawn from published literature and public databases. It includes over 46,650 unique pairs of ORs and their ligands. The database contains information on responses, receptors, molecules, and experiments that have been performed. By analyzing the data, we have found a positive correlation between sequence identity and OR spectrum of recognition. Moreover, the database allowed us to define molecular broadness as the number of receptors activated by a given odorant. It revealed specific odorant families for certain receptors, as well as odorants that activate a large set of different ORs. Additionally, by combining ORs and molecular broadness, we proposed a baseline predictive model with comparable performance to several published machine learning models. Furthermore, we postulate that an "olfactory white percept" are mixtures activating a majority of ORs. Our findings indicate that OR-odorant databases can provide valuable insights into the underlying processes of olfaction, and further research using these databases is likely to yield new discoveries about the function and regulation of the olfactory system.

276 **Processing Complex Nectar Odors In The Mosquito Antennal Lobe**

Jeffrey A. Riffell
University of Washington, Seattle, WA, United States

Mosquitoes are important vectors of the pathogens of disease and locate their hosts and nectar resources using their olfactory system. Odors from nectar sources are complex, comprising tens to thousands of compounds. Unlike host-related behaviors of mosquitoes, comparatively less is understood about the mechanisms involved in nectar-feeding decisions, or how this sensory information is processed in the mosquito brain. Using natural odors from diverse nectar sources, such as fruits and flowers, we demonstrate that olfactory attraction to nectar sources is mediated by the balance of excitation and inhibition in the mosquito's antennal lobe (AL). Many nectar odors emit attractive, aldehyde-rich scents, whereas non-attractive fruits and flowers emit scents dominated by monoterpenes. Using a GCaMP-expressing *Ae. aegypti* line, two-photon calcium imaging experiments in the mosquito AL revealed that coordinated activity between the LC2 (aldehyde-sensitive) and AM2 (monoterpene-sensitive) glomeruli are critical for the representation of attractive odors. Moreover, the AM2 glomerulus is also sensitive to DEET, a mosquito repellent. Lateral inhibition between these two glomeruli corresponds to the level of attraction to the nectar odors. These results demonstrate the behavioral importance of mosquitoes beyond operating as disease vectors and open the door to understanding the neural basis of mosquito nectar-seeking behaviors.

278 **Parallel Projections Of Basal Forebrain Gabaergic Neurons To The Olfactory Bulb**

Pablo S. Villar[2], Ricardo C. Araneda[1]
[1]University of Maryland, College Park, MD, United States, [2]Havard University, Boston, MA, United States

Early olfactory processing is flexibly adjusted by descending feedback projections from multiple brain regions. Among these areas, the basal forebrain (BF) contains varied populations of cells including GABAergic and cholinergic neurons that innervate the olfactory bulb (OB) and regulate its neural output. While the functional roles for cholinergic modulation in the OB has been proposed, less is known about the role of BF inhibition to the OB in odor processing. Here, we use viral tracing and slice electrophysiology in male and female mice acute brain slices to investigate the cellular diversity of the BF GABAergic output neurons and their innervation by the piriform cortex. Whole brain imaging indicated that most top-down GABAergic projections to the bulb concentrate in a BF nucleus called magnocellular preoptic area (MCPO), with a small number of neurons found in nearby areas. The MCPO consisted of at least two non-overlapping populations of neurons, characterized by the expression of the cellular markers somatostatin (Sst) and calretinin (Cr) which differentially targeted the glomerular and inframitral layers, respectively. We demonstrate that fast glutamatergic inputs from the piriform cortex elicited responses in Sst but not Cr BF neurons. These results suggest a high degree of circuit specialization among the BF neuromodulatory neurons that project to the OB. Furthermore, our results provide support for the existence of a long-range feedback loop that can recruit BF GABAergic cells through direct PC glutamatergic inputs that drive a fast feedback inhibition to the OB.

280 **Representations Of Odorant Concentrations And Mixtures In Cortical Projections To The Olfactory Bulb.**

Joseph D. Zak[1,2], Gautam Reddy[3,4], Venkatesh N. Murthy[4,5]
[1]Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL, United States, [2]Laboratory for Integrative Neuroscience, University of Illinois at Chicago, Chicago, IL, United States, [3]Physics & Informatics Laboratories, NTT Research Inc., Sunnyvale, CA, United States, [4]Center for Brain Science, Harvard University , Cambridge, MA, United States, [5]Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA, United States

Sensory systems are organized hierarchically. Early stages format transduced signals and successive processing

steps perform more complex computations to extract sensory representations. However, this hierarchy is broken by cortical projections that descend and terminate in early processing areas. Revealing how these projections contribute to the coding of complex stimuli including odorant concentrations and their mixtures is necessary to understand sensory processing throughout the olfactory system. We expressed the calcium indicator GCaMP6f in the piriform cortex and used multiphoton imaging to measure the stimulus-response properties of cortical projections to the olfactory bulb (OB) in awake and freely breathing mice. We used two sets of odorant stimuli that each revealed surprising aspects of how odorants, and their mixtures, are represented in cortical projections to the OB. First, odorants spanning a concentration range of four orders of magnitude evoked responses in feedback projections that, as a population, reflected concentration invariance. However, at the level of individual boutons, we observed responses that had complex, non-monotonic concentration dependence that favored a select concentration range. We next imaged responses to odorant mixtures that contained between 2 and 12 components. When presented with complex mixtures, the overall activity of cortical projections rarely exceeded the activity evoked by individual mixture components and was representationally distinct from component odorants. As a reference, we imaged the same panel of odorant mixtures in the olfactory epithelium, where we found a strong relationship between representational similarity and mixture complexity. Our studies reveal how behaviorally relevant mixture information is inherited by the OB from the cortex.

282 **Deepnose &Ndash; An Artificial Neural Network Predictive Of Human Olfactory Percepts.**

Sergey Shuvaev, Khue Tran, Khristina Samoilova, Cyrille Mascart, Alexei Koulakov
Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, United States

The olfactory system employs an ensemble of odorant receptors (ORs) to sense molecules and to derive olfactory percepts. We hypothesized that ORs can be considered 3D spatial filters that extract molecular features relevant to the olfactory system, similarly to the spatial-temporal filters found in other modalities. We approximated these filters by training convolutional neural networks (CNNs) to predict human olfactory percepts. We took advantage of the semantic structure of the perceptual data by using Word2Vec dictionary embedding. Our network yields high fidelity perceptual predictions for different olfactory datasets. In addition, our approach allows us to identify molecular features leading to specific perceptual descriptors. Because CNNs are responsive to 3D molecular shapes, our approach predicts different perceptual qualities for different stereoisomers. The architecture of DeepNose relying on processing of several molecules at the same time permits inferring perceptual quality of odor mixtures. We propose that DeepNose network can use 3D molecular shapes to yield high quality predictions for human olfactory percepts and helps identify molecular features responsible for odor quality.

284 **Increasing Transcription Efficiency Enhances Functional Expression Of Human Olfactory Receptors**

Ichie Ojiro[1], Satoshi Ogasawara[2], Takeshi Murata[2], Yuko Terada[1], Keisuke Ito[1]
[1]University of Shizuoka, Shizuoka, *, Japan, [2]Chiba University , Chiba, *, Japan

Humans have ~400 olfactory receptors (hORs) and can distinguish thousands of odorants through a repertoire of responsive hORs. The expression of hORs on the plasma membrane of heterologous cells is essential for the elucidation of hOR response. However, many hORs fail to localize on the cell surface. Here, we examined the effect of the TAR-Tat system, which facilitates the transcription of target genes, in improving the functional expression of hORs. The effectiveness of this system for hOR transcription was examined. The amount of OR1A1 mRNA increased 17-fold with the use of the TAR-Tat system. This result suggests the system is useful for enhancing the transcription level of hORs. Next, the effect of increasing transcription and thereby the expression of hOR on the cell surface was investigated. Cell surface expression of OR1A1 with the TAR-Tat system was 1.5 times higher than without it. Subsequently, 377 hORs were randomly expressed in HEK293 cells and their cell surface expression level was evaluated. In addition to OR1A1, the increase of cell surface expression of many other hORs was observed with the TAR-Tat system. This result indicates that the TAR-Tat system is effective for promoting the cell surface expression of hORs in general. Next, the influence of increasing transcription in a hOR functional assay was analyzed. The responses of 377 hORs against n-hexanal were screened in the presence or absence of the TAR-Tat system. At least 5 hORs were identified as new hORs that respond to n-hexanal by using the system. As the ligands of 3 of these hORs had not have been previously identified. In this study, we succeeded in enhancing the cell-surface expression of hORs by enhancing transcription with the TAR-Tat system and raising the sensitivity of the hOR assay system. This system facilitates the analysis of odorant-hOR interactions.

9:00 - 10:15 AM	Calusa EFGH
Oral Session (Taste/Multisensory)	

Chair(s): Brigit High

9:00 **Structural Basis Of Fructose Recognition By An Insect Gustatory Receptor**

J. Victor T. Gomes, Shivinder Singh-Bhagania, Matthew Cenci, Joel A. Butterwick
Yale University, New Haven, CT, United States

Animals desire sugars for their energy potential and for the pleasurable sensation of tasting sweetness. Despite the importance of sugars to physiological processes as diverse as energy metabolism and neural reward mechanisms, we have yet to determine how taste receptors recognize sweet molecules. Insects use families of gustatory receptors to detect and distinguish sugars, with each receptor activated by different subsets of sweet compounds. Here we show how one receptor, Gr9 from the silkworm *Bombyx mori* (BmGr9), is activated only by a single type of sugar, D-fructose. Using cryo-electron microscopy, we determined structures of BmGr9 alone and when bound to D-fructose. The sugar-binding pocket, located in the transmembrane region of each subunit, collapses to encase D-fructose, allowing the sugar hydroxyl groups to be precisely coordinated by a series of conserved polar amino acids. Although docking studies suggest similarly-sized sugar molecules fit in the pocket, only D-fructose appears capable of engaging a bridge of two conserved aromatic residues that connects the pocket to the ion channel pore. Together, these data support a model in which specific ligand interactions and receptor activation efficacy combine to endow a taste receptor with remarkable specificity for a single sweet chemical.

9:15 **Don Tucker Finalist: Enhancing The Oral Metabolic Signal With Tolbutamide Increases Lick Responses In Rats For Glucose But Not For A Non-Metabolizable Glucose Analog**

Emily C. Hanselman[1], Elizabeth Kaye B. Leonardo[1], Sarah M. Sywanycz[1], Nicholas T. Bello[1], Paul A.S. Breslin[1,2]
[1]Rutgers University, New Brunswick, NJ, United States, [2]Monell Chemical Senses Center, Philadelphia, PA, United States

Glucose stimulates sweet taste receptors, T1R2-T1R3, and an oral metabolic signaling (OMS) pathway in taste receptor cells comprised of glucose transporters (GLUTs & SGLTs), glucokinase, and the ATP-gated potassium channel (K_{ATP}). We seek to determine first whether OMS influences lick responses in rats, and second whether activation of sweet taste and OMS can be manipulated to hyperstimulate licking. Rats show a very strong preference for glucose and saccharin mixture (G+S) and drink their body-weight of this mixture daily. Less is known of the short-term, oral controls of G+S preference and how the OMS contributes to this robust response. Hypothesis: We hypothesize that OMS influences licking for glucose and contributes to appetitive synergy for licking G+S mixtures in rats. Methods: Eight Sprague Dawley rats were given brief access tests with a concentration range of glucose and its non-metabolizable analog, methyl-D-glucopyranose (MDG), from 160 mM to 1.28 M in a series of 30 second presentations via a Davis-rig gustometer. The rats were treated with 5 mM tolbutamide, the K_{ATP} closer, or with vehicle alone immediately prior to testing. Rats were separately tested with G+S and MDG+S. Results: Rats had similar lick responses for glucose and MDG. Pre-treatment with tolbutamide increased licking for glucose, but not MDG. In addition, rats licked twice as much for G+S than for MDG+S. Conclusions: When oral K_{ATP} signaling is enhanced, rats increase licking for glucose but not MDG, which cannot be metabolized. Rats avidly lick for a mixture of G+S in brief access tests, but this effect is negated when glucose is replaced with the non-metabolizable glucose analog MDG. Future investigations will examine the interactions of T1R2-T1R3 signaling and OMS on taste-guided behaviors and neural activity.

9:30 **The Relationship Of Gustatory And Olfactory Activity During Retronasal Olfaction**

Thomas Gray, Donald Katz
Brandeis University, Waltham, MA, United States

The smell and taste systems are highly interconnected, but how activity in olfactory and gustatory circuits impact one another in real-time remains unclear. Previous research has shown that activity in the gustatory cortex (GC) can affect the way odors are processed in the piriform cortex (PC) and that the GC is necessary for distinguishing odors that are perceived through the back of the nose, such as those associated with food while eating. The aim of my current study is to identify the temporal dynamics of information transmitted from GC to PC that might mediate retronasal odor perception by using electrophysiology. By comparing the temporal response profiles of retro- and orthonasal odor responses in the two areas, we can determine if there is a unique relationship between each area's sensory responses in the context of retronasal olfaction. To look at these two regions simultaneously in freely behaving animals we have developed and implemented an easily produced 3D printed drivable electrode array that targets both PC and GC simultaneously. Here we demonstrate preliminary electrophysiological recordings and comparative analyses of GC and PC neuronal populations during retronasal and orthonasal olfaction.

9:45 **Differential Roles Of Ifn-Γ And Tnf In Taste Loss**

Hong Wang[1], Jiang Xu[1], Keiko Yasumatsu[1,2], Masafumi Jotaki[1], Cailu Lin[1], Akihito Kuboki[1], Liquan Huang[1,3], Emad Alnemri[4], Yuzo Ninomiya[1,5], Peihua Jiang[1], Danielle R. Reed[1], Robert F. Margolskee[1]
[1]Monell Chemical Senses Center, Philadelphia, PA, United States, [2]Tokyo Dental Junior College, Tokyo, *,

Japan, [3]Current Address: College of Life Sciences, Zhejiang University, Hangzhou, *, China, [4]Thomas Jefferson University, Philadelphia, PA, United States, [5]Kyushu University, Fukuoka, *, Japan

Inflammation plays important roles in chemosensory loss associated with infection and some other conditions. However, the underlying mechanism of inflammation-associated taste loss is poorly understood. In this study, we focus on investigating the roles of interferon (IFN)-γ and tumor necrosis factor (TNF), two inflammatory cytokines often induced during infection. Previously, we showed that the receptors and signaling components for both IFN-γ and TNF are expressed in taste bud cells. Here we use in vivo and in vitro approaches to determine the roles of these inflammatory cytokines in taste loss. Our results show that induction of either IFN-γ or TNF in taste tissues of transgenic mice results in loss of taste buds and diminished neural and behavioral responses to taste compounds. Induction of IFN-γ leads to massive cell death of taste receptor cells. Cultured taste organoids are also sensitive to IFN-γ-induced cell death. In contrast, adding TNF to cultured taste organoids strongly inhibits taste receptor cell generation but have little effect on cell death. Our results indicate that, although both IFN-γ and TNF contribute to taste loss, their primary mode of action is different. IFN-γ contributes to taste loss primarily by inducing cell death, while the main effect of TNF is on taste cell differentiation. Our study revealed differential roles of IFN-γ and TNF, two critical inflammatory cytokines, in taste loss.

10:00 **Chemosensory Deficits In &Ldquo;Long-Hauler&Rdquo; Subjects Following Covid-19.**

Kym Man[1], Barak Spector[2], Veronica Formanek[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, Head & Neck Surgery, The Ohio State University, Columbus, OH, United States, [3]Division of Biosciences, The Ohio State University, Columbus, OH, United States

Anecdotal evidence suggests prolonged chemosensory deficits occur in some subjects long after recovery from COVID-19. However, little empirical data exist regarding which chemosensory systems (taste, smell, or chemesthesis) may be impacted nor the degree to which they are affected. Presently, we evaluated chemosensory function in control subjects (N=41), self-identified “long-haulers” (LH, N=28) with smell/taste loss >40 days (range: 42-854 days; median: 364 days) and a confirmed COVID diagnosis or with no confirmed COVID diagnosis (“probable long-haulers” [PLH]; N=7), using solutions of quinine (0.1 and 1 mM), sucralose (1 mM), NaCl (100 mM), citric acid (8 mM), and capsaicin (5 ppm) for taste and chemesthesis intensity measurements, scratch and sniff cards for odor identification (ID), and hard candy for aroma and flavor ID and perceived sweet and sour taste intensity. When evaluated with either sniff cards or candy, odor ID was significantly impaired in the PLH ($p<0.05$; $p<0.01$) population only. However, the candy assessments indicated nominal decreases in orthonasal aroma intensity ratings whereas retronasal flavor ratings were significantly (p’s<0.001) affected in both long-hauler groups. Using taste solutions, bitter ratings of 1 mM quinine were significantly ($p<0.05$) lower in LH whereas ratings of sucralose, NaCl, and citric acid were not impacted in either group. Using candy, sweetness ratings were nominally lower in LH and significantly ($p<0.001$) lower in PLH with no observed effects on sourness in either group. Interestingly, compared to the controls, there was a dramatic decrement ($p<0.001$) in the intensity of capsaicin burn in LH and a nominal decrement in PLH. Results suggest that chemosensory loss in long haul subjects is variable and may extend across all chemosensory modalities.

10:15 - 10:30 AM	Calusa Foyer
Coffee Break	

10:30 - 12:30 PM	Calusa EFGH
Carrying taste information to the brain: development, adult plasticity, connectivity and modulation	

Chair(s): Nirupa Chaudhari and Robin Krimm

10:30 **Carrying Taste Information To The Brain: Connectivity, Plasticity, Functional Specialization And Development Of Peripheral Taste Neurons**

Nirupa Chaudhari[1], Robin Krimm[2]

[1]University of Miami Miller School of Medicine, Miami, FL, United States, [2]Univ Louisville School of Medicine, Louisville, KY, United States

While receptors, transduction pathways and cell-cell communication within taste buds has been extensively studied, the peripheral afferent neurons that innervate them are less well studied. Taste buds contain several well-documented cell types. Yet, studies on whether they are uniformly or differentially innervated have been limited. Connectomics based on ultrastructure, among other approaches are beginning to correct the shortfall. Meticulous block-face serial electron microscopic reconstructions allow the visualization of fiber branching and the synaptic interactions of related fibers. Another fascinating aspect of innervation is the question of how existing nerve terminals cope with the continuous turnover of taste bud cells and whether/how selectivity of innervation is maintained with new taste receptor cells. We know there are transcriptionally distinct gustatory neuron types. Yet, does the classification span neurons innervating tastebuds across the oral and pharyngeal epithelia, and how molecularly and functionally similar are such neurons? When, during embryonic development do gustatory neurons acquire their specialized profiles and what transcriptional mechanisms are recruited for this differentiation? These questions and more will be addressed drawing on experimental approaches from 3-D reconstructions, high-resolution time-lapse confocal imaging, and transcriptomic profiling to understand lineage and connectivity, and functional imaging *in vivo*.

10:40 **Serial Section Analysis Of Synapses And Connectivity In Circumvallate Taste Buds Of Mice**

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

Taste buds contain multiple morphologically-distinguishable mature cell types (Type I, II and III) that respond to different tastes, i.e sour (Type III), and sweet, bitter, or umami (Type II). Which cells respond to salty is unclear. Using serial blockface scanning electron microscopy (sbfSEM), we studied five taste buds from the circumvallate papilla in mice. The different cell types utilize different synaptic types: conventional chemical synapses for Type III cells and Channel-type synapses for Type II cells. We found that adjacent Type II and Type III cells never synapse with one another suggesting that any functional interactions between these cell types must be either indirect or non-synaptic. Of the 127 nerve fibers (NFs) in our sample that receive synapses from taste cells, most (70%) only connect to one taste cell, while a few connect exclusively to multiple Type II or Type III cells. A small number (3%) of NFs, found in larger taste buds, connect to both Type II and Type III cells, which does not support the idea of an exclusive labeled-line encoding system for taste. Our study also found variations in the number of synapses per cell/nerve pair and in the number of innervating NFs per taste cell, e.g. a single Type II cell may form synapses with 8 different nerve fiber profiles while other Type II cells in the same bud may form but a single synapse. This variation in degree of synaptic convergence may affect the encoding of taste quality and concentration.

11:10 **Rapid Structural Remodeling Of Peripheral Taste Fibers Is Independent Of Taste Cell Turnover**

Zachary Whiddon, Jaleia Marshall, David Collar, Aaron McGee, Robin Krimm
University of Louisville School of Medicine, Louisville, KY, United States

Taste bud cells are constantly replaced as old cells die and new cells migrate into the bud. The perception of taste relies on new taste bud cells integrating with existing neural circuitry, yet how these new cells connect with a taste neuron is unknown. Do taste neurons remodel to accommodate taste bud cell renewal? If so, how much of the taste axon structure is fixed and how much remodels? We measured the motility and branching of individual taste arbors (the portion of the axon innervating taste buds) over time with two-photon *in vivo* microscopy. Terminal branches of taste arbors continuously and rapidly remodel with concurrent addition and loss. Surprisingly, ablating new taste cells with chemotherapeutic agents revealed that terminal branch dynamics of taste arbors does not rely of the renewal of taste bud cells. Although arbor remodeling was robust in the taste bud, axon structure outside the taste bud was stable. Arbor structural plasticity would permit arbors to locate new taste bud cells, while stability of arbor number could support constancy in the degree of connectivity and function for each neuron over time. We explore this constancy in connectivity by observing individual Penk+ taste arbors and their putative presynaptic partners, Type III taste cells.

11:30 **A New Twist On The Old Tongue Map - Regional Specialization Of The Tongue Revealed By Gustatory Ganglion Imaging.**

Lindsey Macpherson
The University of Texas at San Antonio, San Antonio, TX, United States

Gustatory information is relayed from the anterior tongue by geniculate ganglion neurons and from the posterior tongue by neurons of the petrosal portion of the jugular/nodose/petrosal ganglion complex. Here, we use *in vivo* calcium imaging in mice to compare the encoding of taste information in the geniculate and petrosal ganglia, at single-neuron resolution. Our data support an anterior/posterior specialization of taste information coding from the tongue to the ganglia, with petrosal neurons more responsive to umami or bitter and less responsive to sweet or salty stimuli than geniculate neurons. We found that umami (50 mM MPG + 1 mM IMP) promotes salivation when applied to the posterior, but not anterior, tongue. This suggests a functional taste map of the mammalian tongue where the anterior and posterior taste pathways are differentially responsive to specific taste qualities, and differentially regulate downstream physiological functions of taste, such as promoting salivation.

12:00 **Ribosomal Profiling Of Oral Sensory Neurons Identifies Developmental Mechanisms Of Cell Fate Specification**

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

Geniculate ganglion (GG) oral sensory neurons project via the chorda tympani nerve to innervate taste buds located in fungiform papillae that are distributed across the anterior tongue. Fungiform papillae are multimodal in that chorda tympani nerve fibers respond to all five taste qualities, tactile stimulation of the tongue surface and cold temperature. Our understanding of the different subtypes of GG oral sensory neurons that communicate these varied stimuli to the brainstem, and their role in feeding and the perception of flavor, is rudimentary. Likewise, the molecular mechanisms responsible for cell fate specification of these geniculate oral sensory subpopulations, and the maintenance of their functional connections throughout life, are poorly understood. We used ribosomal profiling to identify genes that are enriched specifically in GG oral sensory neurons. This allowed us to identify receptors and transcription factors that are selectively enriched in these neurons. This method identified the growth factor receptor anaplastic lymphoma kinase (ALK) and the transcription factor early growth response 4 (EGR4) as being highly enriched in PHOX2B+ neurons. Here I will present data describing the role of ALK and EGR4 in the development of oral sensory neurons and taste buds, as well as their function in the diversification of neuronal subpopulations.

10:30 - 12:30 PM	Calusa ABC

Advanced techniques for high-resolution functional MRI and EEG recording in the olfactory bulb and associated olfactory regions in the brain

Chair(s): Jun Hua

10:30 **Advanced Techniques For High-Resolution Functional Mri And Eeg Recording In The Olfactory Bulb And Associated Olfactory Regions In The Brain**

Jun Hua[1,2]

[1]Johns Hopkins University, Baltimore, MD, United States, [2]Kennedy Krieger Institute, Baltimore, MD, United States

Non-invasive neuroimaging techniques have historically been underdeveloped for olfactory regions especially the olfactory bulb compared to the other sensory modalities such as visual and motor regions, which is largely due to the well-known difficulty of obtaining robust MRI signals from olfactory regions near the nasal cavity. Recently, several exciting MRI and EEG techniques have emerged that can measure neuronal activities in the olfactory bulb and associated olfactory regions. In this symposium, several experts with diverse expertise will present the state-of-the-art technologies on this topic. In the first talk, Dr. Kahnt will discuss high-resolution functional mapping of olfactory function in the human brain. In the second presentation, Dr. Luo will introduce a new functional MRI approach that can overcome the well-known signal dropout in conventional MRI methods and measure robust signals in the olfactory bulb and other olfactory regions in the human brain. In the third talk, Dr. Lundström will present a novel EEG method for recording signals from the human olfactory bulb. Finally, Dr. Poplawsky will demonstrate the high-resolution functional MRI methods they developed for the measurement of functional signals from different layers in the olfactory bulb of the rodent brain.

10:40 **Using High-Precision Mapping To Reveal The Structure Of Odor Coding In The Human Brain**

Vivek Sagar[1], Laura Shanahan[2], Christina Zelano[1], Jay Gottfried[3], Thorsten Kahnt[4]

[1]Northwestern University, Chicago, IL, United States, [2]Rhodes College, Memphis, TN, United States, [3]University of Pennsylvania, Philadelphia, PA, United States, [4]NIDA IRP, Baltimore, MD, United States

Odor perception is inherently subjective. Previous work across species has shown that odorous molecules evoke distributed activity patterns in olfactory brain areas, but how these patterns map onto subjective odor percepts has remained unclear. To probe the neural coding scheme linking activity patterns to idiosyncratic odor perception, we conducted a high-precision mapping experiment, collecting fMRI responses to 160 odors from three individual subjects (18 hours of fMRI scanning per subject). We find that activity patterns in piriform cortex (PirC), amygdala and orbitofrontal cortex (OFC) represent odor percepts rather than molecular identity. Moreover, whereas OFC contains fine-grained perceptual representations, PirC and amygdala encode coarser odor percepts. Furthermore, we show that encoding models with perceptual basis functions can predict olfactory fMRI responses to novel odors. Analysis of the encoding weights revealed that the dimensionality of the encoded perceptual spaces increases from PirC to OFC, and that whereas encoding of lower-order dimensions generalizes across subjects, encoding of higher-order dimensions is idiosyncratic. These results provide novel insights into cortical mechanisms of odor coding and suggest that subjective olfactory percepts reside in the OFC.

11:10 **Functional Mri In The Olfactory Bulb And Associated Olfactory Regions In The Human Brain**

Yu Luo[1,2], Jun Hua[1,2]

[1]Johns Hopkins University, Baltimore, MD, United States, [2]Kennedy Krieger Institute, Baltimore, MD, United States

To date, few studies using conventional functional MRI (fMRI) can measure functional signal changes in the olfactory bulb (OB) in humans, mainly due to the well-known susceptibility artifacts caused by the nasal cavity. T2-prepared (T2prep) BOLD fMRI is an alternative approach developed especially for reducing such susceptibility artifacts. Here, functional activation in olfactory-eloquent brain regions, especially the OB, was assessed using T2prep-BOLD-fMRI in healthy participants. The T2prep and conventional EPI-based BOLD fMRI were performed in each subject. The olfactory paradigm started with a stimulus-off period of 60s with odorless mineral oil, followed by three blocks of a stimulus-on period of 60s with Phenyl-Ethyl-Alcohol and a stimulus-off period of 120s with odorless mineral oil. T2prep-BOLD-fMRI showed greater sensitivity in the olfactory bulb, orbitofrontal cortex and the temporal pole. All regions investigated showed a habituation effect in that responses in the 2nd and 3rd blocks were reduced compared to the 1st block. Within the 1st block, all regions showed a bi-phasic pattern with two distinct peaks during the first and second halves of the 1st block. T2prep-BOLD-fMRI showed a good intra-subject reproducibility of the spatial locations of the activated clusters (Dice-coefficients 0.88-0.96) and $\Delta S/S$ (ICC>0.8), comparable to typical BOLD-fMRI measures reported in previous studies. In conclusion, the methodology demonstrated in this study holds promise for future olfactory fMRI studies in the olfactory bulb and other brain regions that suffer from large susceptibility artifacts.

11:30 **Electrobulbogram &Ndash; A Non-Invasive Measure From The Human Olfactory Bulb**

Johan N. Lundstrom
Karolinska Institutet, Stockholm, *, Sweden

Animal studies have demonstrated that the olfactory bulb (OB) is a key node of the olfactory system, and the list

of olfactory tasks in which it is implicated keeps growing. However, little is known about the role and processing of the human OB, mainly due to the dearth of techniques allowing non-invasive measures from the OB in awake and healthy humans. In this talk, I will outline the development, validation, and implementation of a new method, the electrobulbogram (EBG), which enables the non-invasive collection of highly temporally precise measures from the human OB in healthy humans as they process odors. In addition, I will summarize key results from our recent studies that relied on the EBG method to assess the role of the human OB in forming odor percepts as well as its communication with piriform cortex.

12:00 **Contrast-Enhanced Fmri Measures Layer-Specific Neural Activity In The Rodent Olfactory Bulb**

Alexander John Poplawsky[1], Christopher Cover[1,2], Sujatha Reddy[1], Harris B. Chishti[2], Alberto Vazquez[1,2], Mitsuhiro Fukuda[1]

[1]Department of Radiology, University of Pittsburgh, Pittsburgh, PA, United States, [2]Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA, United States

Resolving laminar activation with functional magnetic resonance imaging (fMRI) is becoming increasingly popular. We previously demonstrated that contrast-enhanced, cerebral blood volume-weighted (CBVw) fMRI responses were specific to the sites of increased synaptic activities in individual layers of the anesthetized rat olfactory bulb (OB) with a 100 – 200 μm accuracy, while blood oxygenation level-dependent (BOLD) fMRI responses were not. Specifically, we observed the greatest CBVw responses in the glomerular layer with odor stimulation; while the peak responses shifted to the external plexiform layer and granule cell layer (GCL) when synapses here were targeted with electrical stimulations of the lateral olfactory tract and anterior commissure, respectively. Interestingly, both electrical stimulations targeted the same granule cell population, yet fMRI distinguished excitation of their distal and proximal synapses, respectively. However, it is unknown whether laminar-resolution CBVw fMRI can be achieved in awake mice due to its susceptibility to motion. To examine this, we acclimated mice to the loud fMRI environment and to head fixation using established methods. After motion correction and censoring, we reliably detected distinct activation maps to four different odors in the OB of awake mice, similar to traditional histological maps; and observed more prominent odor-evoked responses in GCL, suggesting that higher-order feedback activity was intact. We also observed layer-dependent attenuation of fMRI responses to repeated odor exposures, similar to other olfactory adaptation studies. Together, noninvasive CBVw fMRI reliably detects layer-specific OB function in awake mice that may be used to examine whole-brain and local circuit function and dysfunction associated with olfactory behaviors.

12:45 - 1:45 PM	Calusa EFGH
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.

1:30 - 7:00 PM	Lunch On Own
Free time	

7:00 - 7:30 PM	Calusa EFGH
Data Blitz	

Chair(s): Yanina Pepino

7:00 **Egr4 Is Critical For Cell-Fate Determination And Phenotypic Maintenance Of Geniculate Ganglion Neurons Underlying Sweet And Umami Taste**

Debarghya Dutta Banik[1,2], Louis J. Martin[1,2], Brian A. Pierchala[1,2]

[1]Indiana University School of Medicine, Indianapolis, IN, United States, [2]Stark Neurosciences Research Institute, Indianapolis, IN, United States

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 (GG) 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 a severe loss of PHOX2B expression in oral sensory neurons of the GG with a concomitant increase in the BRN3A+ pinna somatosensory neurons in *Egr4*$^{-/-}$ mice. Deletion of EGR4 also disrupted the cell fate determination of these neurons resulting in loss of several known subpopulations of GG oral sensory neurons. A significant reduction in the chemosensory innervation of taste buds as well as taste cell number in Fungiform papillae were also observed in the *Egr4*$^{-/-}$ mice. Chorda tympani nerve recordings demonstrated that *Egr4*$^{-/-}$ mice exhibit deficits in responses to sweet and umami taste stimuli. To understand the downstream mechanism of EGR4 function, we performed RNA-seq on the GG from *Egr4*$^{+/+}$ and *Egr4*$^{-/-}$ mice. We found that axon guidance proteins 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 Fungiform papillae. These results indicate that EGR4 plays an integral role in cell fate determination of oral sensory neurons in the GG and controls the expression of the axon guidance molecules required for the proper neuronal innervation and/or synapse formation in taste buds.

7:04 **The Impact Of Social Interactions Over Taste-Related Decisions In Rats**

Roni R. Gerbi, Anan Moran
Tel Aviv University, Tel Aviv, *, Israel

Feeding decisions are critical for well-being and survival and are influenced by different factors such as innate preferences and past self-experience. In addition, external factors such as social interactions are also known to strongly impacts feeding decision-making. So far, studying the mechanism of social influence over feeding decisions in rats had yielded mixed results; rats can learn to prefer a food previously consumed by another rat (guided by olfactory cue) but fail to avoid the same food when the presenter rat shows sickness. Similarly, no social impact was so far identified in conditioned taste aversion (CTA) paradigm, implying that rats ignore the aversive state of others during this type of learning. On the other hand, rats correctly perceive a conspecific state in other behavioral paradigms like in fear conditioning or interacting with a conspecific in pain. We hypothesized that in CTA, the impact of social interactions is shadowed by self-perceived malaise, and can be revealed with more sensitive techniques. To test this we tracked the social behavior of pairs of rats in different CTA conditions. The results show that when two rats share aversive gastric distress following taste consumption, the degree of aversion correlates with the number of grooming events performed on the tested subject. On the contrary, when only the tested rat experiences gastric distress, the correlation between the two measurements disappears. Overall, our results suggest that rats' feeding decisions are influenced by social interactions; an influence that requires both lowering the salience of the self-experience, and performing detailed behavioral analysis to be detected. With this detailed analysis, we can further use this behavioral setup to study the neuronal circuits that underly the social influence.

7:08 **Stimulus Representation In Gustatory Cortex: Quality Or Quantity?**

Martin A Raymond, Max L Fletcher, John D Boughter
University of Tennessee Health Science Center, Memphis, TN, United States

Accumulating evidence from recent studies of Gustatory Cortex suggest that stimulus palatability, or behavioral acceptance of taste stimuli, is a prominent component of gustatory processing and representation. Our own previous studies have found evidence of converging representations of accepted tastes over time, and some additional evidence of a similar convergence of unacceptable taste stimuli. In our current experiment, we sought to further explore this behavior-oriented system of gustatory processing in GC. We used calcium imaging in awake, behaving mice to assess gustatory representations in 8 independent animals during a behavioral paradigm designed to test unanswered questions about the formation and nature of those stimulus representations. We selected four taste stimuli, and then mixed each of them at a highly palatable concentration and a highly unpalatable concentration. This mixed panel allowed us to assess more directly how unpalatable taste stimuli interrelate in gustatory representational space. Additionally, by presenting each animal with both concentrations of 3 of the stimuli for several days, then substituting one of the 3 used stimuli with the 4^{th}, still novel, stimulus, we were able to test how novelty impacts stimulus representation. Finally, we presented the taste panel again such that animals were only allowed to lick each stimulus once per trial, to comprehensively address potential confounds in our interpretation of behavioral acceptance. By analyzing the resulting neural data with tools including multidimensional scaling and classification learning algorithms, this experiment allows us to answer several existing questions left by previous studies and gain greater insight into how GC constructs stimulus representations and generates behavior.

7:12 **A Dopaminergic System Promoting Sniffing**

Natalie L. Johnson[1], Anamaria Cotelo[1], Andy Chavez[1], Minghong Ma[2], Daniel W. Wesson[1]

[1]University of Florida, Gainesville, FL, United States, [2]University of Pennsylvania, Philadelphia , PA, United States

Sniffing is a widely observed behavior reflecting motivational states. For example, rodents sniff when investigating odors, during social interactions, while foraging for food, and even in anticipation of a reinforcer in instrumental tasks. The brain systems mediating this conserved and adaptive behavior are unknown. Here we sought to link displays of sniffing with the dopaminergic (DAergic) system. We hypothesized that DA release in the tubular striatum (TuS, also known as the olfactory tubercle), a component of the ventral striatum receiving both midbrain DAergic input and olfactory sensory input, is integral to sniffing behavior. To first validate that VTA→TuS DAergic input is behaviorally relevant, we used an optical intracranial self-stimulation task and found that DA release into the TuS is reinforcing and supports approach behaviors. Next, using *in vivo* fiber photometry and whole-body plethysmography, we observed that phasic DA release in the TuS is tightly coupled to the display of individual sniff bouts, including both spontaneous and sensory-evoked bouts. Further, bidirectional causal manipulations revealed that while optical stimulation of DA release in the TuS triggers sniff bouts, pharmacological inhibition of DA1 and DA2, but not DA3 receptors, in the TuS reduced both the number and vigor of sniff bouts. Similar-sized effects were not observed in the nucleus accumbens. Together these results implicate DAergic actions within the TuS, a structure which receives dense olfactory input, in the orchestration of sniffing and uncover a system supporting this widely displayed motivated behavior.

7:16 **Cortical-Bulbar Feedback Supports Behavioral Flexibility During Rule Reversal**

Diego E Hernández Trejo[1], Andrei Ciuparu[2], Pedro García da Silva[3], Cristina M Velásquez[4], Benjamin Rebouillat[5], Michael Gross[1], Martin B Davis[1], Raul C Mureşan[2], Dinu F Albeanu[1]

[1]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, United States, [2]Transylvanian Institute of Neuroscience, Cluj-Napoca, *, Rumania, [3]Champalimaud Foundation, Lisbon, *, Portugal, [4]Oxford University, Oxford, *, United Kingdom, [5]Université Paris 8, Paris, *, France

Mice flexibly adjust their behavior to environmental changes and excel at recognizing odorants in complex sensory conditions; however, little we know about: (1)how sudden changes in stimulus contingency modify odorant representations and (2)how changes in odorant representations causally relate to behavioral adjustments. The piriform cortex(PCx) receives sensorial input from the olfactory bulb(OB), and input from associative areas(e.g., orbitofrontal cortex) and sends dense feedback that selectively modulates one of the OB output channels(mitral cells). Therefore, PCx is ideally positioned to integrate sensorial input and behavioral contingencies to modulate OB output in tune with behavioral goals. To study the role of cortical-bulbar feedback(CBF) in supporting flexible behaviors, we trained mice in a Go/No-Go task with rule reversal guided by odor and sound cues. Within the same session, reward contingencies were reversed across blocks of contiguous trials, rewarding either cue type depending on the block rule and the animal decision(report lick). Using multiphoton microscopy, we monitored CBF activity(GCaMP) in mice engaged in the task. CBF boutons exhibited dense and diverse responses that change mirroring the task block structure. The response changes observed after each rule reversal slightly lagged in updating and are correlated to the behavioral switch. Multilayer perceptrons trained to decode behavioral contingency rapidly increased their performance after cue delivery and before the animal's decision. Optogenetic suppression experiments(Jaws) suggest that mice rely on CBF to adapt their behavior after each rule reversal. Our results indicate that CBF multiplexes information about stimulus identity and contingency that is rapidly re-formatted according to changes in the reward contingencies.

7:20 **Enhanced Excitability Of Accessory Olfactory Bulb Mitral Cells Supports Heightened Intermale Aggression**

Kevin J Monk[1, 2, 3], Ian G Davison[1, 2, 3]

[1]Department of Biology, Boston University, Boston, MA, United States, [2]Center for Systems Neuroscience, Boston University, Boston, MA, United States, [3]Neurophotonics Center, Boston University, Boston, MA, United States

Intermale aggression is a highly conserved behavior used to establish dominance, defend territory, and acquire resources. Many factors including socialization and sexual experience affect aggression levels across individuals and within an animal's life. In mice, aggression relies on pheromone detection by the vomeronasal system, but what plasticity mechanisms exist and where in the vomeronasal network they act to support flexible aggression is unknown. Pheromonal cues are integrated in the accessory olfactory bulb (AOB) where mitral cell (MC) plasticity supports other flexible behaviors. Here we address whether and how AOB MC activity supports flexible, intermale aggression through *ex vivo* whole-cell recordings and *in vivo* chemogenetic manipulations. As social isolation increases intermale aggression, we first recorded AOB MCs from mice that were singly- or group-housed, and activated neurons with repeated and prolonged current injections to simulate activity evoked during natural social contacts. We find that AOB MCs of aggressive animals maintain robust spiking activity across repeated activations compared to those of non-aggressive animals. Given that plasticity of male-responsive AOB MCs in females supports sensory memory formation, we hypothesize that intermale aggression is modulated by male-responsive AOB MC excitability. To test this, we have bidirectionally manipulated male-activated AOB neurons with chemogenetic actuators and find that activation of these neurons increases aggression whereas inhibition decreases it. Finally, in ongoing *ex vivo* recordings, we address whether selectively-labeled male-responsive AOB MCs are more likely to show increased excitability. These results demonstrate that plasticity of select AOB MCs is part of the cellular basis for flexible, intermale aggression.

7:24 **Is There A Male Body-Odor Associated With Unexplained Repeated Pregnancy Loss?**

Reut Weissgross[1,2], Liron Rozenkrantz[1,2], Lior Gorodisky[1,2], Stephanie Brener[1,2], Tali Weiss[1,2], Inbal Ravreby[1,2], Liron Pinchover[1,2], Idan Frumin[1,2], Aharon Ravia[1,2], Sagit Shushan[1,2,3], Howard Carp[4], Noam Sobel[1,2]

[1]Department of Brain Sciences, Weizmann Institute of Science, Rehovot, *, Israel, [2]The Azrieli National Institute for Human Brain Imaging and Research, Weizmann Institute of Science, Rehovot, *, Israel, [3]Department of Otolaryngology & Head and Neck Surgery, Edith Wolfson Medical Center, Holon, *, Israel, [4]Department of Obstetrics & Gynecology, Sheba Medical Center, Tel HaShomer, *, Israel

In the Bruce effect, pregnant mice miscarry following exposure to bodily odors emitted from a male stranger. Lesions to the female accessory olfactory system negate this effect. Bruce-like effects have been implicated in other mammals, including primates, and in our previous study (Rozenkrantz et al, eLife, 2020), we found that women who experienced unexplained repeated pregnancy loss (uRPL) displayed altered perceptual and brain responses to men's body-odor (BO), suggesting a possible link between uRPL and the olfactory system. In our current study, we sought to examine the contribution from men by asking whether uRPL-men and Control-men emit different BO. We collected BOs from the spouses of the women who participated in our previous study, using T-shirts worn for two consecutive nights. Twenty-one women with uRPL and 24 control women sniffed and rated 34 BO jars (17 uRPL-men, not their spouse) on a visual analogue scale. We found that uRPL-men were rated as more pleasant, sexually attractive and fertile than control men (rmANOVAs for each parameter but intensity revealed a significant effect of male group (all $F(1,43)>14.24$, all $p<0.001$), no women-group effect or interaction). We then used a PEN3 electronic nose (eNose, Airsense) to sample 37 male BOs (18 uRPL). Using the data from the 10 sensor 80-second time series, a Linear SVM classifier successfully classified the odors to uRPL or Control-men at 69.9% accuracy in a five-fold cross-validation test ($p<0.001$ estimated by repeating the process 1000 times and shuffling the labels). These initial results suggest that uRPL-men's and Control-men's BO have different chemical composition. This may imply a possible contribution of men to the effect we found in our previous study, and combined, they suggest a link between uRPL and the olfactory system.

7:30 - 10:30 PM	Estero Ballroom
Poster Session IV	

201 **Ephrina&Rsquo;S Repel Embryonic Gustatory Geniculate Axons In Vitro And In Vivo And Are Expressed In The Adult Gustatory System.**

Metin Aksu[1], Rohan Jaiswal[1], Angel Munos[1], Anna Grundhoefer[1], Albert J. George[2], David Cho[3], Matthew Russo[4], Randall W. Treffy[5], John Wong[6], Syuzanna Darbinyan[1], Marissa K.L. Pilon[7], M. William Rochlin[1]
[1]Loyola University Chicago, Chicago, IL, United States, [2]Illinois Institute of Technology Research Institute, Chicago, IL, United States, [3]University of Texas Dell Medical School, Dallas, TX, United States, [4]Neuroscience, Rush University Medical Center, Chicago, IL, United States, [5]Medical College of Wisconsin, Milwaukee, WI, United States, [6]Rush Medical College, Chicago, IL, United States, [7]Protein Tech, Chicago, IL, United States

Ephs and ephrins are cell surface proteins that act as ligands and receptors for one another and typically mediate contact-dependent axon repulsion. 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 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. In E14.5 geniculate ganglia, EphA/ephrinA expression varies in intensity and location, with some (EphA5, A6, and A7) restricted to Phox2B+ (oral, mostly gustatory) neurons and others (ephrinA2, A3, and A5) concentrated in Prrxl1+ neurons (aural somatosensory) neurons. In vitro, ephrinA-Fc's repel E18 rat geniculate and trigeminal neurites dose-dependently. Preliminary data indicate that E15.5 Phox2b-Cre::tdTomato mouse geniculate neurites (oral afferents) are also repelled by ephrinA stripes, and suggest that tdTomato-negative neurites were not as strongly repelled. In E15.5 Phox2b-Cre::tdTomato mice lacking ephrinA1, A3, and A4, gustatory axons explore a greater area than in wild type FP in the central region of the tongue. EphA/ephrinA expression is also evident in the adult geniculate ganglion and dorsal lingual epithelium, and in some cases differs from embryonic expression. The variety of Ephs and ephrins expressed in the epithelium at these stages suggests that Eph/ephrin signaling also influences the migration and organization of dorsal lingual epithelial cells.

203 **Egr4 Is Critical For Cell-Fate Determination And Phenotypic Maintenance Of Geniculate Ganglion Neurons Underlying Sweet And Umami Taste**

Debarghya Dutta Banik[1,2], Louis J. Martin[1,2], Brian A. Pierchala[1,2]
[1]Indiana University School of Medicine, Indianapolis, IN, United States, [2]Stark Neurosciences Research Institute, Indianapolis, IN, United States

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 (GG) 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 a severe loss of PHOX2B expression in oral sensory neurons of the GG with a concomitant increase in the BRN3A+ pinna somatosensory neurons in *Egr4*$^{-/-}$ mice. Deletion of EGR4 also disrupted the cell fate determination of these neurons resulting in loss of several known subpopulations of GG oral sensory neurons. A significant reduction in the chemosensory innervation of taste buds as well as taste cell number in Fungiform papillae were also observed in the *Egr4*$^{-/-}$ mice. Chorda tympani nerve recordings demonstrated that *Egr4*$^{-/-}$ mice exhibit deficits in responses to sweet and umami taste stimuli. To understand the downstream mechanism of EGR4 function, we performed RNA-seq on the GG from *Egr4*$^{+/+}$ and *Egr4*$^{-/-}$ mice. We found that axon guidance proteins 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 Fungiform papillae. These results indicate that EGR4 plays an integral role in cell fate determination of oral sensory neurons in the GG and controls the expression of the axon guidance molecules required for the proper neuronal innervation and/or synapse formation in taste buds.

205 **Early-Life Exposure To A Non-Nutritive Sweetener Increases Unconditioned Licking For Fructose With Some Impact On Psychophysically Assessed Fructose Detectability By Adult Male And Female Rats.**

Clare M Mathes[1], Sarah J Terrill[2], Lindsey A Schier[3]
[1]Department of Neuroscience, Baldwin Wallace University, Berea, OH, United States, [2]Department of Neuroscience, Carthage College, Kenosha, WI, United States, [3]Department of Biological Sciences, University of Southern California, Los Angeles, CA, United States

Non-nutritive sweeteners (NNS) are touted as calorie-free sugar alternatives, but their long-term impacts remain unknown. Here we replicated our previous finding that early-life NNS exposure increases brief-access fructose licking in food-restricted adult rats and assessed if this alteration was, even in part, due to differences in fructose detectability. 2 male and 2 female Sprague-Dawley rats from 3 litters were distributed across 2 groups that from postnatal day (PND) 26-77 either received daily home-cage acesulfame potassium (AceK; 0.1%, 15ml/kg)

access (grp NNS) or not (grp CNTL). At >PND250, the rats were trained and tested to lick water and 5 fructose concentrations presented one at a time in 10-s trials across 30-min sessions. When 23-h food restricted, NNS rats licked fructose more avidly than CNTL rats. The rats were then water-restricted and trained in a 2-response operant task to associate one response spout with sampling water and the other with fructose; licking the correct response spout produced a water reinforcer. Once performance was high and stable (n=5/grp), we used a modified method of limits to assess the rats' ability to discriminate the taste of water and 6 fructose concentrations. While percent correct responding across all concentrations did not differ between groups, performance was significantly lower in grp NNS when the lowest 3 perithreshold concentrations were analyzed separately. Collectively, the findings suggest that while early-life AceK exposure increases hedonic-based adult fructose licking, the change occurs without overtly diminishing discrimination of high fructose concentrations and water, but perithreshold discrimination may be dampened. Thus, NNS intake during a critical developmental phase may have lasting effects on multiple aspects of taste function.

207 **Novel Expression And Roles Of Hedgehog Co-Receptor *Gas1* During Embryonic Tongue Development**

Gabrielle C. Audu, Archana Kumari
Rowan University School of Osteopathic Medicine, Stratford, NJ, United States

Sonic Hedgehog (SHH) signaling is vital for embryonic tongue development. Ligand SHH is expressed in taste buds while receptor *Ptch1* and target gene *Gli1* are present in the taste papillae epithelial and stromal cells. SHH binding with PTCH1 also requires the co-receptor GAS1, and loss of *Gas1* leads to many craniofacial abnormalities. However, GAS1 remains understudied in the tongue, impeding our understanding of molecules regulating tongue development. To address this, we first examined the presence of *Gas1* using the $Gas1^{lacZ}$, a knock-in reporter mouse model, at embryonic day (E) 14.5, E16.5, and E18.5. We used $Gas1^{lacZ/lacZ}$ for loss-of-function studies at these three stages. We observed that *Gas1* loss altered tongue size, with mutant tongues being smaller than *Gas1* heterozygous or wild-type controls at all three embryonic stages. We then processed the tongues for anterior tongue cryosectioning. Unlike *Ptch1* expression, *Gas1* is expressed in embryonic taste buds and the entire lingual stroma, including fungiform and filiform papillae, suggesting additional roles for the GAS1 co-receptor in tongue organogenesis. Using H&E at E14.5, we observed stromal enlargement after *Gas1* loss but not with *Gas1* haploinsufficiency, further indicating that *Gas1* functions are not graded. Concerning the role for *Gas1* in axonal repulsion of enteric neurons, we studied nerves using antibody staining for lingual (NF+) and chorda tympani (P2X3+) fibers. Our preliminary data at the E14.5 stage suggest that the loss of *Gas1* activity leads to alterations in the tongue innervation. Our data provide important insight about the potential crucial role of *Gas1* during tongue development and encourage studies to advance our knowledge of stromal regulation of Hedgehog signaling, which remains understudied during tongue development.

209 **Neural Response Of The Developing Chorda Tympani Following Neonatal Lingual Nerve Transection**

Nicholas P. Weber, Suzanne I. Sollars
University of Nebraska, Omaha, NE, United States

Within fungiform papillae, the somatosensory lingual nerve (LN) innervates the perigemmal region surrounding taste buds innervated by the gustatory chorda tympani nerve (CT). Previous studies show that when either nerve is inured, the typical morphology of both papillae and taste buds deteriorate. While gustatory nerve injury can evoke changes in responses of other gustatory nerves throughout development, whether transection of the LN (LX) similarly affects the CT is not known. The current study uses Sprague-Dawley rats to observe the effect of neonatal (10 days of age) LX on CT response to various taste stimuli on the same side as the surgery. Whole nerve electrophysiology was used at adolescent (14–21 days post LX) and adult (> 50 days post LX) developmental periods. Surgery of the LN was performed proximal to the point of bifurcation of the CT, so the CT remained intact. Whole nerve CT responses to a variety of taste solutions were recorded relative to 0.5 M ammonium chloride (NH_4Cl) used as a standard. The CT is highly responsive to sodium, as it innervates taste receptor cells with epithelial sodium channels that can be suppressed by the sodium channel blocker, amiloride. Preliminary data indicate that LX leads to an increase in adolescent CT response to NaCl and sodium acetate (NaAc) solutions at high concentrations (0.5M and 1.0 M). NaCl and NaAc suppression by amiloride is evident and appears to occur to a greater degree at higher concentrations of NaCl in adolescent rats in the LX condition. An increase in adolescent CT response to NaCl following LX may be due to an increase in amiloride-sensitive sodium channels in taste receptor cells. Ongoing studies are delineating these differences across various developmental periods.

211 **Using Single Cell Pseudotime Lineage Tracing To Identify Co-Regulated Genes In Olfactory Neuron Development**

Hsiu-Yi Lu[1], Hiroaki Matsunami[1,2,3,4,5]

[1]Duke University Department of Molecular Genetics and Microbiology, Durham, NC, United States, [2]Duke University Department of Neurobiology, Durham, NC, United States, [3]Duke Cancer Institute , Durham, NC, United States, [4]Duke Institute for Brain Science , Durham, NC, United States, [5]Duke Initiative for Science & Society , Durham, NC, United States

Odor detection in mammals is mediated by odorant receptors (ORs) the largest family of G protein-coupled receptors expressed in the olfactory sensory neurons (OSNs) in the nasal cavity. However, OR - ligand interactions are poorly understood. This is due to the limitation that most OR show little to no cell surface expression (CSE) in non-olfactory cell types. Our lab previously identified Receptor transporting protein 1 (Rtp1) and Rtp2 as chaperones, which enhance the CSE of many but not all ORs in heterologous cells, suggesting additional chaperones functioning in OR trafficking. During OSN development, ORs translated in the

endoplasmic reticulum (ER) in immature OSNs trigger the unfolded protein response (UPR) that induce expression of RTP1 and RTP2, which coincides with UPR downregulation likely due to ORs to be trafficked to the surface. We hypothesize that there are additional chaperones that are co-induced that's important for relieving the UPR in ER to increase CSE of all ORs. Here, we conducted genetic screen to identify chaperones that increases OR CSE. First, we analyzed publicly available olfactory epithelium scSeq data. We found that genes that are abundantly expressed in mature OSNs are not expressed at the same developmental stage. Rtp1 and Rtp2 expression was increased earlier than other mature OSN markers. We next searched for genes that are co-expressed with Rtp1 across the OSN lineage, genes were then cloned and transiently expressed in HEK293T cells. Finally, the CSE is assessed using antibody staining and flow cytometry. Our finding suggests that additional proteins increase OR CSE when co-expressed with RTP1 for a subset of ORs. However, as effect size is moderate, there may be still additional chaperones that that's required to achieve CSE of all ORs in heterologous cells.

213 **Achems Undergrad Finalist: The Relationship Between Spike Response And Calcium Fluorescence Signal In *Drosophila* Olfactory Receptor Neurons**

Yiyi Xiao[1], Shiuan-Tze Wu[1], Yinan Xuan[2], Chih-Ying Su[1]

[1]Department of Neurobiology, University of California San Diego, La Jolla, CA, United States, [2]Department of Electrical and Computer Engineering, University of California San Diego, La Jolla, CA, United States

Insects, including disease vectors, rely on olfaction to identify hosts, find food, and seek mates. Genetically encoded calcium sensors, such as GCaMP, are widely used as proxies for electric activity of olfactory receptor neurons (ORNs) that respond to host or other ethologically relevant odors. However, calcium-induced fluorescence signals are difficult to interpret without first understanding their relationship with the spike responses of a target neuron. Using *D. melanogaster* as a model, we performed simultaneous single-sensillum recording and trans-cuticle antennal calcium imaging to determine the spike-calcium relationship in 16 ORN types, encompassing all four morphological neuronal classes and representing the majority of genetically identified antennal ORNs with known cognate ligands. Our results show that the calcium response dynamic range varies widely across ORN types, with saturation occurring from ~10% to over 200% $\Delta F/F$. Using spike response as the reference, we found that certain ORN types exhibited a broad dynamic range of spike frequencies while having a limited span of calcium response. Last, we observed linear association between spike and calcium response across all examined ORNs, which enabled us to create linear regression models for conversion. Together, our systematic survey revealed unexpected heterogeneity in the spike-calcium response relationships among ORN types and provided useful datasets for subsequent functional comparison among ORNs.

215 **A Genetic Platform For Functionally Profiling Odorant Receptors Ex Vivo With Olfactory Cilia**

Masayo Omura, Eugene Lempert, Paul Feinstein
Hunter College, CUNY Department of Biological Sciences, New York, NY, United States

The molecular basis for odor perception in humans has remained elusive because of the difficulty in studying odorant receptors (ORs) outside of olfactory sensory neurons (OSNs). Efforts toward OR expression and functional profiling have been met with limited success mainly due to the poor efficiency of in vitro cell surface expression. Olfactory cilia contain all the components of the olfactory signal transduction machinery and can be placed into an ex vivo well-plate assay to rapidly measure odor-specific responses. Using our OR gene choice enhancer transgene system for expressing a defined OR[1] in millions of OSNs, we now describe transgenic mice for several human ORs and their isolated cilia reveal 10 to 100-fold more sensitivity as compared to previous in vitro assays. A single mouse can produce cilia for up to 4,000 assays, and isolated olfactory cilia can be stored frozen and thus preserved. We have identified a locus in the genome where a single human OR transgene has landed and is expressed in at least 75% of all OSNs. The identification of this locus allows us to establish a CRISPR-Cas9 based system for the systematic characterization of all functional human ORs. This pipeline offers a sensitive and highly scalable ex vivo odor screening platform that opens the door for decoding human olfaction. ---------- 1. Cell Rep. 2016 Jul 26;16(4):1115-1125. doi: 10.1016/j.celrep.2016.06.047. Epub 2016 Jul 7. MouSensor: A Versatile Genetic Platform to Create Super Sniffer Mice for Studying Human Odor Coding Charlotte D'Hulst , Raena B Mina , Zachary Gershon, Sophie Jamet, Antonio Cerullo, Delia Tomoiaga, Li Bai, Leonardo Belluscio, Matthew E Rogers, Yevgeniy Sirotin, Paul Feinstein PMID: 27396335 .

217 **Constancy Of Olfactory Cilia &Ndash; A Core Principle Of Building A Reliable Chemosensory System?**

Kirill Ukhanov[1,2], Cedric Uytingco[3], Carlos de Celis[1], Dana Shively[1], Chao Xie[1], Dylan Mordeccai[1], Lian Zhang[1], Steven Munger[1,2], Jeffrey Martens[1,2]

[1]University of Florida, Department of Pharmacology, Gainesville, FL, United States, [2]Center for Smell and Taste, Gainesville, FL, United States, [3]10x genomics

Odorant receptors and all essential signal transduction molecules are compartmentalized in the cilia of olfactory sensory neurons (OSNs) where primary detection of odorants occurs. The large number and length of olfactory cilia provide an extensive receptive surface for odor detection. Shortening or loss of olfactory cilia due to environmental factors or disease impairs odor detection, demonstrating that the stability of these organelles is critical for olfactory function. However, it is unclear if olfactory cilia vary in specific ways that affect the fidelity of the odorant receptive field. Using adenovirally assisted ectopic expression of ciliary targeted fluorescent probes, we analyzed intact cilia morphology in live OSNs *in situ*. This unbiased approach revealed a previously unappreciated constancy of cilia length and number, on average 25-30 μm long and 20-25 per neuron across the olfactory epithelium. Despite stochastic variation between neurons, average cilia length and number were also

independent of animal age and sex, genetic background, and even of rodent species. This constancy was maintained across the OE in genetically defined OSNs expressing distinct receptor types M71, I7 or TAAR3. However, a subclass of guanylate cyclase-D-expressing OSNs displayed dramatically shorter cilia on average 12 μm long, suggesting the cilia length but not their number may be controlled by the intrinsic signal transduction machinery. Together, our findings argue that OSNs that use cyclic AMP to transduce odors have similar ciliary morphologies and numbers, allowing them to maintain a consistent physical receptive field. These data provide a basis for understanding structure-function relationship between cilia morphology and odorant detection as a foundation for building a high-fidelity chemosensory organ.

219 **More Spice, Less Salt: How Capsaicin Affects Liking For And Perceived Saltiness Of Foods In People With Smell Loss**

Stephanie R. Hunter, Pamela H. Dalton
Monell Chemical Senses Center, PHILADELPHIA, PA, United States

When people lose their sense of smell, they often find food less enjoyable and alter their diet to increase flavor and eating enjoyment. One dietary alteration that is often reported is adding more salt to foods, and preferring salty foods. Over time, this can lead to excess salt intake and increased risk for cardiovascular disease. Among individuals with a normal sense of smell, the addition of capsaicin to reduced salt dishes has been shown to increase saltiness perception and overall flavor; whether adding capsaicin produces measurable improvements in saltiness perception and food liking has not been studied in those with smell loss. The purpose of this study was to determine 1) whether salt intake in those with smell loss differs from population averages, and 2) whether capsaicin increases flavor and salt taste intensity, and enjoyment of foods in individuals with smell loss. Individuals having confirmed partial or total smell loss for at least 12 weeks rated total flavor, taste quality, and spiciness intensities, and liking of various food samples with different levels of spice and/or salt. 24-hour urine samples were also collected to determine sodium intake. Results indicate that although sodium intake is higher than recommended, those with smell loss do not consume more sodium than population averages (2741 ± 271 mg vs 3039 ± 99 mg, respectively; p=0.3). Adding moderate amounts of capsaicin to soup increased total flavor intensity ($p<0.001$) and salt taste intensity ($p=0.007$) compared to the soup without capsaicin. However, the ability of capsaicin to increase liking differed by food type. Addition of capsaicin can serve as a dietary tool to improve flavor and increase salt taste intensity, and eating enjoyment in people with smell loss.

221 **Sorting Of Odor Dilutions Is A Meaningful Addition To Assessments Of Olfactory Function**

Thomas Hummel[1], Anne Huster[1], Jörn Lötsch[2,3]
[1]Smell & Taste Clinic, Department of Otorhinolaryngology, TU Dresden, Dresden, *, Germany, [2]Institute of Clinical Pharmacology, Goethe-University, Frankfurt a.M., *, Germany, [3]Fraunhofer Institute for Translational Medicine and Pharmacology ITMP, Frankfurt a.M., *, Germany

Background: The categorization of individuals as normosmic, hyposmic, or anosmic from test results of odor threshold, discrimination, and identification may provide a limited view of the sense of smell. The purpose of this study was to expand the clinical diagnostic repertoire by including additional tests. Methods: A random cohort of n = 135 individuals (83 women and 52 men, aged 21 to 94 years) was tested for odor threshold, discrimination, and identification, plus a distance test, in which the odor of peanut butter is perceived, a sorting task of odor dilutions for phenylethyl alcohol and eugenol, a discrimination test for odorant enantiomers, a lateralization test with eucalyptol, a threshold assessment after 10 min of exposure to phenylethyl alcohol, and a questionnaire on the importance of olfaction. Unsupervised methods were used to detect structure in the olfaction-related data, followed by supervised feature selection methods from statistics and machine learning to identify relevant variables. Results: The structure in the olfaction-related data divided the cohort into two distinct clusters with n = 80 and 55 subjects. Odor threshold, discrimination, and identification did not play a relevant role for cluster assignment, which, on the other hand, depended on performance in the two odor dilution sorting tasks, from which cluster assignment was possible with a median 100-fold cross-validated balanced accuracy of 77–88%. Conclusions: The addition of an odor sorting task with the two proposed odor dilutions to the odor test battery expands the phenotype of olfaction and fits seamlessly into the sensory focus of standard test batteries.

223 **Factors Associated With Formal Diagnosis Of Olfactory Dysfunction In The United States**

Bita R. Naimi[1], Emily A. Garvey[1], Stephanie Hunter[2], Alexander Duffy[1], Pamela Silberman[3], Katie Boateng[3], Suz Schrandt[5], Paule V. Joseph[6], Claire Murphy[4], Jenifer Trachtman[2], Pamela H. Dalton [2], Nany E. Rawson[2], Gurston Nyquist[1]
[1]Thomas Jefferson University Hospital, Philadelphia, PA, United States, [2]Monell Chemical Senses Center, Philadelphia, PA, United States, [3]The Smell and Taste Association of North America, Philadelphia, PA, United States, [4]Department of Psychology, San Diego State University, San Diego, CA, United States, [5]ExPPect, LLC, Arlington , VA, United States, [6]National Institute of Alcohol Abuse and Alcoholism, Section of Sensory Science and Metabolism, Bethesda, MD, United States

After olfactory dysfunction (OD) onset, many patients may never receive a formal diagnosis from a provider. This study examines demographic factors correlated with a formal diagnosis of OD. An online survey of patients in the United States with smell and/or taste disorders was administered on April 6-28, 2022. 4,629 participants (12% 18-24 years, 29% 25-39 years, 39% 40-60 years, 20% 60 years or older; 73% female; 88% white) were included in the analysis. Logistic regression was performed to determine factors associated with formal diagnosis of OD. 1,243 (27%) participants had a diagnosis documented in their medical record. Participants aged 25-39 (OR 2.52, CI [1.86, 3.45]), 40-60 (2.56 [1.88, 3.51]), and >60 (1.98 [1.46, 2.72]) were more likely to have a

diagnosis than those aged 18-24 years. Males (1.28 [1.07, 1.52]) were more likely to have a diagnosis than females. Insured patients (1.68 [1.13-2.59]) were more likely to be diagnosed than uninsured patients. Patients with public insurance (0.76 [0.63-0.91]) were less likely to have a formal diagnosis. Patients who saw an otolaryngologist (ENT) (5.83 [4.89-6.96]) and who completed psychophysical smell testing (1.83 [1.47, 2.29]) were more likely to have a diagnosis than those who did not. COVID-19 etiology and symptom onset before the COVID-19 pandemic did not affect the likelihood of obtaining a diagnosis. This study highlights the lack of formal diagnosis of OD among survey respondents, and the importance of objective smell testing and referral to ENT or a trained provider to establish a diagnosis of olfactory dysfunction.

225 **Investigation Of Odor Cue Evoked Craving Responses In Alcohol Use Disorder**

Khushbu AGARWAL[1,2], Valentina Parma[3], Reza Momenan[4], Paule V Joseph[1,2]

[1]National Institute on Alcohol Abuse and Alcoholism, NIH, Bethesda, MD, United States, [2]National Institute of Nursing Research, Bethesda, MD, United States, [3]Monell Chemical Senses Center, Philadelphia, PA, United States, [4]Clinical NeuroImaging Research Core, National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD, United States

Alcohol use disorder (AUD) is a chronic relapsing brain condition. Craving or, desire to drink induced by odors of alcoholic beverages play a predominant role in relapse. To establish the contribution of alcohol odor cues in inducing craving, we must first determine the olfactory abilities of individuals with AUD in existence of contradictory literature; some show impaired olfactory discrimination, identification, or recall, while others preserved olfaction. Further to dissociate the role of olfactory or trigeminal systems in odor cue evoked craving in face of known olfactory deficit we will record the orbitofrontal cortex (OFC) activation patterns of AUD participants using functional near infrared spectroscopy (fNIRS) during the odor cue reactivity task. We describe a cross-sectional proof-of-concept study wherein AUD participants (18-50 years, non-smokers) would first undergo UPSIT for baseline olfactory function. We will then record threshold, intensity, pleasantness, subjective craving (urge to drink on scale 1-7) and OFC activation for odor cues (olfactory/ethanol; trigeminal/cinnamon; neutral/phenyl ethyl alcohol at fifteen geometric dilutions ranging from 1.22ppm-48% v/v). For threshold, odor cues are presented in random triplets (each odor cue for 3 sec) while, for intensity and pleasantness each odor cue will be presented randomly for 3 seconds, separated by an interval of 20sec. To confirm the presence of individual variability in olfactory functioning and its impact on odor cue stimulation of olfactory or trigeminal systems to induce craving response in individuals with AUD, linear mixed effect models will be used to determine group differences (anosmics/hyposmics vs. normosmics) on outcomes (odor cue: thresholds, intensity, pleasantness, subjective craving, and OFC activation pattern).

227 **Anatomical Differences In The Accessory Olfactory Bulb In A Model Of Fragile X Syndrome**

Marcela Navarrete[1], Ricardo C. Araneda[2], Alexia Nunez-Parra[1], Jorge Mpodozis[1]

[1]Universidad de Chile , Santiago , *, Chile, [2]University of Maryland , College Park , MD, United States

Fragile X Syndrome (FXS) is a neurodevelopmental disorder characterized by the absence of the Fragile Mental Retardation Protein. Individuals with FXS show intellectual disability, difficulties in social interaction and atypical sensory perception, among others features. Whether and how the lack of FMRP affects early stages of sensory processing remains poorly explored. Here, we compared the anatomical traits of the olfactory bulbs of *Fmr1*-KO and WT adult mice. We did not find differences between WT and *Fmr1-KO* mice in the main olfactory bulb, a brain region that mostly processes non-social odors. Interestingly, even though the volume of the accessory olfactory bulb (AOB), a brain region that process information of social odors, was not different, the volumetric ratio between the anterior and posterior division of the AOB (aAOB/pAOB) was smaller in the *Fmr1-KO* compared to the WT. This difference was related to a decrease in the volume of the aAOB glomerular layer and to an increase in the volume of the pAOB granular cell layer. Our results suggest that the *Fmr1* mutation leads to specific morphological abnormalities in the initial regions of the olfactory pathway, which could partially explain the atypical social behaviors described in the *Fmr1*-KO mice. To determine whether the anatomical differences found lead to differences in neuronal responses, we are currently examining the neural responses of the AOB circuit in *Fmr1*-KO and WT mice.

229 **Patient Centered Olfactory Research In The Covid Era: Age-Related Differences And Implications For Neurodegenerative Disease**

Claire Murphy[1,2], Stephanie Hunter[3], Nancy E. Rawson[3], Pamela H. Dalton[3], Jenifer Trachtman[3], Gurston Nyquist[4], Katie Boateng[5], Pamela Silberman[5], Suz Schrandt[6], Paule V. Joseph[7], Bita Naimi[4], Emily Garvey[4]

[1]Department of Psychology, San Diego State University, San Diego, CA, United States, [2]Department of Psychiatry, University of California, San Diego Medical School, La Jolla, CA, United States, [3]Monell Chemical Senses Center, Philadelphia, PA, United States, [4]Department of Otolaryngology, Thomas Jefferson University, Philadelphia, PA, United States, [5]Smell and Taste Association of North America, Philadelphia, PA, United States, [6]ExPPect, LLC, Arlington, VA, United States, [7]National Institute of Alcohol Abuse and Alcoholism, Section of Sensory Science and Metabolism & National Institute of Nursing Research, Bethesda, MD, United States

World-wide some 15 million people may suffer from olfactory impairment associated with long COVID. Current treatments and research on new treatments for taste and smell disorders are limited. Actively involving patients in research has the potential to catalyze the dynamic exchange and development of new ideas and approaches to facilitate biomedical research, discovery, and therapeutics. We assessed patients' perceptions of the efficacy of treatments for olfactory impairment with a focus on age using an online questionnaire completed by 5,352

people in the United States. Logistic regression was used to determine predictive variables of reported treatment efficacy for patients 18-24, 25-39, 40-60 and 60+ years old who were treated with nasal steroids, oral steroids, zinc, nasal rinse, smell training, theophylline, PRP, and Omega 3. The most consistent predictor was age, with the majority of those 40-60 and 60+ reporting that nasal steroids, oral steroids, zinc, nasal rinse and smell training were only slightly effective or not effective at all. There were no differences between those who found theophylline or PRP effective, though numbers treated were small. Many of these treatment strategies target regeneration and immune response, processes compromised by age. Findings suggest that older patients may have a more limited regeneration potential in the setting of olfactory impairment and emphasize the need to include patients of all ages in clinical trials. Older adults with olfactory impairment are at increased risk for Alzheimer's disease (AD). We speculate that olfactory impairment associated with long COVID introduces the potential for a significant rise in AD. Long COVID-associated olfactory impairment increases the urgency for translational and clinical research on novel treatment strategies.

231 **The Association Between Depth Of The Olfactory Sulcus, Age, Gender And Olfactory Function: An Mri-Based Investigation In More Than 1000 Participants**

Zetian Li[1], Hanani A. Manan[1,2,3], Hanna Heitmann[1], Veronica Witte[4], Kerstin Wirkner[5], Steffi Riedel-Heller[4], Arno Villringer[4], Thomas Hummel[1]

[1]Smell & Taste Clinic, Depatrment of Otrohinolaryngology, Technische Universität Dresden, Dresden, *, Germany, [2]Functional Image Processing Laboratory, Department of Radiology, Pusat Perubatan Universiti Kebangsaan Malaysia (PPUKM), Kuala Lumpur , *, Malaysia, [3]Department of Radiology and Intervency, Hospital Pakar Kanak-Kanak (Specialist Children Hospital), University Kebangsaan Malaysia, Kuala Lumpur , *, Malaysia, [4]Institute of Social Medicine, Occupational Health and Public Health, University Leipzig, Leipzig, *, Germany, [5]Leipzig Research Centre for Civilization Diseases, Leipzig University, Leipzig, *, Germany

Objective: The present study aimed to investigate the relationship between olfactory sulcus (OS) depth and olfactory function considering age and gender and to provide normative data on OS depth in a population with normal olfactory function. *Materials and methods*: OS depth was obtained using T1 magnetic resonance imaging scans. Participants (mean age ± *sd* = 57 ± 16 years, ranging from 20-80 years) were screened for olfactory function using the Sniffin' Sticks Screening 12 test. They were divided into an olfactory dysfunction group (n = 604) and a normosmia group (n = 493). Participants also completed questionnaires measuring depression, anxiety and quality of life. *Results*: The right OS was deeper than the left side in all age groups. On the left side, women had deeper OS compared with men, exhibiting a higher degree of symmetry in left and right OS depth in women. Normative data for minimum OS depth was 7.55 mm on the left and 8.78 mm on the right for participants aged between 18 and 35 years (n = 144), 6.47 mm on the left and 6.99 mm on the right for those aged 36-55 years (n = 120), and 5.28 mm on the left and 6.19 mm on the right for participants older than 55 years (n = 222). *Conclusion*: Considering the limited resolution of the presently used T1 weighted MR scans and the nature of the olfactory screening test, the olfactory function was found to be largely determined by age, while OS depth explained only minor portions of its variance.

233 **A Bayesian Approach To Measure Trigeminal Thresholds.**

Sarah Brosse[1], Jason Steffener[2], Benoit Jobin[3], Oliver Fortier-Lebel[3], Johannes Frasnelli[1,4]

[1]Department of Anatomy, Université du Québec à Trois-Rivières, Trois-Rivières, QC, Canada, [2]Faculty of health science, University of Ottawa, Ottawa, ON, Canada, [3]Department of psychology, Université du Québec à Trois-Rivières, Trois-Rivières, QC, Canada, [4]Research Center, Sacré-Coeur Hospital of Montreal, Montreal, QC, Canada

The trigeminal system is a chemical sense involved in the perception of freshness, pungency, pain, tickling and burning when we smell odors. The measurement of trigeminal sensitivity has the potential to improve the early diagnosis of Parkinson's disease. Trigeminal sensitivity is currently measured by a standard method: the Trigeminal Lateralisation Task (TLT). However, this task has limitations in terms of duration (30 min) and validity that do not allow it to be introduced in a clinical setting. The objective of this study is to evaluate the effectiveness of a new algorithm to calculate trigeminal sensitivity, the QUEST, and to compare it to the standard method (TLT), a semi-quantitative measure of trigeminal sensitivity. More specifically, the TLT includes a total of 40 stimulations with a constant duration of 500 ms whereas our new task presents stimulations whose duration is determined by a Bayesian algorithm thus allowing to considerably reduce the number of stimulations and to obtain a perceptual threshold for each nostril. This QUEST method is very promising since it has already been used to measure the olfactory and gustatory threshold in previous studies. We hypothesized that the QUEST method will correlate with the standard method, will be shorter than the standard method, will discriminate good from poor locators, and will define a perceptual threshold. For this purpose, we recruited 20 younger (18-35 years old) and 20 older (55+ years old) participants who completed the two tasks in a randomized order. The results of the two methods for each group of participants will be compared and discussed in light of the existing literature. In summary, this study highlights a new, shorter and more accurate method of measuring the trigeminal system that can be used in a clinical setting.

235 **Can Humans Smell Tastants?**

Shuo Mu, Eleonora Vissers, Markus Stieger, Sanne Boesveldt
Division of Human Nutrition and Health, Wageningen University,, Wageningen, *, Netherlands

Although general consensus suggests that tastants have no smell, there are limited indications that humans are able to perceive tastants via orthonasal olfaction. This study aims to (a) explore whether humans can

discriminate between solutions of basic tastants and water through orthonasal and retronasal olfaction, (b) and if so, to examine what volatile odor compounds (VOCs) underlie the discrimination ability. Solutions of five basic tastants (sucrose, sodium chloride, citric acid, monosodium glutamate, quinine dissolved in water, with concentrations of 25g/100g, 3g/100g, 5g/100g, 1g/100g, and 0.0083g/100g respectively) and two fatty acids (oleic and linoleic acid dissolved in mineral oil, with concentrations of 40g/100g for both solutions) were prepared. Triangle discrimination tests were performed (n=41 in duplicate) to assess whether the tastant solutions can be distinguished from the blanks (solvents) through ortho- and retronasal olfaction. Interestingly, participants were capable of distinguishing all tastant solutions from blank through orthonasal olfaction ($p<0.05$ for quinine vs blank, $p<0.01$ for the other six comparisions) . Only sucrose, sodium chloride, oleic acid, and linoleic acid could be distinguished from the blank by retronasal olfaction ($p<0.05$ for sucrose vs blank, $p<0.01$ for the other three comparisons). Participants indicated for sucrose solutions that olfactory discrimination was based on perceiving a sweet smell. For all other tastant solutions, olfactory discrimination was not associated with smelling a specific taste quality. Determination of the VOC composition of the headspace is ongoing and will shed light on these intriguing findings and help to explain why and how humans can smell tastants.

237 **Olfactory Detection Of Maltrin, A Maltodextrin, In C57Bl/6J Mice.**

Elizabeth A. Hamel, Ellie Williams, Alan C. Spector, Adam Dewan
Florida State University, Tallahassee, FL, United States

Maltodextrin solutions have been shown to be highly palatable to rodents and appear to generate a taste percept that is distinct from other carbohydrate stimuli such as sugars. While it is believed that taste drives consumption in these rodent models, the question remains as to whether olfaction contributes to the behavioral responses seen to such stimuli. Here, we tested the perithreshold olfactory sensitivity of head-fixed mice (C57BL/6J, n = 7) in a Go/No-Go conditioning procedure to Maltrin M580, a maltodextrin (mean DP = 6.2). Mice had a head-fixation bar surgically implanted and following recovery were water-restricted (1ml/day) for a minimum of 14 days prior to training. Head-fixed mice were trained in a precision olfactometer to report the detection of odor and then tested on 1 Maltrin concentration a day (0.125-32% w/v) in descending order. Volatiles underwent a 10x air dilution before being presented to the animal. Psychometric detectability curves were derived for each animal (mean $R^2 = 0.96$). The $\log_{10}$ mean ± se EC_{50} (operationally defined as threshold) was -0.264 ± 0.07 (corresponding to 0.56% Maltrin) *before* the 10x air dilution of the odor headspace. These results show that mice can detect Maltrin, or some contaminant, via smell and highlight the chemosensory complexity of this stimulus. Typical maltodextrin taste tests in rodents include concentrations ≥1% that, based on these results, are detectable by olfaction. Accordingly, signals from the olfactory system may play a role in guiding behavioral responses to maltodextrins in mice in addition to the contribution of gustatory processes. We are currently conducting further behavioral tests in efforts to distinguish between the relative contribution of olfactory and gustatory signals in the motivated responsiveness of mice to Maltrin.

239 **Tracking Covid-19 Variants With Scentinel, A Rapid Smell Test**

Valentina Parma[1], Benjamin Schalet[2], Anne Zola[3], Stephanie Hunter[1], Edith Adjei-Danquah[1], Micheal Kallen[3], Emily Ho[3], Gregory Smith, Jr[3], Chad Achenbach[3], Richard Gershon[3], Pamela H. Dalton[1]
[1]Monell Chemical Senses Center, Philadelphia, PA, United States, [2]Amsterdam Universitair Medische Centra, Amsterdam, *, Netherlands, [3]Northwestern University Feinberg School of Medicine, Chicago, IL, United States

SCENTinel, a rapid multifunction smell test assessing odor detection, intensity, and identification, is a candidate tool to enable population surveillance of smell disorders. To examine its potential, we carried out a cross-sectional study on a sample seeking outpatient SARS-CoV-2 testing at Northwestern Medicine and University of Pennsylvania sites throughout the COVID-19 pandemic (April 2021 - July 2022). From the original sample of 2691 participants, 1979 (74%) were included in the final analysis and matched with existing medical records that had time-matched SARS-CoV-2 PCR test results. The sample includes 61% women, 75% white, age: 50±16 years old; 127 (6.9%) tested positive for SARS-CoV-2 infection during periods of dominance by several virus variants (n mixed = 28; n delta=28; n omicron=71). The overall SCENTinel score shows a moderate negative correlation with SARS-CoV-2 positive PCR test results, $r(693) = -0.26$, $p < 0.001$, implying that failing the SCENTinel test is associated with having a positive SARS-CoV-2 test result. Overall, percent agreement between SCENTinel score and PCR test result (84.5%), specificity (90%), and negative predictive value (94%) were high; sensitivity was low (13%). Logistic regressions showed odor intensity was a significant predictor beyond self-reported symptoms, including self-reported smell loss. Taking into account the number of symptoms, if a participant failed the odor intensity subtest, they were 17.94 times more likely to test positive for SARS-CoV-2 (95% CI = [4.36, 75.82]). SCENTinel was able to track SARS-CoV-2 positivity rate in the population during the time when Mixed and Delta variants were dominant, but not during Omicron. We conclude that SCENTinel is a tool that can be rapidly deployed in the population to track smell function.

241 **Transient Receptor Potential Channels In Ciliates?**

Wade E. Bell
Virginia Military Institute, Lexington, VA, United States

Transient receptor potential (TRP) channels are composed of a broad family of ion channels that allow passage of various cations into cells under a wide variety of stimuli. They are associated with behaviors generated by hot peppers (capsaicin), temperature sensitivity, vision in insects, and many other activators. Of particular interest is the role that TRP-V1 channels play in pain reception and taste *Paramecium* are excitable cells that respond to small molecules produced by plants or bacteria. Initial studies by our lab show that they are responsive to appropriate levels of TRP channel ligands such as capsaicin and ruthenium red. Specifically, we used motion analysis to assess swimming speed and frequency of turning, both well-documented

indicators of *Paramecium* membrane potential. Capsaicin, a TRP-V1 activator, caused a significant reduction in swimming speed of *Paramecium* in a test buffer. Cells exposed to capsaicin also turned more frequently than control cells. In contrast, cells exposed to ruthenium red, a TRP channel antagonist, swam straighter and at a higher speed than controls. In order to further analyze effects of TRP channel agonists and antagonists on *Paramecium*, we did live cell calcium imaging on treated cells. Capsaicin treated cells showed an expected calcium influx upon initial treatment. Over time, calcium levels were reduced, indicating possible desensitization. We continue to do live cell ion imaging to assess the effects of ruthenium red and other antagonists on intracellular calcium levels. Further studies on pharmacology and TRP channel genomic homologies in *Paramecium* should provide interesting evolutionary comparisons for both conservation of channel characteristics and deviations from vertebrate norms.

243 **Molecular Characterization Of The Intestinal Tuft Cells Using The Intestinal Organoids Derived From Non-Human Primates**

Akihiko Inaba[1, 2, 3], Ayane Arinaga[4], Keisuke Tanaka[5], Ken Iwatsuki[4], Hiroo Imai[2]

[1]Grad. Shc. of Sci., Kyoto University, Kyoto, *, Japan, [2]Center for the Evolutionary Origins of Human Behavior, Kyoto University., Aichi, *, Japan, [3]JSPS Research Fellow, Tokyo, *, Japan, [4]Apli. Biosci., Tokyo University of Agriculture, Tokyo, *, Japan, [5]Genome Research Center, Tokyo University of Agriculture, Tokyo, *, Japan

Intestinal epithelium indirectly contacts the external environment; however, it is continuously exposed to various substances in the intestine that can be beneficial and harmful to the body. To maintain homeostasis, intestinal epithelial cells (IECs) exert multiple sensing mechanisms against nutrients, chemicals, and microorganisms. Tuft cells, an atypical epithelial cell-type found in many organs including the intestine, are known to be taste-like chemosensory cells (or solitary chemosensory cells) because these cells express molecules involved in the taste-signal transduction. Recently, intestinal tuft cells have been found to activate group 2 innate lymphoid cells (ILC2) by secreting interleukin (IL) -25 and leukotrienes at the early stage of parasite infection which is essential to exclude parasites from the gut. Although many studies using mice have shown that intestinal tuft cells are key sentinels to regulate the innate immune system in the gut, whether the same mechanism exists in primates has not been investigated. Recently, we generated intestinal organoids from macaque monkeys (Rhesus macaques and Japanese macaques) to analyze the intestinal chemosensory cells of primates *in vitro*. We have shown that tuft cell marker genes reported in mice, such as *POU2F3*, *TRPM5*, and *CHAT*, were highly expressed in the organoids induced by Th2 cytokines such as IL-4 and IL-13 by the transcriptome analysis. In addition, acetylcholine accumulation in the IL-4 / IL-13-treated organoids was detected by LC-MS. Here, we report on our current work that the fluorescent protein labeling of tuft cells in the organoids using Cas9-mediated genome editing. We will discuss the chemosensory function of tuft cells as well as the latest technique to label this unique cell-type in primates.

245 **Transcriptomics Profiling In T1R2 Null Muscle Fibers**

Jordan E. Boyd, Joan Serrano , Carter Mason , Ian S. Brown , George A. Kyriazis
The Ohio State University , Columbus , OH, United States

Sweet taste receptors (STRs,T1R2/T1R3) were discovered in the taste buds of the tongue but have been found in other tissues such as the pancreas and intestine where they function as nutrient sensors and regulate endocrine function. STRs are also expressed in skeletal muscle where they are closely associated with oxidative type 1 fibers and may have a role in oxidative function. Accordingly, muscle-specific genetic ablation of T1R2 (mKO) caused increases in muscle mass, muscle fiber size, improved oxidative capacity and mitochondrial function. To understand the role of STRs signaling in skeletal muscle, we performed transcriptomic analysis in WT and mKO mice. Myogenin-Cre mice were crossed with HA-tagged RPL22 (Ribo-Tag) to express hemagglutinin (HA) in myocytes only (Myo-Cre x Ribotag fl/fl; WT). These mice were subsequently crossed with T1R2 fl/fl mice to generate T1R2-KO mice with tagged myocyte ribosomes (Myo-Cre x Ribotag fl/fl x T1R2 fl/fl; mKO). The solei RNA from 5 WT and 5 mKO mice was immunoprecipitated and analyzed with a mouse Clariom S array. Several pathway modifications are present in the mKO model compared to the WT. Down-regulation of electron transport chain/oxidative phosphorylation and most cytoplasmic ribosomal proteins ranked as the most significantly altered pathways. T1R2 ablation also altered the gene expression repertoires of most GPCRs while the GPCR signaling pathways trended ($p=0.09$) to significance due increased expression of Gi-coupled phosphodiesterases. Altered signaling pathways included WNT, EGF, MAPK, IL17 and chemokine, while m-TOR, toll-like, delta notch, androgen and estrogen trended to significance ($p<0.09$). These signaling adaptations may support some observed phenotypic changes in mKO mice, such as the increased muscle mass, fiber size and overall metabolism.

247 **Isothiocyanates In Brassica Vegetables Impact Glucagon Like Peptide-1 Secretion, Potentially Through Intestinal Transient Receptor Potential Ankyrin 1 And Bitter Taste Receptors**

Anqi Zhao[1], Elizabeth Jeffery[1,2], Michael Miller[1,2]

[1]Division of Nutritional Sciences, University of Illinois at Urbana-Champaign, Urbana, IL, United States, [2]Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, Urbana, IL, United States

The activation of intestinal bitter taste receptors (T2Rs) and transient receptor potential ankyrin 1 (TRPA1) have been found to improve host glucose homeostasis and reduce food intake, mainly through inducing the secretion of gut hormones. Brassica vegetables are rich in glucosinolates (GSLs), which upon hydrolysis, produce isothiocyanates (ITCs) with a bitter and pungent flavor. We hypothesized that ITCs may interact with the intestinal T2Rs and/or TRPA1 to induce the secretion of glucagon-like peptide-1 (GLP-1). To test the hypothesis, murine enteroendocrine cell line STC-1 cells were treated with allyl isothiocyanate (AITC), phenethyl isothiocyanate (PEITC), benzyl isothiocyanate (BITC), sulforaphane (SF), sinigrin (SN), or vehicle (0.1%

DMSO) for 1 hour at 6.25, 12.5, and 25.0 μM for active GLP-1 measurement. Mechanisms of ITCs inducing GLP-1 secretion were tested by inhibiting intracellular calcium pathways and knockdown of Tas2r138 or Trpa1 gene. The in vivo study was performed by randomizing C57BL/6J mice (n=8) to receive a sequence of a one-dose gavage of AITC/SN/SF/saline, separated by a one-week wash out period. Serum GLP-1 was measured. In vitro studies showed that all tested ITCs increased GLP-1 secretion from STC-1 cells compared to vehicle at all tested levels (p <0.05), whereas SN showed no impact. Calcium pathway inhibitors significantly reduced GLP-1 secretion in AITC/SF treated cells compared to AITC/SF only (p <0.05). Knockdown of Trpa1 significantly reduced GLP-1 secretion induced by both AITC and SF, whereas knockdown of Tas2r138 reduced GLP-1 secretion induced by AITC, but not SF. In vivo study results are under analysis. This study may provide evidence for a novel therapeutic mechanism explaining brassica vegetables improving glucose homeostasis and weight management.

249 **Achems Undergrad Finalist: Glucagon-Like Peptide-1 Receptors In The Gustatory Cortex Influence Food Intake**

Milayna M. Kokoska, Amanda M. Dossat, Jessica Whitaker-Fornek, Sarah E. Sniffen, Aishwarya S. Kulkarni, Erica S. Levitt, Daniel W. Wesson
Department of Pharmacology and Therapeutics, University of Florida, Gainesville, FL, United States

The gustatory region of the insular cortex (GC) processes taste information in manners important for taste-guided behaviors, including food intake itself. In addition to oral gustatory stimuli, GC activity is also influenced by physiological states including hunger. The specific cell-types and molecular mechanisms that afford the GC with such abilities are unclear. Glucagon-like peptide 1 (GLP-1) is produced by neurons in the brain whereafter it can act upon GLP-1 receptor-expressing (GLP-1R+) neurons found in several brain regions. In these brain regions, GLP-1 receptor (GLP-1R) agonism suppresses homeostatic food intake and dampens the hedonic value of food. Here, we report in mice of both sexes that cells within the GC express *Glp1r* mRNA and further, by *ex vivo* brain slice recordings, that GC GLP-1R+ neurons are depolarized by the selective GLP-1R agonist, exendin-4 (Ex-4). Next we found that chemogenetic stimulation of GLP-1R+ neurons, and also pharmacological stimulation of GC-GLP-1Rs themselves, both reduced homeostatic food intake. When maintained on a high-fat diet, obese mice exhibited impaired food intake responses when Ex-4 was administered into the GC. Yet, when obese mice were switched to a low-fat diet, the effect of GC Ex-4 was restored – indicating that GC GLP-1R influences may depend upon palatability of the food. Together, these results provide evidence for a specific cell population in the GC which may hold roles in both homeostatic and hedonic food intake.

251 **Don Tucker Finalist: Targeting Bitter Taste Receptor 14 To Kill Head And Neck Squamous Cell Carcinoma**

Zoey A Miller[1, 2], Jennifer F Jolivert [1], Ray Z Ma[3], Sahil Muthuswami[1], Ryan M Carey [1], Robert J Lee[1]
[1]Department of Otorhinolaryngology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, United States, [2]Pharmacology Graduate Group, Perelman School of Medicine, Philadelphia , PA, United States, [3]Harriton High School, Bryn Mawr, PA, United States

Within head and neck squamous cell carcinomas (HNSCCs), oral and oropharyngeal squamous cell carcinomas (SCCs) affect ~34,000 people in the US each year. Patients face a 50% 5-year survival rate and an overall decline in quality of life (QOL) due to morbidities of current treatments. Novel targeted therapies are needed for SCCs to prolong survival and to maintain QOL. Due to the oral localization of SCCs, bitter taste receptors (T2Rs) have sparked interest as new therapeutic targets. T2Rs are a subset of G-protein coupled receptors (GPCRs) that induce an intracellular calcium (Ca^{2+}) response when activated. T2R14, one of 25 T2R isoforms, has implications in extraoral cancers, including breast and pancreatic. However, its role in HNSCCs remains unknown. Here, we show that T2R14 is expressed in HNSCC cell lines: SCC 47, FaDu, and RPMI 2650. We found that T2R14 agonists lidocaine, thujone, and flufenamic acid trigger intracellular Ca^{2+} release in HSNCC cells. 6-methoxyflavanone (6-MF), a T2R14 agonist, inhibits this Ca^{2+} response. Furthermore, lidocaine in particular reduces NADH metabolism via XTT assay, indicating poor cellular health. It also decreases mitochondrial potential as measured by JC-1 dye and stimulates production of superoxide species. These compounds ultimately induce apoptosis via caspase 3 and 7 cleavage. Co-incubation with 6-MF inhibits apoptosis induction. Drivers of this cell death may be mitochondrial Ca^{2+} overload and/or proteasome inhibition, as T2R14 activation causes both mitochondrial Ca^{2+} influx and accumulation of poly-ubiquinated proteins. Taken together, T2R14 agonists could function as alternative/complementary therapies due to their pro-apoptotic effects in HSNCC cells. Further work is warranted to understand T2R signaling in HNSCC and normal surrounding epithelia.

253 **Chat Is A General Marker For Skin And Mucosal Merkel Cells**

Ranhui Xi, Mamoon Ali, Marco Tizzano
University of Pennsylvania, School of Dental Medicine, Philadelphia, PA, United States

Merkel cells are oval-shaped mechanoreceptors essential for light touch sensation and found right below the epidermis in the skin of vertebrates in organized clusters in touch-sensitive regions, such as tips of digits and whiskers, and in structures called “touch domes” associated with hair follicles. Keratins (KRT) 20, 8, 18, and 19 are specific markers for Merkel cells in skin, with KRT20 considered the more specific of these markers. ChAT gene encodes for choline acetyltransferase, an enzyme that synthesizes the neurotransmitter ACh. In ChAT-tau-GFP mice, the GFP signal is widely expressed in cholinergic nerve fibers in the brain and peripheral nervous system, as well as sensory taste-like tuft cells in the airway and gut. In this study, we discovered that

mechanosensory Merkel cells specifically express ChAT-GFP signal in the keratinized epithelium of the mouse foot pad, fingertips, whiskers, skin, and hard palate epithelium. Merkel cells expressing ChAT-GFP cells were often found in clusters or groups and in contact with NFH antibody-labeled nerve fibers. Immunofluorescence staining confirmed that ChAT-GFP signal was co-expressed with the Merkel cell's marker KRT8. Merkel discs expressing ChAT-GFP were observed in the vibrissae or facial whiskers and in touch domes in both glabrous and hairy skin. Despite recent studies showing that Merkel discs are the main sites of mechanotransduction in response to tactile stimuli, the underlying molecular mechanisms and neurotransmitters responsible for tactile signaling in Merkel discs remain largely obscure, with possible candidates including norepinephrine, serotonin, glutamate, and ATP. The specific expression of ChAT in Merkel cells may suggest that acetylcholine is a possible Merkel cell-produced neurotransmitter involved in tactile sensations.

255 **The Organization Of Taste And Visceral Responses In The Mouse Gustatory Thalamus**

John D Boughter, Martin A Raymond, Lianyi Lu, Keval Patel, Max L Fletcher
University of Tennessee Health Science Center, Memphis, TN, United States

In rats, it is well established that gustatory information is represented in the activity of neurons found in the most medial portion of the ventral posteromedial medial (VPM) thalamus. This area is typically classified according to cytoarchitecture as the parvocellular division of the VPM (i.e., the VPMpc), and it includes a population of neurons that project directly to the gustatory cortex (GC). Almost no studies exist examining the organization of gustatory responses in the mouse VPMpc. We used neuroanatomical and imaging techniques to characterize this crucial relay in the B6 mouse. First, neural tracing of the brainstem-VPMpc and VPMpc-GC pathways suggested a medial location for this nucleus from about -1.9 to -2.2 relative to Bregma. Second, mice were stimulated with taste compounds through IO cannulas or given a visceral stimulus (i.p. LiCl) and processed for Fos expression. Fos was expressed in the region pinpointed by neural tracing, although cell counts did not vary according to stimulus, and patterns of Fos+ cells evoked by either oral or visceral stimuli overlapped spatially. In a third experiment, we imaged taste-evoked activity in GCaMP6-expressing cells in VPMpc via head-mounted miniscopes in awake, actively behaving mice. Mice licked a panel of basic taste stimuli and water in brief trials. A similar percentage of cells in the VPMpc responded significantly to each basic taste stimulus, although more NaCl-best cells (17%) were found than any other type. Neurons were relatively narrowly tuned, with a mean entropy of 0.29. Collectively, these studies help describe the VPMpc as a region involved in both taste and visceral processing in the mouse.

257 **Don Tucker Finalist: The Role Of The Laterodorsal Tegmental Nucleus In Taste-Based Motivation For Metabolically-Distinct Simple Sugars In Mice**

A-Hyun Jung[1], Leah J. Wootton[2], Badruddin Mahamed[3], Kieran Heung[2], Amelia Cave[4], Jack Brown[2], Benjamin Phan[2], Lindsey A. Schier[1,2]

[1]Neuroscience Graduate Program, University of Southern California, Los Angeles, CA, United States, [2]Human and Evolutionary Biology, Department of Biological Sciences, University of Southern California, Los Angeles, CA, United States, [3]Quantitative Biology, Department of Biological Sciences, University of Southern California, Los Angeles, CA, United States, [4]Health and Human Sciences, Department of Sociology, University of Southern California, Los Angeles, CA, United States

Glucose and fructose (G&F), two common dietary sugars, both bind to the sweet taste receptor but are metabolized differently in the body. Rodents come to prefer the taste of glucose over fructose after regular G&F consumption, suggesting these sugars can be discriminated based on oral cues. Yet, whether this is accompanied by alterations in the reinforcing efficacy of one or both sugars and how these discernable cues are processed in the central gustatory neuroaxis remain unclear. First, to identify brain regions involved in the rapid discrimination of G&F, food-restricted male C57BL6/J mice (n=24) received glucose only or alternating G&F solutions (Sugar-exposed, SE) for 18 days (0.316/0.56/1.1M, 1 sugar/day). Then, they were offered 300 licks (1µl/lick) of 1.1M glucose or fructose before termination. Mapping of c-Fos immunoreactivity in key brain areas revealed that the Laterodorsa