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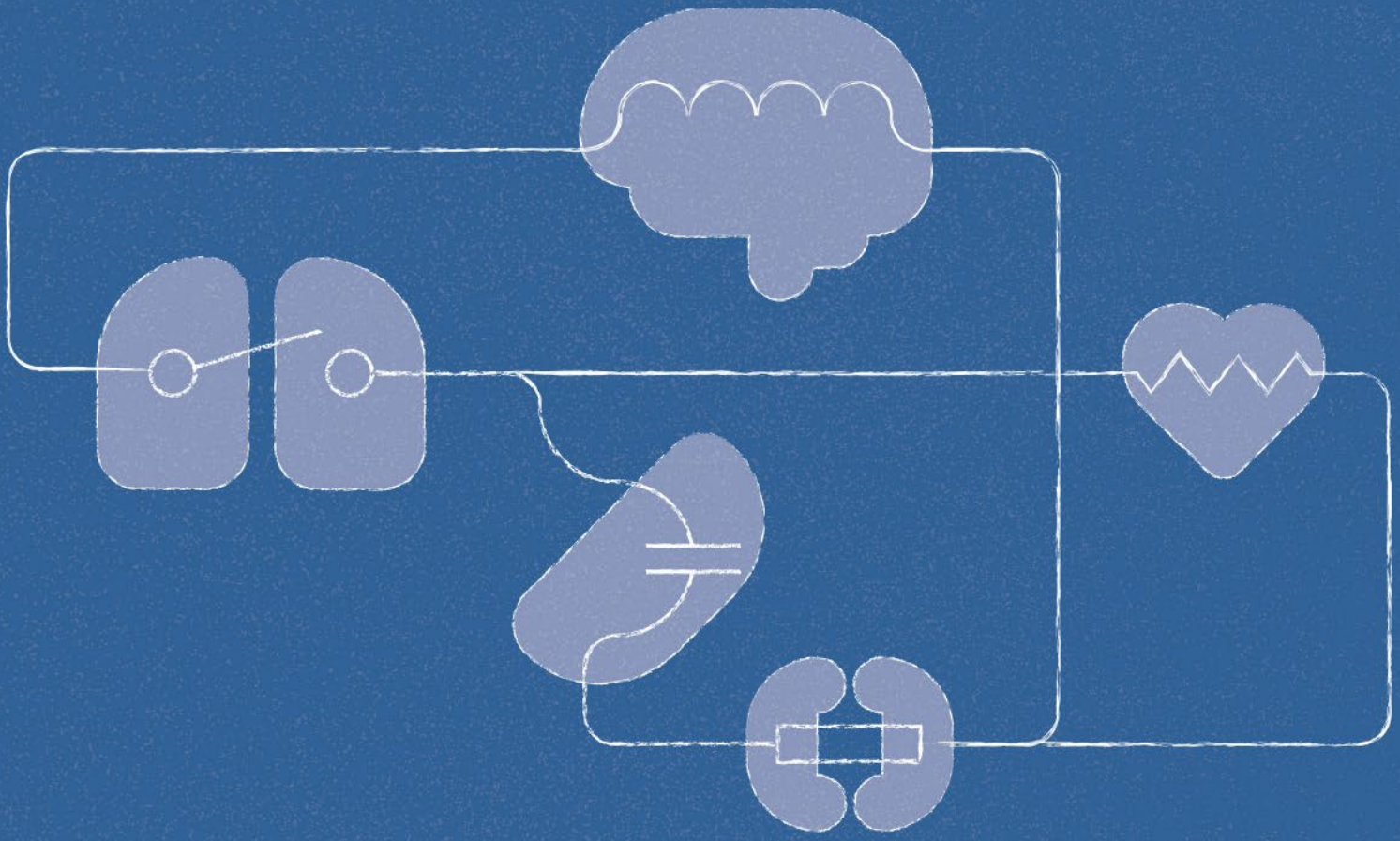

2nd Annual **NIH Investigator Meeting for Interoception Research**

November 11, 2023

Full Meeting Summary

2nd Annual NIH Investigator Meeting for Interoception Research
National Center for Complementary and Integrative Health
Bethesda, Maryland
November 11, 2023

This meeting highlighted recent advances in interoception research relevant to the National Institutes of Health (NIH) Blueprint for Neuroscience Functional Neural Circuits of Interoception Initiative. Invited speakers focused on basic and human subjects research related to functional neural circuit analysis of interoception.

Opening Remarks
Helene M. Langevin, M.D., Director, National Center for Complementary and Integrative Health (NCCIH)
Walter Koroshetz, M.D., Director, National Institute of Neurological Disorders and Stroke (NINDS)

Dr. Langevin opened the meeting by emphasizing that pursuing whole person health requires recognizing that the body's different systems are deeply interconnected. Interoception—the two-way conversation between the brain and internal organs—is integral to whole body health. Understanding how we sense and respond to signals from the internal world is critical to many integrative health therapies and practices. Dr. Koroshetz provided an overview of the NIH Blueprint for Neuroscience Research, which aims to accelerate transformative discoveries across NIH in brain function in health, aging, and disease. A workshop on interoception in April 2019 highlighted this emerging area of science. In 2021, NIH awarded funds to seven projects, totaling $18.15 million over 5 years, to a new effort focused on interoception across multiple organs and pathways. Results of some of these studies have already published.

Program Updates
Wen G. Chen, M.MSc., Ph.D., NCCIH

Dr. Chen thanked the working group, planning committee, and logistics team and welcomed the junior investigators. She provided data showing a five-fold increase in interoception research funded by NIH from 2018 to 2023, which has yielded increases in interoception publications. Four current funding opportunities are available across a variety of health concerns. Importantly, a new study section is dedicated to the neuroscience of interoception and chemosensation and the Assisted Referral Tool can assist applicants in finding the most suitable study section for their research. Finally, the Keystone Symposia is supporting a conference series, "Interoception: Neural Sensing and Control of Organ Function," to launch in spring 2025.

Keynote Address: Neural Mechanisms of Social Homeostasis
Kay Tye, Ph.D., Salk Institute for Biological Studies
Moderator: Dana Schloesser, Ph.D., Office of Behavior and Social Science Research (OBSSR), NIH

Dr. Tye began by stating that homeostasis is at the core of interoception. Social homeostasis is mediated by the brain and explains how we relate to, adapt to, and interact in society, both individually and collectively. The critical nodes within any homeostatic system are a detector (i.e., a receptor that detects change), a control center that compares the change to a set point, an effector (e.g., muscles, glands), and in the case of social homeostasis, feedback. Social homeostasis occurs both in the individual brain and in the social group. The notion of a social homeostatic system came to Dr. Tye when her laboratory found that just one day of social isolation in mice produced increases in synaptic strength and neural activity of

dopamine neurons in response to a social stimulus. This accidental discovery of a neural substrate for a loneliness-like state opened up the opportunity for further study. If there is a cellular substrate for an unpleasant need state that drives motivation to correct it (e.g., hunger, thirst, loneliness), there must be an upstream effector system to detect and control that deficit. Tye and colleagues found that specific dopamine neurons are necessary for promoting rebound sociability following a short period of isolation in mice. Further, the degree to which these neurons modulate behavior was predicted by social rank, suggesting that rank is also a necessary component of a social homeostasis circuit. This effect was reproduced in humans, which showed that acute social isolation evokes midbrain craving responses that are similar to hunger. Ascertaining the effector in the homeostasis circuit provides an entry point at which one can work backward to find the control center.

Humans seek social contact for the rewards it brings (prosocial) or to avoid loneliness (aversive). Social contact becomes a positive or negative valence stimulus when it is deficient or in excess, respectively. For example, acute isolation leads to a rebound reaction when the isolation ends. In comparison, chronic isolation leads to set-point adaptations in which reintroduction to the previous social optimum is experienced as a surplus, leading to aggressive, avoidant behavior. All social species have shortened lifespans when socially isolated, and in humans, even just perceived loneliness is correlated with shorter lifespans, increased mood disorders, heart disease, inflammation, and cancer.

The "Pain Overlap Theory" proposes that the experience of social and physical pain potentially share some of the same underlying processing systems in the brain. This has been seen in human studies in the insular cortex and anterior cingulate cortex, which are critical to emotional awareness and detecting signals from the organs systems. This has also been demonstrated in the mouse using Social LEAP (SLEAP), a system for multi-animal pose tracking to quantitively assess behavior. However, we do not know the specific neural substrates where two types of pain overlap in circuitry. What is it in the homeostasis circuitry driving these responses, and what are the time points at which the set point adapts from a prosocial to an aversive response? Dr. Tye described two models being tested to explain how social pain can have a sustained influence on physical pain. The first is spatial convergence, where social and physical pain activate the same pain responsive ensembles. Alternatively, a second possibility is through temporal convergence, in which experiences of social pain trigger the release of neuromodulators, and the gradual accumulation of these neuromodulatory signals during social pain recruit more neurons to the pain-responsive ensemble. To explore these models Dr. Tye observed mouse behaviors in real time during the "#FOMO (Fear Of Missing Out) Task," which is a new paradigm for social pain. Social exclusion was found to increase behavioral responses to physical pain. They speculate that endocannabinoid dynamics play a protective effect on these two types of pain and could be a neuromodulatory mechanism to unite social and physical pain. Current focus is on the control center and the detection node, which are key to the concept of social interception. In response to a question about whether Dr. Tye has assessed somatic pain, she responded that the focus has been on the affective components of pain; that is, the behavioral responses to social or physical pain rather than detection of the nociceptor threshold.

Session One: Flash Talks by Junior Investigators
Moderators: Emmeline Edwards, Ph.D., NCCIH and Olga Tjurmina, Ph.D., National Heart, Lung, and Blood Institute (NHLBI)

Dr. Edwards described the process for this critical capacity-building program. Of 120 abstracts submitted, six were selected based on specific criteria. Efforts were made to ensure gender and ethnic diversity. Dr. Tjurmina introduced each investigator.

Mapping the Gut-Brain Neural Circuitry
Minel Arinel, B.Sc., Duke University

Ms. Arinel's research focuses on understanding how gut-mediated signals are encoded across the gut-brain neural circuitry. Enteroendocrine cells (EECs) in the gut epithelium are known to send enteric information directly to the brain through synaptic connections with vagal neurons. The larval zebrafish is an ideal and simple model system to study this gut-brain communication as its transparency provides access to the whole system at single-cell resolution. To investigate how different types of nutritional information are processed, Ms. Arinel developed a gut-mediated nutrient preference assay. Nutrients from the gut were collected through gavage under a two-photon microscope. Lateralized encoding in vagal ganglia, spatiotemporal encoding across the brain, and photostimulation of EECs determined how nutrients in the gut affect feeding behavior. This research demonstrated how interoceptive stimuli are sensed along the gut and communicated to the brain through the vagus nerve.

Neuromodulation Devices You Can Eat
Khalil Ramadi, Ph.D., New York University

Dr. Ramadi provided the rationale for developing an ingestible electronic device for gastric neural/hormonal modulation through electrical stimulation. Gastric electrical stimulation (GES) is approved for the treatment of gastroparesis. Despite its widespread use, GES has not been found to enhance gastric emptying despite patient-reported decreased nausea and vomiting. This neuromodulation outcome was hypothesized to be based on direct neuronal excitation via stomach-brain vagal afferent pathways. A primary hypothesis was that GES improves nausea and vomiting by increasing plasma ghrelin (the "hunger hormone"). Dr. Ramadi and his team hypothesized that mucosal stimulation could directly induce the release of ghrelin from the gastric mucosa via endoscopic stimulation. They developed an ingestible electronic device, the surface of which, called FLASH (fluid-wicking capsule for active stimulation and hormone modulation), enabled mucosal engagement and stimulation despite the presence of mucus and gastric juices. This ingestible electroceutical can modulate systemic levels of neurohormones in a vagal-dependent manner without necessitating wireless power transfer or communication. In a swine model, the effects of mucosal electrical stimulation were directly controlled by varying electrical stimulation parameters. Devices were ingested and safely excreted without breaking apart and causing no adverse effects.

Interoception Biases Decision Making on an Approach-Avoidance Conflict Task
Michael Cardenas, B.S., University of Arizona

Mr. Cardenas opened by noting that everyday decision making frequently relies on the weighing of multiple conflicting considerations. Choices made reflect the tradeoff between a motivation to approach appetitive stimuli and avoid aversive stimuli (approach-avoidance conflict). Internal and external variables can alter how an organism evaluates risks or costs and potential benefits. The contribution of interoceptive signals to influence approach-avoidance decision making beyond mere communication of homeostatic needs has not been described. He hypothesized that behavior on an approach-avoidance conflict task would shift toward avoidance when the visceral state is dominated by sympathetic versus parasympathetic tone. To test this hypothesis, rhesus monkeys performed an approach-avoidance conflict task after being administered glycopyrrolate, a parasympatholytic drug that does not cross the blood-brain barrier. In all animals tested, glycopyrrolate increased avoidance behavior, but had no effect on approach behavior. This indicates that a sympathetic-dominated visceral state communicated through

interoceptive afferents is sufficient to modify the behavioral response of the animals. This finding lays the groundwork for neurophysiological studies to assess the neural mechanisms by which interoceptive signals can bias decision making, particular involving complex social behavior.

Differences in Functional Connections and Impairments in Heart-Based Interoception in Parkinson's During Stress and Exercise
Senegal Alfred Mabry, B.A., M.P.A., Cornell University

Mr. Mabry described the key symptoms of Parkinson's disease (PD); worsening voluntary motor function and cell death in the dopamine-producing substantia nigra. However, symptoms begin earlier with invisible autonomic visceromotor changes and can occur years before the brain visibly changes. In addition, although people with PD are at a greater risk of cardiovascular disease (CVD), CVD risk factors such as smoking history and past myocardial infarction decrease the likelihood of PD diagnosis. The role of powerful psychosocial risk factors for CVD in pathogenesis and comorbidity between CVD and PD is not well understood. Recent efforts have focused on the interoceptive aspects of these factors. Observations of people with PD engaged in a cardiopulmonary exercise intervention to improve their motor-gait symptoms provided a clue in that they reported feeling no change in their heart rate despite recorded increases. Mr. Mabry described a study to assess whether those with PD have impaired interoceptive awareness, and if so, why. People with PD participated in a high-intensity cardiopulmonary exercise intervention to improve their motor-gait symptoms. They rated their perceived level of exertion and how strongly they could feel their heartbeat before, during, and after each training session. As observed clinically, they appeared to struggle with feeling changes in their heart rate during exercise. Participants also performed a stress task. They reported changes in their perceived stress and ability to feel their heart-based interoceptive signals during the task's baseline, stress, and recovery periods. Those with PD perceived stress from the task at the same level as healthy controls; however, they could not feel their heartbeat during the baseline, stress, and recovery periods despite recorded increases. They hypothesized that those with PD have differences in insula functional connectivity during stress, which was shown to be the case based on imaging.

Neuro-Immune Interactions and Interoception in Prodromal Parkinson's Disease
Le Zhang, Ph.D., Yale University

PD patients present with multiple motor and nonmotor symptoms, including in the gastrointestinal system. Immune cells serve as a major body-to-brain connection in this disease. Dr. Zhang hypothesized that in a subset of patients, PD is initiated by an autoimmune event involving α-synuclein in the gut, and interactions among the immune system and the peripheral and central nervous systems establish the disease in the brain. Rapid eye movement (REM) sleep behavior disorder (RBD) is a preclinical state to PD. Dr. Zhang hypothesized that progression of RBD and PD pathology is also initiated by an autoimmune process involving α-synuclein-specific T cell activation, which is followed by neuroimmune interactions through interoception that establish PD pathology in the brain. A T cell receptor (TCR) found in cerebrospinal fluid is a unique nucleotide sequence on the same T cell clone. TCR sequencing can serve as a powerful tool for tracking the same clonal expanded T cells across organs. Dr. Zhang tested the hypothesis by integrating neuroimmunology, genomics, and microbiome approaches in patients with RBD and PD. Immune cells were profiled and generated the first human single-cell cerebrospinal fluid (CSF) atlas of RBD and PD. Characterization of CSF myeloid cells showed significant cell type proportion changes with increased CSF-specific monocytes in RBD and PD and enriched tumor necrosis factor (TNF) signaling pathways. CSF changes in RBD were compared to those in multiple sclerosis (MS), and, strikingly, decreases in TNF pathways were found in MS. This suggests that anti-TNF therapy might

prevent PD, given that TNF inhibitors worsen MS and anti-TNF lowers PD risk in inflammatory bowel disease. Comparisons of blood, CSF, and gut immune populations could elucidate the underlying mechanisms of immune regulation in PD and identify the role of the gut-brain axis in regulating immune responses in RBD and PD.

Persistent Dyspnea: Insights From Invasive Human Recordings of Respiratory Related Brain Oscillations During Respiratory Challenges
Jose L. Herrero Rubio, Ph.D., Feinstein Institutes for Medical Research

The past few years have witnessed an increase in the incidence of respiratory diseases, ranging from acute to chronic syndromes and related to higher levels of pollutants/allergens and the effects on breathlessness of other factors. In addition, something called "screen apnea" has been described in which people spend excessive time in front of a screen, not moving, experiencing anxiety, and being unaware that they are holding their breath. These disruptions in breathing and awareness sensations alter the way the brain processes ascending respiratory signals. Previous research by Dr. Herrero Rubio used neuronal activity to track the breathing cycle throughout widespread cortical/limbic sites, so-called "breathing above the brain stem." Those findings suggested a fundamental role of breathing-related oscillations in driving neuronal activity and provide insight into the neuronal mechanisms of interoceptive attention. In current research, Dr. Herrero Rubio recorded respiratory-related brain oscillations (RRBOs) in epilepsy patients implanted with intracranial electrodes in cortical and subcortical areas and engaged in a task that induced dyspnea. When the airways were partially obstructed by the experimental loads, increased RRBOs were observed in the lateral olfactory and posterior cingulate cortices. In comparison, the sensorimotor cortex exhibited comparable increases during both load and nonload trials. These findings suggest that the sensorimotor cortex may play a pivotal role in monitoring the current behavioral state, while the olfactory and cingulate cortices may encode the current effort. By mapping the primary respiroceptive cortices in humans, his team has shown that distinct brain areas are engaged in the motor awareness versus the effort associated with breathing, and that the respiration rate can be controlled volitionally. This has implications for finding the underlying mechanisms and management of breathlessness. Future efforts will aim to map secondary respiroceptive cortices in humans, identify affective components of dyspnea, and develop a realistic model of dyspnea.

Session Two: Technology, Translation, and Reverse Translation in Interoception Research
Moderators: Todd S. Horowitz, Ph.D., National Cancer Institute (NCI) and Olujimi Ajijola, M.D., Ph.D., University of California, Los Angeles

From Microbial Membrane Proteins to the Heart-Brain Connection
Karl Deisseroth, M.D., Ph.D., Stanford University

As a psychiatric resident, Dr. Deisseroth became interested in how a primary cardiovascular condition could lead to a psychiatric disorder (e.g., an increased heart rate might itself induce anxiety or fear responses). Precise, noninvasive modulation of electrochemical signals in the heart in vivo could facilitate studies of physiology and interoceptive signaling in this phenomenon. Research on the channelrhodopsin (ChR) genes identified ChRmine as the model member of the pump-like ChR protein family, with properties of monovalent-cation selectivity, unusually large photocurrents, exceptional red-shift, speed, and extreme light-sensitivity. This finding facilitated new opportunities in optogenetics, such as the control of specific mammalian perceptions at single-cell resolution and the use of noninvasive light delivery to study body-wide cellular communication. In 2021, Ritchie Chen et al. demonstrated the use of ChRmine to achieve transcranial photoactivation of defined neural circuits, including midbrain and

brainstem structures with great precision. Dr. Deisseroth collaborated with colleagues Chen and Brian Hsueh (2023) to optically evoke tachycardia enhanced anxiety-like (apprehensive) behavior in the mouse in a context-dependent manner (i.e., risk), showing that both central and peripheral processes are involved in the development of emotional states. The posterior insular cortex was identified as a potential mediator of bottom-up cardiac interoceptive processing. Optogenetic inhibition of this brain region reduced the anxiety-like behavior that was induced by optical cardiac pacing. The team then searched for a better tool. Kalium ChRs (KCRs) have received attention as potential inhibitory optogenetic tools. Dr. Deisseroth and colleagues designed a new KCR with increased K+ selectivity, which has provided key new advantages for in vivo optogenetics research. These high-resolution tools for controlling and mapping specific well-defined elements in intact and fully-assembled biological systems provide an opportunity to gain a deeper understanding of a heart-to-brain axis of communication that is precisely and causally relevant to setting internal affective/emotional states.

Perturbing the Rhythms Within: Cardiorespiratory and Gastrointestinal Insights Into Psychiatric Disorders
Sahib Khalsa, M.D., Ph.D., Laureate Institute for Brain Research

Human studies using interoceptive perturbation are enriching our grasp of the neural circuits of interoception and laying groundwork that holds promise for tailored approaches to psychiatric treatment. Despite the fact that interoception is crucial for human health, it has been missing from the clinical vocabulary because there have been few methods of studying it until the advent of functional imaging. The hypothalamic-pituitary-adrenal (HPA) axis is key to interoceptive stress signaling and attention has focused primarily on cortisol versus adrenergic signaling. Dr. Khalsa and colleagues have used the adrenalin analogue isoproterenol as a visceral psychophysical approach to study interoception. Isoproterenol increases sympathetic arousal and the conscious experience of cardiorespiratory sensations in a dose-dependent manner. They hypothesized that there would be a dose-dependent correlation between the continuous subjective ratings of isoproterenol-related interoceptive sensations and the neutral physiological response to isoproterenol, as measured by the change in heart rate. For more than two decades the laboratory has conducted isoproterenol perturbation studies of cardiac interoception across various psychiatric disorders. In one, they explored whether individuals with generalized anxiety disorder (GAD) show abnormal physiological, perceptual, or neural responses during peripheral β-adrenergic stimulation with isoproterenol. In a randomized trial, female patients with GAD exhibited hypersensitivity to this adrenergic stimulation as well as greater interoceptive sensation and, based on imaging, showed diminished ventromedial prefrontal cortex activity compared with healthy participants. The GAD patients showed a selective hypoactivation of the ventromedial prefrontal cortex (vmPFC). This suggests that the vmPFC might be a treatment target for those with GAD. These findings converge with those reported by Dr. Deisseroth. They raise additional questions about why silencing insula, but not the vmPFC, ameliorates interoceptive anxiety-like behaviors in mice and why the vmPFC is more relevant to cardiac interoceptive processing in humans with GAD. A second study evaluated whether central autonomic network alterations in anorexia nervosa (AN) occur following peripheral adrenergic stimulation. Relative to a healthy control, adrenergic stimulation caused widespread whole-brain functional connectivity reductions in the AN group between central autonomic network regions and motor, premotor, frontal, parietal, and visual brain regions. This suggests that dysfunctional processing of interoceptive signaling may contribute to affective and body image disturbance in this disorder.

Switching organ systems, Dr. Khalsa described research that identified neural responses to gastrointestinal sensation using a minimally invasive mechanosensory probe by quantifying brain, stomach, and perceptual responses following the ingestion of a vibrating capsule. He is currently

applying this method in eating disorder inpatients in the search for interoceptive markers of illness severity and prognostic indicators of treatment outcome. These findings have led to research to explore the role of PIEZ02 as a primary mediator of human gastroduodenal mechanosensation and find which peripheral neural pathways drive the conscious sensation. Additional research has found causal evidence for the processing of bodily self in the anterior precuneus, which could provide clues to interoceptive signaling. Collectively these studies propose that manipulating peripheral organ signals can illuminate how interoceptive circuits influence symptom perception in psychiatric disorders and lay groundwork for tailored approaches to treatment, particularly for anxiety and eating disorders.

Advancing Methods and Technologies To Monitor and Modulate Couplings Between the Human Brain and Stomach
Todd Coleman, Ph.D., Stanford University

Dr. Coleman's research spans from developing fundamental information theory and machine learning techniques to developing technologies to monitor and modulate the physiology of nervous systems in the brain and visceral organs. Just as the EKG and EEG have transformed cardiovascular and brain research and care, cutaneous electrogastrography (EGG) is a noninvasive technique for recording the gastric myoelectric activity using electrodes placed on the abdominal surface overlaying the stomach. It can detect spatial features of the gastric slow wave, which makes it attractive for diagnosing abnormalities in gastric motility. Dr. Coleman's research has shown that, importantly, spatial electrical patterns correlate with symptom severity, which makes this tool clinically useful. For example, in a study of functional dyspepsia and gastroparesis, Dr. Coleman and colleagues found spatial slow-wave abnormalities in 44 percent of subjects with foregut symptoms. Subjects with a higher percentage of slow waves with aberrant propagation direction had a higher total gastroparesis index score and more severe abdominal pain. This suggests that the slow wave is due to an afferent signal. However, patients with severe symptoms have symptoms that cannot be explained by gastric myoelectric dysfunction score alone. They hypothesized that gastric myoelectric dysfunction is not a prominent disease etiology in this group. The team then developed the EGG in the form of a novel wearable technology that can assess the slow wave patterns of the stomach over time to better understand the associations between spatial patterns and symptoms. Recording of the heart during sleep served as a way to study the parasympathetic nervous system alongside the gastric slow wave. They were able to identify autonomic and gastric myoelectric biomarkers throughout the day that differentiate patients with gastroparesis, diabetics without gastroparesis, and healthy controls, specifically around sleep and meals. A next step was to develop noninvasive measures of gut-brain electrical coupling. Using high-resolution magnetoencephalography (MEG) and EGG, Dr. Coleman and colleagues were able to visualize greater coupling in the resting state relative to working memory. Coupling was modulated by satiation and cognitive states in the left frontal central brain region, and the strength of coupling correlated with behavioral performance speed during working memory tasks. Finally, to test whether it is possible to modulate the slow wave patterns of the stomach to treat gastric conditions, a novel, inductively powered, gastric pacing technology was developed. Altogether, these results highlight the role of gut-brain interactions in cognition and demonstrate the feasibility of these recordings using scalable sensors.

Translating Gut-Derived Reinforcement
Dana Small, Ph.D., McGill University/Yale School of Medicine

Dr. Small's research focuses on understanding how sensory, metabolic, and neural signals are integrated to determine food choices and how the dysregulation of these systems contributes to the development of obesity, diabetes, and cognitive impairment. Rodent work by Han et al. (2018) found that when lipids

bind to receptors in the upper intestine, a reinforcing signal is generated that ascends to the brain via the vagus nerve to release dopamine. A gut-to-brain neural circuit establishes vagal neurons as an essential component of the reward neuronal circuitry, linking sensory neurons in the upper gut to striatal dopamine release (i.e., gut-induced reward). Under physiological conditions, the pathway is lipid sensing. It can be blunted by habitual intake of high-fat food, and this is associated with reduced dopamine release and shifting preference away from low-fat foods. Administration of oleoylethanolamide (OEA), a lipid messenger that is depleted by high fat diet, reverses these effects, suggesting a new gut-brain therapeutic target for weight loss. To assess how the brain encodes the reward value of food in humans, Dr. Small and colleagues combined fMRI and a novel PET method to assess activation and dopamine release in response to palatable food intake. They found an immediate orosensory response and a delayed post-ingestive dopamine release. Furthermore, they identified brain areas where dopamine release reflected the desire to eat, highlighting the interactive role of the brain and periphery to reinforce food intake in humans. A subsequent study aimed to determine in healthy-weight individuals whether frequent exposure to a subtle high-fat, high-sugar diet over 8 weeks causes shifts in fat preference or alters the neural response (reward circuits) during exposure to palatable food. The investigators found evidence for dopamine signaling of post-oral reinforcement. In addition, a short-term high-fat dietary intervention decreases preference for low-fat foods and enhances responses to food cues and prediction error coding, indicative of altered dopamine signaling. In a pilot study it was found that OEA administration improves weight loss outcomes on a behavioral weight loss intervention, but only in those who are habitual consumers of a high-fat diet. These findings support the translation of a lipid-sensing gut-derived reinforcing pathway from mice to humans and the potential for a gut-brain target for weight loss and other conditions, such as alcohol use disorder.

Cardiac Interoceptive Training Enhances Insula Connectivity and Reduces Anxiety
Sarah Garfinkel, Ph.D., University College London

Autism spectrum condition (ASC) is a neurodevelopmental condition displaying difficulties in emotion recognition in self and others, comorbid anxiety, and sensory changes. Dr. Garfinkel and others have hypothesized that emotional deficits expressed by individuals with ASC may originate in impaired interoceptive processing. This hypothesis does not fit logically with clinical observations that individuals with ASC report a heightened sensitivity to internal bodily sensations. Relatedly, interoceptive awareness (one's perception of one's own interoceptive performance) does not always correspond directly to accuracy. Dr. Garfinkel noted that ASC individuals will display impaired interoceptive accuracy while at the same time showing heightened belief in their interoceptive ability. Further, because anxiety is the most common comorbidity in ASC, it is notable that interoceptive ability has significant implications for anxiety. Research has shown that anxiety is associated with enhanced interoceptive sensibility; that is, a tendency for people with anxiety to believe that they are interoceptively proficient. Further, precision in bodily signals can aid the regulation of emotions, and anxiety has been associated with interoceptive error. Dr. Garfinkel described a randomized superiority clinical trial of cardiac interoceptive training (Aligning Dimensions of Interoceptive Experience ([ADIE]). ADIE combines two modified heartbeat detection tasks with performance feedback and physical activity that transiently increases heart rate. The study compared ADIE with an exteroceptive control condition. It demonstrated that cardiac interoceptive training can enhance interoceptive accuracy and significantly reduce anxiety in both neurotypical and autistic individuals. Reductions in anxiety were maintained after 1 year. Increases in interoceptive accuracy were associated with enhanced insula connectivity with the agenesis of the corpus callosum (ACC) and the ventromedial prefrontal cortex (vmPFC). Participants reported a reduction of sensory overload, enhanced regulation, and heightened general body awareness. Together, these results suggest that trait interoceptive accuracy is malleable, with observable changes in insula

connectivity and clinically meaningful reductions in anxiety in autistic adults. Novel therapeutic approaches that target interoceptive mechanisms offer promise for the treatment of other mental health conditions.

Discussion

In response to a question, Dr. Garfinkel said her team has not yet looked at physiological data from the trial but have collected brain metrics and heart rate variability. She added that training did have an unexpected outcome—those who became more accurate in their interoception also became less aware, suggesting that they were relying more on external factors than internal. In response to another question, she said that her team has begun computer modeling to assess whether there are predictive factors that would facilitate a precision medicine approach to these patients.

Dr. Deisseroth replied to a question about whether those with anxiety and tachycardia will have less anxiety if treated for the tachycardia. He believes that could be so but more study is needed. A commenter added that there is a difference between effects on heart rate in acute versus chronic anxiety, which must be considered. Dr. Deisseroth added that while his intervention causes ventricular contraction, it is not yet clear whether those signals are sent back to the brain.

Session Three: Brain–Multiorgan System Connections
Moderators: Julia Berzhanskaya, Ph.D., NHLBI and Mark L. Andermann, Ph.D., Harvard Medical School

Role of PIEZO2 in Interoception
Ardém Patapoutian, Ph.D., Scripps Research and Howard Hughes Medical Institute

Proteins and ion channels that sense mechanical force are hypothesized to play critical roles in sensing touch and pain (somatosensation), sound (hearing), and shear stress (cardiovascular function), among others. Years of research have elucidated the wide-ranging role of the ion channel PIEZOs in somatosensation and interoception, involving: touch sensation; proprioception; pain (tactile allodynia); mechanical itch; respiration; baroreception; urination; and gut motility. Despite knowing this, very little was known about the role of mechanotransduction at the molecular level as the last major sensory. Dr. Patapoutian's research has shown that PIEZO1 and PIEZO2 are mechanically activated ion channels that mediate touch perception, proprioception, and vascular development. PIEZO1 displays a three-bladed propeller shape with a curved transmembrane region containing at least 38 transmembranes, more than any other protein in the genome. PIEZO2 in particular is required for proprioception as demonstrated in a mouse model. This was reproduced in the human; PIEZO2-deficient humans lack touch sensitivity and proprioception, and serious lack of coordination. Moreover, the identity of the ion channels involved in sensing mechanical force remained elusive. The Patapoutian laboratory identified PIEZO1 and PIEZO2, mechanically activated cation channels that are expressed in many mechanosensitive cell types. Genetic studies established that PIEZO2 is the principal mechanical transducer for touch, proprioception, baroreception, and bladder and lung stretch, and that PIEZO1 mediates blood-flow sensing, which impacts vascular development and iron homeostasis. Clinical investigations have confirmed the importance of these channels in human physiology. Co-investigator Dr. Ye Li studied how adipose tissues communicate with the CNS to maintain whole-body energy homeostasis. The traditional view is that circulating hormones secreted by the fat convey the metabolic state to the brain. In addition, somatosensory neurons of the dorsal root ganglia (DRG) innervate adipose tissue. Sensory ablation in the mouse was equivalent to activation of the sympathetic nervous system. Similar results were found in mice on a high-fat diet despite more mechanical stimulation in this model. PIEZO2 is expressed in fat-

innervating neurons at almost the same level as in skin. This suggests an important role of the innervation by DRG of adipose tissues, not mechanosensation as was assumed, and could enable future studies to examine the role of sensory innervation of disparate interoceptive systems. Future efforts will focus on: what mechanical forces are at play in fat; what sensory neurons are sensing; and the role of sensory neurons in other internal organs. Other cells and the role of vasodilation are also being considered as the source of mechanosensation.

Modulation of Spinal Pathways Involved in Interoception in Patients With Chronic Pain
Julie Pilitsis, M.D., Ph.D., M.B.A., Florida Atlantic University Schmidt College of Medicine

Chronic pain has been estimated to affect about 50 million adult Americans and is a leading cause of long-term disability. It is a condition clearly related to interoception, but better tools are needed to study the associations. We know that in chronic pain the pathways and mechanisms in interoceptive awareness shift and that acute pain can lead to chronic pain, altering all of the acute responses and pathways. The autonomic nervous system is known to be involved in interoception. The role of the somatic nervous system, particularly through spinal pathways, is less clear. Alterations that occur in chronic pain disease states compound the understanding of that role. Further, the tridimensional aspects of pain that occur in rodent models and those experienced by humans are likely to differ. Spinal cord stimulation (SCS) is a U.S. Food and Drug Administration–approved neuromodulation treatment to relieve chronic refractory pain. Dr. Pilitsis said that perioperatively, it offers an opportunity to learn more about pain especially because currently used subjective metrics (e.g., pain scales) and objective measures (e.g., functional imaging) are ineffective in measuring pain and interoception in patients. Relying on a patient database of those diagnosed with back pain and scheduled for SCS surgery for chronic pain, Dr. Pilitsis and colleagues collected data through intraoperative neuromonitoring, which has yielded a large amount of information for selected muscles that are being stimulated with this device. Prior research showed the efficacy of DRG stimulation to decrease pain. Dr. Pilitis's research has demonstrated how modulation of the DRG and dorsal columns in a swine model and in humans influences pain behavior and brain electrophysiology. Specifically, low-intensity, focused ultrasound (versus electrical stimulation) was used to show modulation of the DRG in a neuropathic swine model. In addition, high-resolution EEG was used to show the effects of spinal cord stimulation on pain processing in humans. Ongoing research will center on how spinal cord stimulation and evoked motor potentials elicited in the lower extremities can be used to create a somatotopy of the dorsal thoracic spinal cord.

Research Evaluating Vagal Excitation and Anatomical Links (REVEAL) in Humans
John Osborn, Ph.D., University of Minnesota

The vagus nerve conveys information from the brain to most organs in the body and vice versa. Its dysregulation underlies many pathological conditions, and we are just now discovering ways to modulate it to treat them. The activation or blockade of the vagus nerve produces central and peripheral multiorgan physiological responses. Cervical vagus nerve stimulation (VNS) through an implant is clinically approved for treating drug-resistant epilepsy and depression and for poststroke recovery. Despite more than 100,000 patients receiving treatment with VNS and numerous studies in various animal models, the physiological effects on peripheral organs in humans is poorly understood. REVEAL co-principal investigator Dr. Osborn described the protocol for the study, which involves global research on the effect of VNS on four key systems: autonomic nervous, cardiovascular, immune, and metabolic. The response of these systems to both acute (minutes) and chronic (months) VNS are being studied in 144 participants already being treated for treatment-resistant depression or drug-resistant epilepsy. A subset of participants with implanted VNS devices will receive a wide range of tests to measure

physiological, molecular, imaging, genetic, and neural responses to VNS, along with many other experimental and computational outcomes. Functional magnetic resonance imaging (fMRI) of the brain will be conducted before and after implantation and during VNS. Imaging of moment-activation responses to VNS will then be used to determine whether upstream central nervous system modulation is key to changes observed in the peripheral organ systems. Interleaved fMRI acts as a window to the direct, in-the-moment effects of VNS on brain activity and interoceptive processes. Precision functional mapping will be used to map the multimodal networks involved in interoception and allostasis. A supplemental study led by Dr. Ziad Nahas will investigate the effects of VNS on central autonomic network and interoception, based on his prior work showing a right insula and middle prefrontal cortex relationship to antidepressant response and time. It will investigate how chronic VNS brings about dynamic changes in large-scale brain network functional connectivity proportional to the observed changes in autonomic nervous system activity.

From Body to Brain: The Coding Logic of Interoception in the Vagus Nerve Interoception
Rui Chang, Ph.D., Yale School of Medicine

The ability to timely and precisely sense changes inside the body's organ systems is critical for survival. Vagal sensory neurons (VSNs) form an important body-to-brain connection through the vagus nerve, navigating visceral organs along the body's rostral-caudal axis and diving across the organ's surface-lumen axis into appropriate tissue layers. The vagus nerve is a major interoceptive system shown to play a major and complex role in memory and cognition, heart rhythm and blood pressure, neurogenerative disease, respiratory function and rhythm, digestion and food intake, and glucose metabolism. Although the brain can discriminate numerous body signals through VSNs, the underlying coding strategy at the systems level is poorly understood. Dr. Chang's research aims to understand the coding architecture of the vagal interoceptive system, recognizing that three independent key features of interoceptive signals form the visceral organ, the tissue layer, and the sensory modality. Multiple state-of-the-art technologies were developed, including unique projection barcodes for each organ composed of exogenous nucleotide sequences (Projection-seq) and vagal calcium imaging-transformed fluorescence in situ hybridization (vCatFISH), to precisely determine neuronal identity based on innervations and response patterns. The research demonstrated that VSNs code visceral organs, tissue layers, and stimulus modality—key features of interoceptive signaling—in different dimensions. Multiplexed projection barcodes were used to profile large-scale, single VSN cells from seven major organs and revealed a visceral organ dimension of differentially expressed gene modules that coded organs along the body's rostral-caudal axis. Another tissue layer dimension with gene modules coded 26 VSN-ending locations along the organ's surface-lumen axis. Calcium imaging was used to guide spatial transcriptomics, which showed that VSNs were organized in functional units that sensed similar stimuli across organs and tissue layers, constituting a third stimulus modality dimension. Together, the three independent, feature-coding dimensions specified many parallel VSN pathways that combine to facilitate complex and efficient VSN projection in the brainstem. This research highlights a novel multidimensional coding architecture of the mammalian vagal interoceptive system for effective signal communication.

Extraocular Light Sensing via the Opsin GPCRs OPN3 and OPN5 Regulates Energy Homeostasis
Richard Lang, Ph.D., Cincinnati Children's Hospital Medical Center

The availability of photons from the sun has created opportunities for evolution to generate adaptive, light-sensing mechanisms. The obvious examples are the visual system, which allows object identification through decoding of radiant photons, and the circadian system, where physiologic rhythmicity can be entrained by light detection in the eyes. Dr. Lang's laboratory has been focused on the possibility of

other extraocular light-sensing pathways in mammals and has been investigating the roles of encephalopsin (OPN3) and neuropsin (OPN5). The opsins are photon-detecting G-coupled receptors. They are proteins that bind to light-reactive chemicals to facilitate vision, phototaxis, circadian rhythms, and other light-mediated responses of organisms. OPN3, OPN4, and OPN5 are nonvisual short wavelength opsins. Except for the past 200 years, the primary source of photon ligands was the sun, which established a certain evolutionary pathway. Two light-sensing pathways regulate eye vascular development, suggesting that light could be a development timing clue, mammalian deep tissue light sensing is possible, and opsins work in pairs. Recent analysis has shown that OPN3 and OPN5 mediate extraocular light sensing in mice. OPN5, a violet-sensitive opsin is expressed in preoptic area neurons that function as deep-brain photoreceptors. OPN3 is found in white adipocytes that directly respond to violet-blue photons. These two light-sensing tissues function in a regulatory circuit that controls many metabolic parameters, including body temperature. This suggests a new mechanism for regulation of energy metabolism in which intimate coupling to the sunlight cycle is a key feature. Light stimulation of preoptic area OPN5 neurons downregulates sympathetic nervous system output to brown adipose tissues and thereby suppresses body temperature. In contrast, blue-light stimulation of white adipocytes promotes an increase in body temperature by enhancing the lipolysis pathway and the production of free fatty acids used as heating fuel. Finally, sensory ganglia express OPN3 and OPN5 and their expression as a feature of many sensory neurons, suggesting that the sensory ganglia are light sensing. These findings demonstrate that the entire mammalian system is set up to sense light systemically as a component of normal physiology integrated with the circadian system. Disruption of this response might be behind several disorders, some of which might be susceptible to photon as a drug.

Discussion

Discussion focused on:

- Further exploration of innervation of the visceral organs and how that affects interoception
- How similar signals adapt to the local environment and its receptors
- Whether increasing exposure to blue light is contributing to increases in both metabolic disease and myopia
- The need for neurons to keep spatial information as redundant to the VSNs
- Understanding the interactions of the somatosensory, sympathetic, and parasympathetic systems as they underlie interoception
- Whether VNS can be targetable to specific cell types or organs
- How to advance human applications to differentiate neuronal subtypes for specific stimulation
- How modelling of human data can identify the parameters for each cell type or organ

Closing

Dr. Chen thanked the participants and speakers and encouraged ongoing discussion at the poster session and beyond.