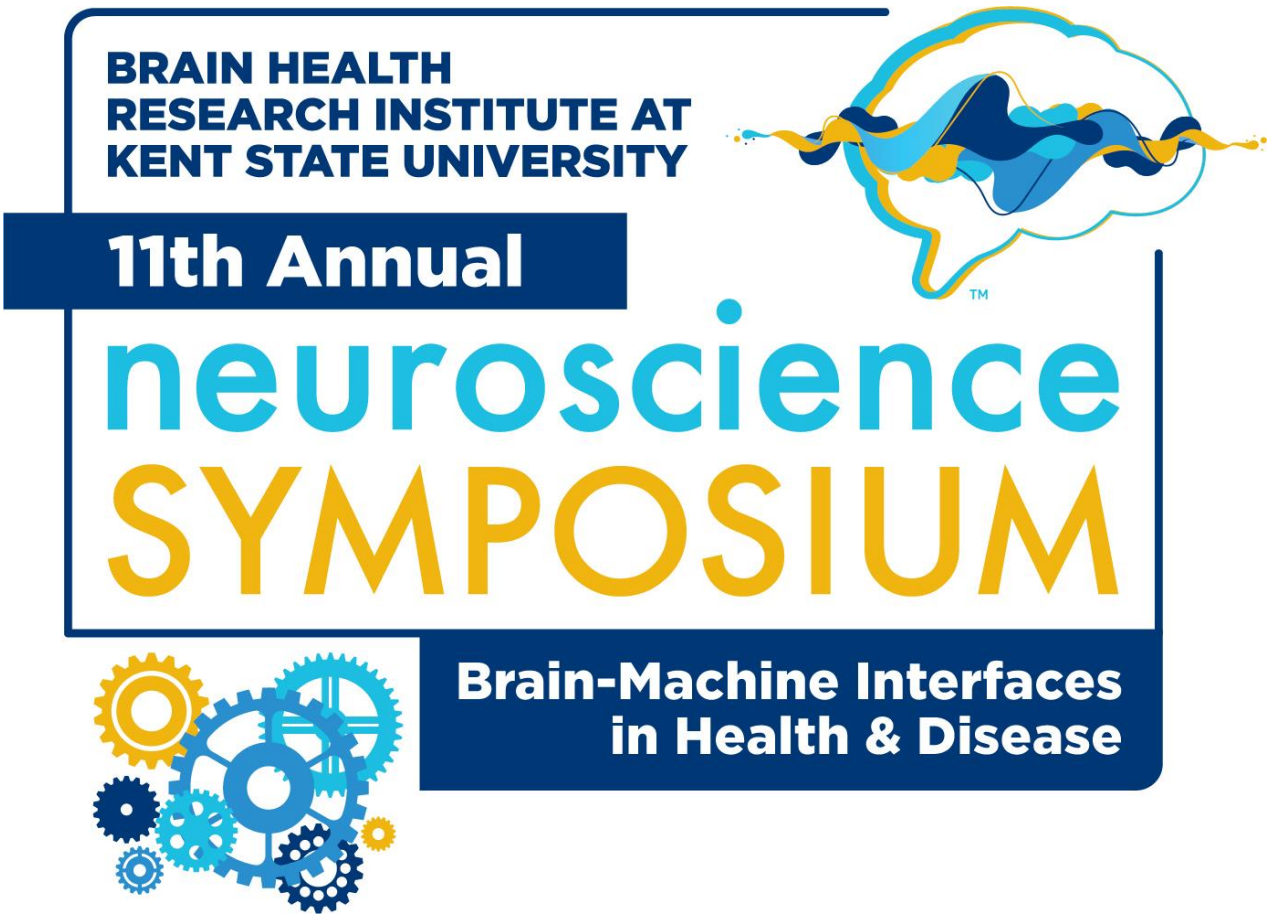

POSTER ABSTRACTS BOOKLET

BHRI 11TH ANNUAL NEUROSCIENCE SYMPOSIUM:

"BRAIN-MACHINE INTERFACES IN HEALTH & DISEASE" – OCT. 26-27, 2023

TABLE OF CONTENTS:

Page 12 – **Ya'el Courtney***, yaelcourtney@g.harvard.edu, Harvard University

A Choroid Plexus Apocrine Secretion Mechanism Alters Fetal Cerebrospinal Fluid Proteome and Instructs Cortical Development

Page 13 – **Elizabeth Cousins***, ecousins@kent.edu, Kent State University

Burden and Positive Aspects of Caregiving: Cluster Profiles of Dementia Caregiving Experiences

Page 14 – **Kendra Dillon***, kdillo15@kent.edu, Kent State University

Loss of Classical Progesterone Signaling in Kisspeptin Cells Does Not Impair Negative Feedback Regulation of the GnRH Pulse Generator in Mice

Page 15 – **Sierra Duncan***, sdunca18@kent.edu, Kent State University

Comparing neuron and glial densities in the cerebral cortex and hippocampus of humans and chimpanzees

Page 16 – **Anna Duraney***, aduraney@kent.edu, Kent State University

Investigating the role of proopiomelanocortin (POMC) neurons in impaired steroid hormone feedback in polycystic ovary syndrome

Page 17 – **Chloe Edmonds***, cedmonds@neomed.edu, Kent State University, Northeast Ohio Medical University

ANESTHETIZATION OF TRIGEMINAL AFFERENTS SIGNIFICANTLY ALTERS FEEDING PHYSIOLOGY IN INFANT PIGS

Page 18 – **Thywill Ettey***, tettey@kent.edu, Kent State University

Dopamine D3 receptor activation of the spinal ejaculation generator facilitates sexual reflexes

Page 19 – **Hunter Frey-Burkart***, hfreybur@kent.edu, Kent State University

Effects of Acute and Chronic Corticosterone Injections on Immune Sensitivity to E. coli in Rats

Page 20 – **Kylie Gannon***, kgannon4@kent.edu, Kent State University

Exposure to Acceptance and Commitment Psychoeducation Reduces Mental Health Stigma in Veterinary Healthcare Teams

Page 21 – **Mia George***, mcg135@case.edu, Case Western Reserve University, Cleveland Clinic Lerner Research Institute

Coherence Across Cortical and Muscular Nodal Points During Deep Brain Stimulation for Essential Tremor Revealed Using Magnetoencephalography

Page 22 – **Salan Ghaju***, Sghaju@kent.edu, Kent State University

Ingestion of Sub-chronic Non-lethal Cyanotoxin Levels Disrupts the Stress Response

Page 23 – **Roman Giacomino***, rgiacomi@kent.edu, Kent State University

Mouse Muscle Thermogenesis in Response to Predator Odors: A Multilayer Analysis

Page 24 – **Phoenix Green***, pgreen18@kent.edu, Kent State University

Expression of enkephalin and opioid receptors in the spinal ejaculation generator after chronic spinal cord injury in male rats

Page 25 – **Alexandra Haupt***, ahaupt2@kent.edu, Kent State University

Disrupted oxytocin signaling in the posterior paraventricular thalamus increase anxiety-like and depression-like behaviors with no effect on maternal behavior

Page 26 – **Alliana Hite***, ahite4@kent.edu, Kent State University

Will mice work harder for salty + sweet, or just sweet, snacks?

Page 27 – **Phaedra Keller***, pnorrell@kent.edu, Kent State University

Investigating the effects of early-life programming on neurodegenerative disease risk

Page 28 – **Macit Emre Lacin***, lacinm@ccf.org, Cleveland Clinic Lerner Research Institute

Rodent Pupillary Dynamics During a Reversal Learning Task

Page 29 – **Aiswarya Mukundan Nair***, amukunda@kent.edu, Kent State University

Exploring role of ADAR editing dysregulation in neurological manifestations of COVID-19

Page 30 – **Alyssa Novak***, anovak17@kent.edu, Kent State University

Kisspeptin/Neurokinin B/Dynorphin (KNDy) neurons in mice exhibit robust reciprocal synaptic connectivity that may mediate synchronization of the population

Page 31 – **Kemal Ozdemirli***, ozdemik@ccf.org, Cleveland Clinic Lerner Research Institute

High Throughput Analysis of Pupillometry Data Using Various Deep Learning Based Architectures

Page 32 – **Karlee Patrick***, kpatri14@kent.edu, Kent State University

Pain in the Context of Virtual Neuropsychological Assessment of Older Adults

Fanan Aboalrob*, Zack Knauss, Devin Mueller

Integrative Physiology and Neurobiology, Biological Sciences Department, Kent State University

Acquisition of fentanyl seeking in adolescence following maternal separation is sex and hormone dependent.

The opioid epidemic is a major health crisis in the U.S. resulting in 107,622 deaths in 2022. Early Childhood Neglect (ECN) accounts for up to 75.3% of all child maltreatment cases in the US annually and coincides with susceptibility to opioid use disorder (OUD); survivors are twice as likely to be prescribed opioids and 4.5 times more likely to develop OUD. Thus, we assessed the effects of maternal separation (MS), a potent form of ECN, on seeking behavior during adolescence and adulthood using a rat model of fentanyl-induced conditioned place preference (CPP). Sprague Dawley rat pups (n=65; male=23, female=42) were cross fostered at birth and received 3-hour daily MS on P2-P18 or were allowed to receive full maternal care. Rats were assigned to adolescent (P32) or adult (≥P50) place conditioning in a three-chamber apparatus for eight days under one of four conditions: 1) control - saline (1 ml/kg, s.c.), 2) control – fentanyl (5 ug/kg, s.c.), 3) MS – saline, or 4) MS – fentanyl. Extinction testing was conducted 24-hours post-conditioning for three days during adolescence and for eight days and then weekly in adulthood until day 91 or until extinction criteria were met. We found that MS enhanced the magnitude and persistence of fentanyl seeking in adolescent and adult males as compared to control non-MS rats (p<0.05). In contrast, MS impaired the formation of fentanyl seeking in adolescent and adult female rats as compared to control non-MS rats (p<0.05). Thus, MS induced a significant but sex-dependent alteration in the expression of fentanyl seeking during adolescence and adulthood. To determine the role of sex hormones in the observed sex differences, we assessed whether masculinizing females with 1mg/kg testosterone propionate on P1 would result in male-like responses to fentanyl in adolescence and adulthood. Indeed, masculinized females showed enhanced fentanyl seeking following MS during adolescence compared to control females (P<0.05) and matched the responses of males that had MS. These trends continued into adulthood. Importantly, MS suppressed exploratory behavior and locomotion in females suggesting MS exacerbated anxiety. These findings are consistent with human studies on the effects of ECN on the propensity and persistence of OUD.

Najmah Al Ramel*, Marwan Shalih, Jennifer McDonough

Biological Sciences Department, Kent State University

The Role of the Neuronal Mitochondrial Metabolite NAA in Myelination.

Multiple sclerosis (MS) is an inflammatory neurodegenerative disease of the central nervus system (CNS) resulting in progressive neurological disability. This disability results from demyelination or nerve damage disrupting the communication between the brain and the body. It has been demonstrated that the neuronal mitochondrial metabolite N-acetylaspartate (NAA) is decreased in the MS brain, but the significance of reduced NAA to MS pathology is not clear. It is known that NAA mediates crosstalk between axons and oligodendrocytes. NAA is made in neurons and taken up by oligodendrocytes where it is catabolized by ASPA to acetate and L-aspartate. We have previously shown that NAA catabolism is involved in regulating metabolic and gene expression changes that are characteristic of myelinating oligodendrocytes. Deleting the N-acetyltransferase-8-like (NAT8L) enzyme that synthesizes NAA causes less compact myelin and sensorimotor disability in mice. We have hypothesized that NAA is involved in maintenance of myelin. In the present study, we used Nat8l-/- mouse model at the age of one year to test demyelination. Using fluorescent immunohistochemistry for WT and Nat8l-/- mouse brains, we evaluated myelin basic protein (MBP), and we also evaluated SMI-32, a marker for axons. We also used CARS microscopy to quantitate myelin lipids. Our data show that there is less myelin in Nat8l -/- mice in corpus callosum compared to WT mice. These data suggests that NAA catabolism in oligodendrocytes is necessary for maintaining proper myelination during aging in the CNS.

Anna Anello*, Nafees Mahmood, Brittany Smith, Angela Ridgel, Kwangtaek Kim

Computer Science Department, Kent State University

Haptic Mixed Reality Button Press for Parkinson's Rehabilitation.

Parkinson’s disease (PD) is a neurodegenerative disorder that primarily causes motor function deficits such as tremors, bradykinesia, and abnormal gait or posture. These deficits are the result of a decrease in the production of dopamine by the substantia nigra in the brainstem. Exercise and rehabilitation can help to improve motor deficits and potentially slow the progression of the disorder. The aim of this study is to develop and test an immersive upper limb rehabilitation system that uses a stylus haptic (force feedback) device and mixed reality glasses to simulate a 3D button push task that has previously been shown to improve the hand tremors of individuals with Parkinson’s. Individuals with Parkinson’s disease (N=9) and healthy controls (N=22) were asked to push three different (small, medium, large) virtual buttons using the haptic stylus and complete 12 button pressing trials, with six trials on each hand as fast as possible. The virtual button click system tracked the hand movements of participants and measured task completion times. The hand motion trajectory of each person with Parkinson’s disease was compared to the average trajectory of healthy participants. Y-axis synchronization was used to determine the correlation for each button-to-button trajectory. A low correlation indicates that the individual with Parkinson’s disease had more severe tremors. Individuals with Parkinson’s had an average difference of almost 2 seconds when the button sizes differed in comparison to healthy controls. Additionally, qualitative usability data using NASA TLX demonstrated that this new immersive system provides a practically usable and personalizable interface based on a severity level of hand tremors. Individuals with PD reported a medium level of mental demand and those with tremor reported a medium level of physical demand. Healthy controls while felt no physical demand. The differences in performance between individuals with PD is most likely due to the severity of their condition. Future studies will incorporate activities of daily living into the simulation and real-time personalized feedback.

Kyle J. Backston*, Leah C. Brenneman*, Roman A. Giacomino, Eric M. Mintz, Colleen M. Novak, Emily N. Welch

Department of Biological Sciences, Kent State University

Temporal map of hepatic Fgf21 shows rhythmic expression driven by the suprachiasmatic nucleus in mice.

The hepatokine fibroblast growth factor 21 (FGF21) is a member of the unique subclass of fibroblast growth factors that function as endocrine signaling molecules. Since its discovery, FGF21 has been implicated in physiologic responses related to fasting, insulin, glucose, and fatty acid metabolism, energy balance, body weight, and the regulation of macronutrient preference, primarily through signaling in the central nervous system (CNS). Within the CNS, the obligate co-receptor for FGF21, beta klotho (KLB), is densely expressed in the suprachiasmatic nucleus of the hypothalamus (SCN). This localization suggests an overlap between the FGF21 signaling axis and the circadian timing system. Previous work from our lab has shown that Klb is rhythmically expressed in the SCN. Given that food is a potent zeitgeber for the liver, and circulating FGF21 levels are influenced by metabolic stress, we sought to map the temporal expression of hepatic Fgf21 under both ad libitum and 4-hour time-restricted feeding conditions. Using quantitative real-time PCR on mouse liver tissue that was collected at four time points spanning 24 hours, we demonstrated that Fgf21 is rhythmically expressed under both ad libitum and 4-hour daytime time-restricted feeding conditions (ZT6-ZT10) when maintained on a 12:12 light/dark cycle. Additionally, there is no significant difference between the phase of the Fgf21 rhythm under ad libitum or 4-hour time-restricted feeding conditions, suggesting that the SCN regulates the temporal expression of hepatic Fgf21. Consequently, we have uncovered a novel interaction between the SCN and the FGF21 signaling axis that could act as a mechanism through which the central circadian clock influences metabolic homeostasis.

Robin Bearss*, Richard Piet

School of Biomedical Sciences, Neuroscience, Kent State University

Activation of Glutamate Receptors Increases Activity of Female Kisspeptin Neurons.

Kisspeptin (kiss1) neurons in the arcuate nucleus (ARN) stimulate gonadotropin releasing hormone (GnRH) neuron activity and GnRH release, which subsequently promotes luteinizing hormone (LH) release from the anterior pituitary gland. ARNkiss1 neurons co-release kiss1, neurokinin B, dynorphin A, and glutamate. ARNkiss1 neurons are thought to coordinate their activity through reciprocal connections, generating episodic bouts of activity and driving GnRH pulsatile secretion. Ionotropic glutamate receptor antagonists suppress ARNkiss1 neuron coordinated activity and pulsatile LH release. Although this suggests glutamatergic neurotransmission onto kisspeptin neurons, little is known about the impact of glutamate receptor activation on kisspeptin neurons. We sought to examine the effect of ionotropic AMPA and NMDA receptors, and group 1 metabotropic glutamate receptors (mGluRs) on ARNkiss1 neuron activity in female mice. We used calcium imaging to monitor the activity of kiss1 neurons in brain slices. We utilized Kiss1-Cre x GCaMP6f mice that express GCaMP6f selectively in kiss1 neurons. Coronal brain slices obtained from female diestrus mice containing the ARN were placed in a recording chamber. Changes in individual kisspeptin neuron fluorescence were measured using epifluorescence microscopy to estimate variations in intracellular calcium concentrations ([Ca2+] i) as a proxy for electrical activity. Kiss1 neurons were exposed to either AMPA (10µM), NMDA (50µM), or DHPG (50µM). On average, applications of AMPA, DHPG, or NMDA to female ARNKiss1 neurons transiently increased normalized fluorescence by 38.6 ± 1.6% (161 cells, 6 slices, 4 animals), 7.6 ± 0.7% (111 cells, 7 slices, 4 animals), and 15.0 ± 1.1% (103 cells, 6 slices, 4 animals), respectively. The proportion of cells activated for each drug was 100 (n=161), 94 (n=111), and 100% (n=103) for AMPA, DHPG, and NMDA application, respectively. This indicates that activation of ionotropic AMPA and NMDA receptors as well as group 1 mGluRs increases [Ca2+] i in female ARNkiss1 neurons and, thus, stimulates activity in these cells. Further investigation into the role of endogenous glutamatergic signaling and into the respective role of glutamatergic receptors in regulating kiss1 neuron activity is needed.

Joseph Bedont*, Anna Kolesnik, Pavel Pivarshev, Dania Malik, Cynthia Hsu, Aalim Weljie, Amita Sehgal

Department of Biological Sciences, Kent State University

Chronic sleep loss sensitizes Drosophila melanogaster to nitrogen stress.

Chronic sleep loss profoundly impacts metabolic health and shortens lifespan, but studies of the mechanisms involved have focused largely on acute sleep deprivation. To identify metabolic consequences of chronically reduced sleep, we conducted unbiased metabolomics on heads of three adult Drosophila short-sleeping mutants with very different mechanisms of sleep loss: fumin (fmn), redeye (rye), and sleepless (sss). Common features included elevated ornithine and polyamines, with lipid, acyl-carnitine, and TCA cycle changes suggesting mitochondrial dysfunction. Studies of excretion demonstrate inefficient nitrogen elimination in adult sleep mutants, likely contributing to their polyamine accumulation. Nitrogen stress accumulation likely underlies the broadly enhanced toxicity of high dietary nitrogen load from protein in chronically sleep-restricted Drosophila, including both sleep mutants and flies with hyper-activated wake-promoting neurons. Together, our results implicate nitrogen stress as a novel mechanism linking chronic sleep loss to adverse health outcomes-and perhaps for linking food and sleep homeostasis at the cellular level in healthy organisms.

Christopher Blank*, Emily Welch, Salan Ghaju, Xiaozhen Mou, Wilson Chung, Colleen Novak
Department of Biological Sciences, Kent State University

Effects of Chronic Oral Administration of Microcystin on Body Temperature and Composition in Mice.

Cyanobacteria, commonly known as blue-green algae, are a prevalent public health concern found in many freshwater sources, including the Great Lakes. Lake Erie, a fresh water source for nearly 12 million people, is the shallowest of the five Great Lakes, making it rich in nutrients that drive excessive growth of blue-green algae, leading to the development of harmful algal blooms (HABs). One of the most common species of cyanobacteria found in Lake Erie HABs is Microcystis aeruginosa, which produces the toxin microcystin. Microcystin is a known hepatotoxin in humans, and pre-clinical in vivo studies have shown effects on other organs and tissues including the kidney, heart, lungs, reproductive system, nervous system, and the immune system. New evidence, including the results of this study, also suggests an impact on neural centers affecting the stress axis and energy balance. We implanted male and female day with a temperature-sensitive transponder and administered a low dose of microcystin or water every other day orally for 22 days. Post administration, we measured body temperature non-invasively at 15-minute intervals for 1 hour, and then once at 2 hours, 4 hours, 8 hours, and 24 hours. We recorded body weight daily and lean and fat mass using Echo-MRI prior to day 1 and on day 22 to assess changes in body composition. Our results demonstrate that chronic exposure of mice to microcystin did not significantly alter body weight or composition (p=0.XX). However, there was a significant decline in body temperature in microcystin-treated mice compared to controls (p<0.05). This decline was observed immediately following administration of microcystin and persisted through the following 24-hour period, indicating a possible immune response that could lead to other physiological consequences, such as changes in metabolism via stress response mechanisms. Further research is warranted to explore the broader implications of the observed effects on neural centers, stress axis, and energy balance.

Eric Brown*, Phoenix Green, Rachel Rice, Jacob Corey, Lique Coolen

Brain Health Research Institute, Kent State University

Characterization of the Mouse Spinal Ejaculation Generator.

Nearly all men with spinal cord injury (SCI) have difficulty ejaculating without invasive medical intervention. An incomplete understanding of the mechanisms underlying SCI induced anejaculation has limited potential treatment options. Ejaculation is a reflex completely controlled by a group of cells in the L3/L4 spinal cord called lumbar spinothalamic (LSt) cells which comprise the spinal ejaculation generator (SEG). Changes to excitatory and neuromodulatory synaptic inputs to the SEG have been strongly implicated in SCI induced anejaculation using rat models. To further probe the identity of these synaptic inputs, it is necessary to use genetic tools only available in mice, however the mouse SEG remains to be fully characterized. While LSt cells exist in mouse, the extent to which they regulate ejaculation remains untested. First, we used IHC against galanin, a marker for LSt neurons, to confirm that the location and distribution of the SEG in mouse is similar to rat. We also used IHC against androgen and estrogen receptors to confirm the sexually dimorphic nature of the SEG in mouse. Next, we used RNAscope to show that following ejaculation, LSt cells in male mice express fos, a marker of neural activation. We next asked whether activation of LSt cells is required for ejaculation to occur. To test this, we delivered a cre-dependent AAV encoding the inhibitory DREADD hM4di to the lumbar spinal cord of galanin-cre mice to specifically drive expression in LSt neurons. Starting several weeks post-surgery, males were given either saline or CNO prior to pairing with hormone primed ovariectomized female mice. We hypothesize that by inhibiting the activation of LSt neurons with CNO, we will increase the latency to ejaculation while numbers of mounts and intromissions will remain unaffected. This result would reaffirm the necessity of LSt neurons in ejaculation during normal mating behaviors, and thus confirm the suitability of the mouse in investigating the neural regulation of ejaculation. Future investigations will then use a rabies-virus mediated strategy to label synaptic partners of Lst neurons in galanin-cre mice.

Erin Burke*, John Gunstad, Phillip Hamrick
Psychological Sciences Department, Kent State University

Examining the Association Between Cognitive Function and Word Frequency in Young-Old and Old-Old Adults.

Recent work suggests that changes in word frequency (WF), the rate at which a given word appears in spoken language, may be a sensitive marker for conditions like mild cognitive impairment and Alzheimer's disease. However, it is unknown whether WF is also associated with normal cognitive changes seen in aging. To investigate this possibility, the current study compared WF in healthy Young-Old (50-69 years) and Old-Old (70-89 years) groups and examined their association to neuropsychological test performance. Speech transcripts of 70 healthy older adults (OA) from an expository speech task were analyzed. Stratified subgroups were created for 38 Y-O and 32 O-O to explore possible age-related differences. For each participant, subject-level WF was computed for all words using WF values from the COCA. Group differences in WF and cognitive performance were evaluated by independent samples t-tests, while correlations between WF and neuropsychological test performance were evaluated with Pearson correlations. The Y-O group exhibited lower average WF values [t (68) = -2.3, p = 0.024] and performed better on tests of executive function [DSS, t(68) = 2.66, p = 0.01] and language [Animal Fluency, t(68) = 2.07, p = 0.042] than the O-O group. Within the combined sample, WF correlated with measures of attention [DSF, r = .26, p = 0.027], and episodic memory [Craft Story-21 Immediate recall, r = .27, p = 0.027]. The Y-O group produced words that were on average lower frequency and exhibited better cognitive performance compared to the O-O group, consistent with the notion that age-related cognitive decline is associated with changes in word retrieval in speech. However, findings from correlation analyses run counter to this hypothesis and suggest that better attention and memory was associated with use of higher frequency (i.e., more commonly used) words. The exact explanation for these findings is unclear and may reflect methodological factors, such as the nature of the speech task or intention from study participants. Future work should look to replicate these findings using different speech tasks with other variables associated with WF (e.g., semantic distinctiveness) to better understand the impact that age-related cognitive changes have on linguistic characteristics.

William Butke*, Joshua Comport, Salan Ghaju, Colleen M. Novak, X Mou, Wilson Chung
Department of Biological Sciences, Kent State University

Impact of Cyanobacterial Harmful Algae Toxins on Neuropeptide Y in the Mouse Brain.

With a worldwide increase in cyanobacterial harmful algal blooms (cHABs) in freshwater, the prevalence of cyanotoxins, such as microcystin leucine-arginine (MC-LR), has dramatically increased in the last decade. Due to the increasing human exposure to MC-LR in drinking water, it is vital to understand the impact that this toxin has on brain function. In our studies, ingestion of (sub)chronic non-lethal MC-LR levels elevated the stress hormone corticosterone (CORT) in mice. Earlier studies have also shown that elevated stress hormones may inhibit the expression of neuropeptide y (NPY), the primary orexigenic, or hunger, hormone. Therefore, we hypothesized that ingestion of (sub)chronic non-lethal MC-LR level may also disrupt NPY signaling. In our first experiment, young adult C57BL6 male mice were separated between two groups: water (H2O) control (n = 9), and 50 µg/kg MC-LR body weight (b.w.) (n = 6). Animals were gavaged every other day for 21 days. Following RNA isolation, NPY mRNA expression was measured using RT-qPCR. There was no significant change in MC-LR induced NPY mRNA expression (p = 0.06). Here we will present data from our second experiment were young adult C57BL6 male and female mice were separated between four groups: male water (H2O) control (n = 5), female H2O control (n = 5), male MC-LR 50 µg/kg body weight (b.w.) (n = 5), and female 50 µg/kg body weight (b.w.) (n = 5). C57BL6 male and female mice were gavaged every other day for 21 days. Brain tissues were collected and processed for NPY immunohistochemistry to measure NPY-immunoreactivity in the arcuate nucleus (ARC) of the hypothalamus. These studies are aimed at whether MC-LR disrupts brain regions known to control mouse food intake and serves as a first step towards determining whether cyanotoxin exposure may alter both mouse and human energy balance following exposure.

Matthew Campbell*, Donovan Brown, Riya Jacob, Caroline Nitirahardjo, Ayesha Tariq, Sarah Melen, Dr. Patrick T. Kang, Dr. Angelo DeLucia, Dr. William Lynch, Dr. Helen Piontkivska

Biological Sciences Department, Kent State University

Understanding viral neuropathogenesis in COVID-19: expression of SARS-CoV-2 Spike and Nucleocapsid genes elicits differential transcriptome changes in host cells.

Growing evidence suggests that damage due to SARS-CoV-2 infections is not limited to the respiratory tract tissues but extends to a broad set of neurological and neuropsychiatric, cardiac, and vascular complications, often grouped under the umbrella term of “long COVID”, or post-acute sequelae of SARS-CoV-2. Yet, our understanding of such multisystem manifestation remains limited, including the unresolved question of whether the virus causes direct cellular damage or instead indirectly damages neural cells via immune/neuroimmune activation. Notably, such multi-system sequelae is not limited to SARS-CoV-2, but has been documented for a variety of enveloped viruses. For many of these viruses, pathogenesis is associated with their viral envelope glycoprotein genes (Env), which have conserved structural domains needed for viral membrane-cell membrane fusion. To address the question whether viral Env genes can cause direct changes in neural cells, through recognition as foreign by innate immune mechanisms, we have engineered neural stem cells (C17.2, and primary mTmG NSCs) and human induced pluripotent stem cells (healthy donors 1 and 2 iPSCs) with viral Env vectors (+/- Adv-GFP, -N, -S, and PB-TRE-GFP/Spike) and characterized the transcriptomic changes associated with the expression of Env genes. The results show that expression of Spike gene and/or protein perturbs multiple pathways in these cells, with the innate immune pathways, particularly interferon signaling pathways, significantly overrepresented among dysregulated genes. Likewise, among differentially expressed genes are those involved in nervous system development, offering insights into potential mechanistic impact of viral infection on neural cells.

Ya'el Courtney*, Josh Head, Neil Dani, Elizabeth Yimer, Frederick Shipley, Towia Libermann, Maria Lehtinen

Neurobiology, Harvard University

A Choroid Plexus Apocrine Secretion Mechanism Alters Fetal Cerebrospinal Fluid Proteome and Instructs Cortical Development.

During embryonic brain development, cerebral cortical neurons form from neural progenitor cells that divide along the brain's ventricles and in contact with cerebrospinal fluid (CSF). CSF is produced largely by the choroid plexus (ChP), an epithelial tissue in each ventricle whose cells secrete CSF and its contents, including instructive factors like IGF-2. The ChP also serves as a blood-CSF barrier, protecting the brain from infection and inflammation. The cultivation of proper CSF composition is crucial to healthy development. Indeed, CSF aberrations are increasingly implicated in neurodevelopmental disorders including autism spectrum disorder (ASD), hydrocephalus, and schizophrenia. Despite its lifelong role in titrating CSF contents, ChP secretory machinery is poorly understood. Advances in imaging approaches enabled our discovery that, in addition to vesicular exocytosis, the ChP displays a high-capacity regulated exocytosis mechanism called apocrine secretion. We developed a toolbox using highly expressed G-protein coupled serotonin receptor 5HT2C to evoke and interrogate apocrine secretion using standard biochemical approaches, multi-photon imaging, expansion microscopy, and electron microscopy. We demonstrate this mechanism's functionality during embryonic development, confirm that these events alter CSF composition in vivo, and report findings on their contents. We further provide evidence that this altering this secretion process negatively affects cortical brain development and adult behavior. This mechanism may be sensitive to stressors like maternal inflammation and environmental teratogens, with resulting perturbations leading to impaired brain development.

Elizabeth Cousins*, Karlee Patrick, John Martin, Jennifer Drost, Christopher Was, Mary Beth Spitznagel

Department of Psychological Sciences, Kent State University

Burden and Positive Aspects of Caregiving: Cluster Profiles of Dementia Caregiving Experiences.

Objectives: Although caregiver burden (strain experienced when caring for an ill person) is common in the context of dementia caregiving, the caregiving role is linked to beneficial outcomes too. Individuals reporting higher positive aspects of caregiving (positive caregiving experiences such as such as a sense of personal or spiritual growth, feelings of competency) tend to exhibit lower burden relative to those reporting few. The goal of this retrospective review of outpatient memory clinic medical records was to demonstrate whether and how constructs of burden and positive aspects of caregiving coexist within individual caregivers, and to explore potential contributors to caregiver profiles created based upon these constructs. Method: Cluster analysis was conducted on 225 caregivers meeting inclusion criteria. Multinomial logistic regressions examined cluster predictors. Results: Results suggested a three-cluster solution: a High Burden group, a High Positive Experiences group, and a Low-Moderate Experiences group showing low burden and moderate positive experiences. Greater behavioral problems predicted belonging to the High Burden cluster. Greater care recipient dependence predicted belonging to the High Positive Experiences cluster while greater independence predicted the Low-Moderate Experiences cluster. Conclusion: Findings suggest that burden and positive aspects of caregiving are not simultaneously present in caregivers at high levels. Supportive caregiver interventions might be tailored to profiles demonstrated here. Future research should investigate other potential contributors to experiences of burden and positive aspects of caregiving.

Kendra Dillon*, Dayanara Lohr, Alyssa Novak, Aleisha Moore

Department of Biological Sciences and Brain Health Research Institute, Kent State University

Loss of classical progesterone signaling in kisspeptin cells does not impair negative feedback regulation of the GnRH pulse generator in mice.

Mammalian reproductive function depends on the ability of progesterone to suppress pulsatile gonadotropin-releasing hormone (GnRH) secretion in a homeostatic negative feedback loop. Impaired progesterone negative feedback is associated with polycystic ovary syndrome (PCOS), the leading cause of female infertility worldwide. However, the mechanism by which negative feedback occurs is largely unclear. Previous research identified that cells upstream from GnRH neurons expressing the classical progesterone receptor (PGR) are required. However, the identity of these cells and the mechanism by which they reduce GnRH pulsatile secretion are unknown. In this study, we aimed to address the hypothesis that PGR expressed by a neuronal population recently defined as the GnRH pulse generator, cells expressing Kisspeptin, Neurokinin B, and Dynorphin (KNDy cells), is required for progesterone negative feedback. To achieve this, we assessed negative feedback regulation of LH pulsatility in mice where PGR has been conditionally knocked out from kisspeptin cells (KPRKO mice) and quantified the expression of the arcuate KNDy peptides kisspeptin and dynorphin, which are excitatory and inhibitory to GnRH secretion, respectively. KPRKO mice are subfertile, producing significantly fewer litters (p<0.05) and pups per litter (p<0.05) compared to WT controls. However, ELISA-based measurement of LH in serial blood samples collected from the tail-tip of mice every 6 mins for 2 hours determined that loss of PGR in KNDY cells does not alter total or baseline LH release, nor the frequency or amplitude of LH pulses when compared to wildtype controls (p>0.05). Further, RNAscope analysis detected no change in the number of cells expressing kisspeptin and dynorphin mRNA in the arcuate nucleus (p>0.05, student t-tests), nor the number of kisspeptin and dynorphin mRNA transcripts within KNDy cells (p>0.05,). This data suggests that progesterone signaling in kisspeptin cells through PGR is not essential for negative feedback regulation of LH pulses and does not contribute to the infertile phenotype of KPRKO mice. These results indicate that either non-classical mechanisms are sufficient to maintain negative feedback, or that a yet unidentified PGR-positive cell population is involved.

Sierra R. Duncan*, Emily L. Munger, William D. Hopkins, Patrick R. Hof, Chet C. Sherwood, Mary Ann Raghanti, and Melissa K. Edler

Anthropology Department, Kent State University

Comparing neuron and glial densities in the cerebral cortex and hippocampus of humans and chimpanzees.

Comparative primate studies are critical for understanding the neuroanatomical correlates of human-specific cognition, aging, and neurodegenerative disease processes. In the present study, we aimed to gain insight into how two fundamental cell types, neurons, and glia, respond to aging in humans and one of their closest living relatives, chimpanzees. The dorsolateral prefrontal cortex (DLPFC), middle temporal gyrus (MTG), entorhinal cortex (EC), and hippocampus are regions vulnerable to age-related changes and are involved in higher-order cognitive processes that include task switching and configuration, inhibition, language, sensory integration, and memory formation and retrieval. We used unbiased stereology to quantify neuron density (Nv) in the DLPFC (layer III), MTG (layer III), EC (layer II), and CA1 and CA3 (pyramidal layer) in humans (n = 14, 2-88 y) and chimpanzees (n = 46, 12-62 y) across the lifespan. In addition, glia densities (Gv) and glia to neuron (G: N) ratios were collected in the DLPFC, MTG, and EC. Chimpanzees possessed higher Nv in the DLPFC and MTG, while humans exhibited higher Nv in the CA1 and CA3 (p values ≤0.01). EC Gv was greater in chimpanzees compared to humans (p≤0.01). Regional variation in Nv was noted with chimpanzees exhibiting lower DLPFC and MTG Nv compared to the EC and hippocampus (p values ≤ 0.03). In humans, Nv was significantly higher in the CA3 than the EC and CA1 (p≤0.01). No sex differences were detected in either species. These data differ from an earlier study with a smaller cohort of chimpanzees (n = 28, 12-62 y), in which we identified mild age-related Nv decreases in the chimpanzee hippocampus. This discrepancy is likely due to the inclusion of several younger animals in the current report. Conversely, these data support a previous analysis that found the human hippocampus had higher Nv compared to chimpanzees and aligns with aging studies in humans in which no changes with age were identified in the CA1 or CA3. These preliminary findings may hint at potential underlying evolutionary mechanisms responsible for differences in memory and sensory processing in humans and chimpanzees. Greater hippocampal Nv could be partially responsible for increased plasticity in humans that allows for enhanced cognitive processes.

Anna Duraney*, Alyssa Novak, Aleisha Moore

Department of Biological Sciences, Kent State University

Investigating the role of proopiomelanocortin (POMC) neurons in impaired steroid hormone feedback in polycystic ovary syndrome.

The most common cause of infertility in people with ovaries is polycystic ovary syndrome (PCOS). Patients with PCOS exhibit an increase in the pulsatile release of gonadotrophin-releasing hormone (GnRH) from neurons in the hypothalamus and luteinizing hormone (LH) from the pituitary gland, which drives the downstream ovarian symptoms of PCOS. Elevated GnRH/LH release is resistant to inhibition by the ovarian steroid hormone estradiol, showing that PCOS is a state of impaired steroid hormone-negative feedback. Although the mechanism underlying impaired feedback is unclear, cells in the arcuate nucleus containing proopiomelanocortin (POMC) are of interest due to impaired steroid hormone negative feedback in mice when estrogen receptor alpha (ERα) is knocked out from this cell population. Therefore, we hypothesized impaired estradiol negative feedback in PCOS is caused by a reduction in ERα expression in POMC cells. To test this, we utilized a mouse model of PCOS induced by prenatal androgen (PNA) exposure. Pregnant B6 mice were injected subcutaneously with either the androgen dihydrotestosterone (250µg) or a sesame oil vehicle on embryonic days 16, 17, and 18. Prenatal vehicle-treated (PNV) and prenatal androgen (PNA)-treated offspring were studied as adults between 60-80 days of age. Fluorescent immunohistochemistry was performed using coronal brain slices collected from perfusion-fixed PNV and PNA mice to label for POMC and ERα. Z-stack images of immunolabeling were collected using an FV3000 confocal microscope. The number of POMC-immunoreactive cells with and without ERα was manually counted in ImageJ software, and the percentage of POMC cells containing ERα quantified. No differences in the number of POMC cells were detected in the rostral and middle arcuate regions between PNV and PNA mice (p>0.05, students t-test). However, a significant increase in the percentage of POMC cells with ERα was seen in the rostral arcuate of PNA mice compared to PNV mice (p<0.05, students t-test), indicating a surprising increase in the sensitivity of POMC cells to estrogen in PCOS-like mice. The effects of increased ERα at POMC cells in the PCOS mouse model are still unclear, and ongoing studies aim to examine how POMC affects elevated pulsatile GnRH/LH release in PCOS.

Chloe E. Edmonds*, Kaitlyn N. Robbins, Elizabeth G. Dvorak, Sarah A. Sheldon, Brianna M. Bish, Ava Tang, Stephen P. Howe, Rebecca Z. German

Doctoral Neurosciences Program, School of Biomedical Sciences, Kent State University; Department of Anatomy and Neurobiology, Northeast Ohio Medical University

ANESTHETIZATION OF TRIGEMINAL AFFERENTS SIGNIFICANTLY ALTERS FEEDING PHYSIOLOGY IN INFANT PIGS.

Introduction: Infant feeding depends upon brainstem integration of afferent signals from a variety of cranial nerve branches. One such cranial nerve is the trigeminal, as its mandibular division (CN V3) provides sensory innervation to the lower lip and teeth. However, the mechanism by which afferent signals from CN V3 modulate infant feeding physiology remains unclear. Here we use an infant pig model to investigate the sensorimotor relationships between CN V3 sensation and oropharyngeal function. Methods: We acquired six infant pigs at ten days postnatal and trained them to feed on bottles with custom silicone nipples. Pigs underwent sterile surgery at 15-16 days postnatal to insert electromyographic (EMG) wires into digastric (DG), a jaw depressor. After at least 24 hours of recovery, we used synchronous biplanar video fluoroscopy and EMG recording to collect feeding data prior to and following bupivacaine anesthetization of two branches of CN V3, the inferior alveolar and mental nerves. Variables of interest included suck rate, the number of sucks per swallow, area under the curve (AUC, a measure of muscle activity) and duration of firing of DG, kinematic excursions of the tongue and jaw, and milk extraction delay (time from initiation of sucking to milk extraction). Results: Sucking rate did not change following nerve anesthetization, though the number of sucks per swallow increased. Digastric AUC and duration of firing per suck decreased significantly with anesthesia. Excursions of the anterior and middle tongue were unaffected, while excursion of the jaw increased. The milk extraction delays significantly increased following anesthetization. Conclusion: The observed changes in motor outputs, kinematics, and feeding behaviors indicate a significant role of CN V3 sensory information in modulating infant feeding. These afferents may therefore offer an ideal target in the design of sensory interventions for infants with feeding deficits. Future work should consider the potential effects of mandibular nerve stimulation or anesthetization on feeding efficiency and airway protection.

Thywill Ettey*, Rachel Rice, and Lique M. Coolen
School of Biomedical Sciences, Kent State University

Dopamine D3 receptor activation of the spinal ejaculation generator facilitates sexual reflexes.

Spinal cord injury (SCI) has a devasting effect on ejaculatory function. Ejaculation is a reflex controlled by the spinal ejaculation generator (SEG) located in the lumbar spinal cord. In male rats, mice, and humans, the SEG comprises a cluster of neurons known as lumbar spinothalamic (LSt) cells, which integrate sensory inputs generated during sexual activity into autonomic and motor response, leading to ejaculation. SEG is influenced by supraspinal inputs from brainstem and hypothalamus areas, including serotonin and dopamine inputs. The current study aims to test the role of dopamine on ejaculation by localizing dopamine receptors in the SEG and determining effects of dopamine agonists on ejaculation. Fluorescent in situ hybridization was used to test the expression of dopamine receptors in LSt cells. Spinal cord tissues of male rats (n=11) were hybridized using RNAscopeTM probes specific for galanin as a marker for LSt cells together with Drd 1, Drd2, and Drd 3 genes. Confocal microscopy and Fiji image-analyses showed that LSt cells co-expressed mRNA for Drd1 (37%), Drd2 (80%), or Drd3 (52%) with no effects of SCI. We previously showed that D2/3 receptor agonist 7-OH-DPAT facilitates ejaculation in sham controls and restores ejaculatory reflex in SCI rats. Here, we test the hypothesis that D3 receptors are crucial by examining effects of the specific dopamine D3 agonist Pramipexole in control rats. Adult male rats received a complete spinalization at T6-T7, and bulbocavernosus muscle (BCM) activities were recorded after systemic injections of saline (n=8), PPX (0.1 (n=8); 0.3 (n=9); or 1.0 (n=8) mg/kg) or PD 128907 (n=7; 0.1 mg/kg) and after a subsequent dorsal penile nerve (DPN) stimulation. BCM activities were analyzed for number of bursts, events, and latency to first burst. Results showed that injections of PPX or PD 128907, but not saline, triggered increased BCM bursting activity. However, effects of PD 128907 group were significantly higher compared to PPX groups, suggesting that both D2 and D3 receptor activation triggers ejaculation. Current studies are examining effects of PPX in SCI male rats. Together, these findings suggest that dopamine receptor agonist can be considered for treatment options of ejaculatory dysfunction following SCI.

Hunter Frey-Burkart*, Destiny Ezeliora, John Johnson
Department of Biological Sciences, Kent State University

Effects of Acute and Chronic Corticosterone Injections on Immune Sensitivity to E. coli in Rats.

The etiology of depression is complex, with much of it yet to be understood. In recent years, it has been thought that the immune system may contribute to the development of depression. Proinflammatory cytokines are early response communicators of the immune system, which are released in response to infection and bind to receptors on peripheral nerves to communicate with brain regions involved in mediating sickness behaviors such as decreased motivation and lethargy. Previous studies demonstrate that exposure to stress sensitizes inflammatory responses that may increase the risk of depression. Studies here investigated whether acute and chronic injections of corticosterone (CORT), a prominent stress hormone, sensitize inflammatory responses to Escherichia coli (E. coli) in rats. Adult male and female rats were injected subcutaneously with 2.5mg/kg CORT or vehicle for either 1 day (Acute) or 7 days (Chronic). Twenty-four hours after the last CORT injection, animals were challenged with either intraperitoneal Saline, 2.5x106 CFU, or 2.5x107 CFU E. coli and animals were euthanized 2h later for measurement of TNF-a, IL-1b, and IL-6 in blood, liver, spleen, and brain. We found that Acute CORT did not sensitize immune response in either male or female rats, but Chronic CORT enhanced IL-1b production in the blood and liver of females compared to males. These results indicate that repeated elevations in circulating CORT, such as what occurs during chronic stress, has the propensity to enhance cytokines responses, particularly IL-1b, in females to ultra-low inflammatory triggers. These results are consistent with elevated inflammatory responses being a risk factor for depression and that women are at increased risk of depression.

Kylie Gannon*, John Martin, Elizabeth Cousins, Christopher Was, Mary Beth Spitznagel
Department of Psychological Sciences, Kent State University

Exposure to Acceptance and Commitment Psychoeducation Reduces Mental Health Stigma in Veterinary Healthcare Teams.

Background: High rates of psychological distress are present in veterinary healthcare professionals, yet fewer than half of those with serious distress seek treatment. Elevated mental health stigma has also been demonstrated in the field and may underlie treatment reluctance. Recent work from our group utilizing evidence-based psychoeducation that provides coping skills for difficult veterinary client situations showed reduced stress and burnout in veterinary teams. Although the goal of that training was to decrease occupational distress, we hypothesized that exposure to this evidence-based framework could yield reduced mental health stigma as a byproduct. Methods: 143 veterinary healthcare providers were randomized to Intervention (n=72) or Control (n=71) groups. Intervention participants completed 3 weekly 1-hour Acceptance and Commitment Training sessions. Self-reported mental health stigma was measured at Pre-Test, Post-Test, and 1-month Follow-Up. Results: A longitudinal growth curve model was set with these three time points to determine intercept and slope, with condition (intervention vs. control) as a fixed covariate. All fit indices suggest good model fit, $\chi2$ (4) = 3.24, p = .52, $\chi2$ /df = .81, RMSEA = .00 (90%CI [.00; .12]), SRMR = .03, CFI = 1.00. Condition predicted slope such that those who received the intervention reported less mental health stigma over time relative to the control group, β = -.28, p = .009. Condition did not predict intercept, β = .12, p = .57, indicating that the two conditions did not differ in initial mental health stigma. Slope and intercept were not significantly associated (r = -.25 p = .62) suggesting that initial levels of mental health stigma were not associated with rate of change over time. Conclusions: Exposure to evidence-based psychoeducation reduced mental health stigma in veterinary providers. Workplace training may be an avenue for decreasing mental health stigma in this at-risk group. Limitations of this work include that veterinary providers self-selected for participation; results may reflect attitudes of individuals who were more psychologically open at baseline. Future research should examine this question in larger samples with a lower potential for sampling bias.

Mia George*, Madeline D. Porter, David Escobar, Ph.D., Andre G. Machado, MD, Ph.D., Kenneth B. Baker, Ph.D.

Neurosciences, College of Arts and Sciences, Case Western Reserve University; Department of Neuroscience, Cleveland Clinic Lerner Research Institute.

Coherence Across Cortical and Muscular Nodal Points During Deep Brain Stimulation for Essential Tremor Revealed Using Magnetoencephalography.

Essential tremor (ET) affects 5% of adults over 60. The main symptom of ET is a bilateral action tremor at 4-8 Hz in the upper extremities that often worsens over time and spreads to other areas of the body. Its pathophysiology is thought to involve circuits interconnecting the cerebellum, thalamus, and sensorimotor cortex. We seek to understand changes in the functional connectivity of these brain areas in ET patients with implanted deep brain stimulation (DBS) systems, a standard treatment for ET. A well-established method of assessing functional coupling is through coherence, a measurement that represents the co-occurrence of neural oscillations' frequency over time and can reveal interactions between regions of the brain and the periphery. To that end, we are first assessing the functional connectivity between the cortex and the hand and arm muscles in 5 ET patients in the DBS-off condition. We collected magnetoencephalography (MEG) and electromyography (EMG) data during a wrist extensor task, designed to induce postural tremor, and a pinch task, designed to induce kinetic tremor. We computed the power spectral density (PSD) of the MEG and EMG signals, as well as the corticomuscular coherence (CMC) between the two signals using a custom MATLAB (MathWorks, Natick, MA) script. EMG recordings were loaded into MATLAB and plotted for visual inspection to select epochs of muscle activation when the patient was performing the task. Magnitude squared coherence was calculated using MATLAB, and significance was computed between active and resting state across trials using a permutation test with replacement. Preliminary results align with previous literature in that several patients show increased EMG PSD, MEG PSD, and CMC within the tremor frequency band. Additionally, we are assessing which of the two motor tasks results in higher coherence levels and subsequently represents a better task for evaluating tremor-evoked coherence. These preliminary results will inform a new study designed to evaluate the deep cerebellar nuclei as a potential DBS target for ET.

Salan Ghaju*, Pakuwal Evance, Gessiane Pereira da Silva, Rafaela Takeshita, Xiaozhen Mou, Wilson Chung

Department of Biomedical Sciences, Kent State University

Ingestion of Sub-chronic Non-lethal Cyanotoxin Levels Disrupts the Stress Response.

Cyanobacterial harmful algal blooms (cHABs) are a major recurring environmental problem that is harmful to living organisms. CyanoHABs produce various types of cyanotoxins, chiefly amongst them is microcystin leucine arginine (MC-LR). Humans and other organisms exposed to MC-LR contaminated water or food chain may have severe detrimental ramifications on health. We found that MC-LR affects the stress response, which in mammals is regulated by the hypothalamus-pituitary-adrenal (HPA) axis. In the presence of a stressor, paraventricular nucleus (PVN) neurons secrete corticotropin-releasing hormone (CRH) to stimulate adrenocorticotropic hormone release from the anterior pituitary into general circulation which in turn induces the release of adrenal glucocorticosteroids. Previous studies established that the abnormal HPA function is a hallmark of mood disorders, such as anxiety and depression. Here we tested the hypothesis that ingestion of sub-chronic non-lethal MC-LR levels causes extended stress response activation. Therefore, we gavaged young adult male C57BL6 mice with water (vehicle; n = 9) or MC-LR (50 microgram/kg b.w) (p.o) every 2 days for 21 days. Animals were sacrificed on day 22. Our analysis of blood corticosterone levels, the number of PVN neurons with long-term activity marker delta-FOSB, CRH, AVP and GR mRNA expression indicated that ingested MC-LR caused an extended activation of the HPA activity. Overall, we conclude that sub-chronic MC-LR exposure results in an extended HPA response that may contribute to stress-related disorders, such as depression and anxiety.

Roman Giacomino*, Dinah Walter, Guensu Jang, Christina Watts, Colleen Novak, Ph.D.

Department of Biological Sciences, Kent State University

Mouse Muscle Thermogenesis in Response to Predator Odors: A Multilayer Analysis.

Skeletal muscle thermogenesis, or the heat produced by muscles, has a direct correlation with the amplification of energy expenditure. In previous studies, our lab has used predator odor (PO) as thermogenic stimuli in mice and rats. By quantitatively measuring the change in skeletal muscle thermogenesis in response to different POs, we can answer questions involving sex-based differences, effects of repeated exposure to stimuli, and the qualitative advantages of some POs over others. To address these questions, remote temperature transponders were used to monitor the skeletal muscle temperature in mice while in the presence of 3 different predator odors- ferret odor, cat odor, rat odor—and a control odor. Prior to thermogenesis assessment, the mice were habituated to the testing conditions to decrease the stress-related thermogenic effect of the procedure itself. Then, the mice were tested in three separate trials per odor, in a randomized order. During the four rounds of habituation, females had elevated temperature readings for a longer period than males. In contrast, female mice, unlike males, decreased their thermogenic response upon repeated exposure to the control odor. Little difference between sexes was found for the rat and ferret odors, which elicited robust muscle thermogenesis in both males and females. Upon repeated exposure, cat fur provoked less of a thermogenic effect in both males and females. Overall, all 3 POs caused an increase in skeletal muscle thermogenesis, but ferret odor caused the greatest increase, while control odor caused only a minimal increase. Based on this, we can conclude that female mice require additional habituation to prevent excessive muscle thermogenesis in response to testing conditions. Without additional habituation, we might mistakenly conclude that there is a sex difference in the muscle thermogenic response to predator threat. Also, ferret odor causes the most robust and consistent increase in skeletal muscle thermogenesis in comparison to the other POs tested. Future energy expenditure studies using predator odors should consider using ferret odor, as it is still effective over multiple trials.

Phoenix Green*, Alexandra Enders, Autumn Redd, Eric Brown, and Lique Coolen
Department of Biological Sciences, Brain Health Research Institute, Kent State University

Expression of enkephalin and opioid receptors in the spinal ejaculation generator after chronic spinal cord injury in male rats.

Spinal cord injury (SCI) in men and chronic mid-thoracic contusion injury in male rats impair ejaculation. Ejaculation is controlled by a spinal ejaculation generator (SEG) consisting of a lumbar spinothalamic (LSt) neurons. LSt cells control ejaculation through release of neuropeptides via projections to autonomic and motor neurons. LSt cells express enkephalin, controlling ejaculation via mu (MOR) and delta (DOR) opioid receptors. but location of these receptors in SEG is unknown. Here, the expression of MOR and DOR within LSt cells was examined after mid-thoracic contusion (n=5) or sham (n=6) injury in male rats using fluorescent in-situ hybridization (RNAscope). Results showed high expression of MOR in LSt neurons (76%) and lower expression of DOR (42%). 39% of LSt cells expressed both MOR and DOR. Preganglionic cells in central autonomic nucleus, intermediolateral cell column, and sacral parasympathetic nucleus also expressed MOR (53%, 39%; 25% resp.), DOR (54%, 33%; 27% resp.), or both (31%, 19%, 9% resp). SCI had no effect on receptor expression in any cell type. RNAscope analysis of enkephalin expression in LSt cells demonstrated no effect of SCI on enkephalin, while galanin was reduced. These results suggest that enkephalin released from LSt cells can influence ejaculation via interconnections and postsynaptic receptors on LSt soma. Enkephalin may also act on presynaptic receptors expressed on LSt axons, and/or on postsynaptic receptors in autonomic preganglionic cells. Finally, lack of changes of opioid receptors after SCI further supports development of opioid receptor agonists as a potential treatment for sexual dysfunction after SCI.

Alexandra Haupt*, Shannah Witchey, Heather K. Caldwell

School of Biomedical Sciences, Kent State University

Disrupted oxytocin signaling in the posterior paraventricular thalamus increases anxiety-like and depression-like behaviors with no effect on maternal behavior.

Oxytocin (Oxt) signaling via its receptor, the Oxtr, is important for the onset of maternal care. Previous work from our lab has found that genetic disruption of Oxtr expression in the nucleus accumbens (NAcc) shell results in a robust pup abandonment phenotype. However, it is unclear whether or not this observation is due to the disruption of the Oxtr exclusively in the NAcc shell or if knocking down Oxtr in other brain regions would result in a similar outcome. Thus, we chose to disrupt the expression of Oxtr in the posterior paraventricular thalamus (pPVT), due to its role in modulating stress response and avoidance behaviors, both of which can be involved in a dam's reaction to pups. We hypothesized that that the disruption of Oxtr in the pPVT would not impair the onset of maternal care or maternal behaviors. In order to test this hypothesis, adult female Oxtr flox/flox females were intracranially injected with either AAV2/2CMVCRE-wtIRESeGFP or AAV2/2CMVeGFP (control) in the pPVT. Females were then paired with C57BL/6J males until they were noticeably pregnant and then observed for the onset of maternal care. If maternal behavior was initiated, the latency to retrieve the first pup, latency to retrieve all the pups, time spent off the nest, time spent licking/sniffing, time spent self-grooming, and time spent rearing as well anxiety-like and depression-like behaviors were evaluated. Brains were collected and site checks were performed to verify the accuracy of the viral injections. While dams injected with AAV2/2CMVCRE-wtIRESeGFP displayed increased self-grooming behaviors during the maternal care test, as well as decreased time spent in the center of the open field test, and decreased time spent swimming in the forced swim test, there were no disruptions in the onset of maternal care and maternal behaviors appeared normal. These results support the assertion that Oxtr expression specifically within the NAcc shell, but not the pPVT, is necessary for the onset of maternal behavior in female mice.

Allianna Hite*, Lauren Scrimshaw, Cameron Russell, Marissa Nicodemus, Elayna Hallal, Jasmin Beaver, Aliyah Ross, Brady Weber, Lee Gilman
Department of Psychological Sciences, Kent State University

Will mice work harder for salty + sweet, or just sweet, snacks?

Americans consume on average nearly twice as much salt per day than recommended by the World Health Organization. Many health concerns are associated with consuming too much salt like increased risk of hypertension, heart disease, and stroke, but people continue to consume more salt than they should. Why? This is an understudied topic in mammals. To advance understanding of this phenomenon, we used mice to examine how hard they were willing to work for sweet reinforcers that had varying levels of salt. Mice were never food restricted at any point in the experiment. Both male and female C57BL/6J mice were first trained on fixed ratio (FR) schedules of reinforcement with just sweet reinforcers (i.e., 0% salt). Then mice were moved to a progressive ratio (PR) schedule, initially with sweet reinforcers. Once mice were stably responding to the PR schedule, mice were transitioned to sweet reinforcers containing 1, 2, or 4% salt. After PR stability at one level of salt, mice were moved back to 0% salt reinforcers before moving up to the next salt percentage. The mice went through this progression (0 ◊ 1 ◊ 0 ◊ 2 ◊ 0 ◊ 4) twice to evaluate how repeated exposure to increasing salt levels impacted the effort mice expended to obtain reinforcers. We found that males quickly worked harder for 0% salt reinforcers following previous exposure to reinforcers with lower levels of salt (1% and 2%). In contrast, effort of females to obtain 0% salt reinforcers did not change until they had been exposed to 4% salt reinforcers. These data indicate that consumption of salty + sweet reinforcers increase the appetitive value of subsequently encountered sweet reinforcers, through a process that has a lower salt threshold in males than females. Future studies are needed to identify what brain circuitry drives consumption of reinforcers when there is no physiological need for calories, and how prior salt consumption augments the willingness to work harder for sweet reinforcers.

Phaedra R. Keller*, Brad T. Casali, Erin G. Reed
School of Biomedical Sciences, Kent State University

Investigating the effects of early-life programming on neurodegenerative disease risk.

Microglia, the brain's resident immune cells, play a central role in many neurodevelopmental processes, such as synaptic pruning and guiding axonal growth cones. Brain masculinization is also facilitated through microglial involvement and is crucial to the normal development of male sexual behaviors. The masculinization of brain and behavior (mediated by estradiol, or E2) is accompanied by changes in the microglial transcriptome, which, in early life, favors a pro-inflammatory state in males, though this increased inflammation is short-lived, with females exhibiting increased inflammatory markers compared to males beginning in early adolescence. This increase in inflammation in females is persistent and this life-long exposure to increased inflammation is thought to be linked to the increased risk of developing a neurodegenerative disease in female patients. The current study aims to investigate the role of sex hormones in microglia-mediated inflammation and neurodegenerative disease processes across the lifespan, through the use of a previously established masculinization paradigm in 5xFAD mice.

Macit Emre Lacin*, Kiersten Hawk, Shreya Guha, Kemal Ozdemirli, Murat Yildirim

Neurosciences, Cleveland Clinic Lerner Research Institute

Rodent Pupillary Dynamics During a Reversal Learning Task.

Pupillary dynamics is linked to noradrenergic and cholinergic neuromodulator systems, which play a role in arousal, uncertainty, exploration-exploitation preference, and task engagement. Previous studies have implicated neuromodulator brain stem centers and phasic pupil responses in suppressing the impact of prior expectations and biases on decision-making. In this study, we investigated how phasic, task-related rodent pupil size changes correlate with rule switches in a deterministic reversible learning task. We combined pupillometry, behavioral measurement, and computational modeling to probe into the interaction between task-related pupil arousal, choice biases, and exploration-exploitation preferences over a month-long reversal training. Computational analysis of mouse behavioral measures showed that mice largely deviated from the optimal “win-stay, lose-shift” strategy and displayed a mix of different behavioral modes during individual sessions, which we inferred by building a block-wise hidden Markov model. Further analysis showed that these behavioral modes can be explained by model-free and inference-based learning. In parallel, we plotted the pupil size changes during individual sessions by employing a deep-learning-based algorithm (DeepLabCut) and custom scripts. Analysis of pupil dynamics across the training days for animals that display inference-based learning modes at later stages (high-performers) showed that the variance of pupil size during the sessions diminishes as the animal transitions to the expert level. This change in variance was not observed in animals that failed to transition to high-learning modes (low-performers). These results suggest that the high-performers may stop displaying task-related pupil arousal, possibly owing to their transition to predominantly inference-baseds learning strategies at later stages. Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by repetitive behavior and impaired behavioral flexibility. Several studies suggest ASD individuals may have altered pupil responses during cognitive tasks. We plan to train a mouse model of ASD (PTEN) in our reversal learning task and measure phasic and tonic pupil responses over the course of training.

Aiswarya Mukundan Nair*, Helen Piontkivska
Department of Biological Sciences, Kent State University

Exploring role of ADAR editing dysregulation in neurological manifestations of COVID-19.

COVID-19 pandemic, caused by the SARS-CoV-2, has resulted in a significant number of confirmed cases and deaths. Although it is primarily a respiratory illness, a significant portion of affected individuals develop neurological and neuropsychiatric conditions that can persist for a long period after initial infection. However, the molecular mechanism underpinning neurological manifestations remains elusive. Our study aims to explore one possible mechanism of pathogenesis, dysregulation of ADAR editing in the host transcriptome due to activation of innate immune response. We hypothesize that dysregulation of post transcriptional ADAR editing in neural genes underlies neurological manifestations of COVID-19. When SARS-CoV-2 enters a cell, it triggers an interferon response, leading to a cascade of downstream signaling resulting in the activation of interferon-stimulated genes, including ADARp1. ADARs are enzymes that modify RNA molecules by deaminating adenosine residues in double-stranded RNA and can act on both viral and endogenous RNAs. These editing events are highly dynamic and are regulated in a normal state, but during viral infections, this balance may be disrupted due to overexpression of ADARp1. Several neural genes hosting ADAR editing sites have been experimentally validated, and dysregulation of editing in these genes have been causally linked to development of neurological diseases. In this study we examined the impact of SARS-CoV-2 infection on the expression of ADARp1 and host ADAR editing patterns focusing on editing changes in a list or previously validated editing sites on key neural genes. To achieve this, we used a customized computational pipeline to analyze whole blood RNA sequencing data from 45 individuals sampled across three different stages of SARS-CoV-2 infection in CHARM study (Sauerwald et al., 2022). Our results showed elevated expression of ADARp1 and global changes in RNA editing levels during infection. Importantly, we observed that while ADARp1 expression levels returned to pre-infection levels in post-infection samples, the editing levels did not. We further aim to explore changes in editing levels in previously identified ADAR editing sites hosted in neural genes to get mechanistic insights into neurological manifestations.

Alyssa Novak*, Aleisha Moore
Department of Biological Sciences, Kent State University

Kisspeptin/Neurokinin B/Dynorphin (KNDy) neurons in mice exhibit robust reciprocal synaptic connectivity that may mediate synchronization of the population.

The pulsatile release of gonadotropin-releasing hormone (GnRH) and the subsequent release of luteinizing hormone (LH) are critical for mammalian fertility. This GnRH/LH release is controlled by a pulse generator comprised of cells upstream from GnRH neurons. In recent evidence, imaging the activity of cells in the arcuate nucleus that express kisspeptin, neurokinin B and dynorphin (KNDy) in freely behaving mice has shown the population produces episodic activity with synchronized firing events directly preceding each pulse of LH. It is hypothesized that synchronization between KNDy cells is mediated via direct KNDy-to-KNDy synaptic input, however, an assessment of these anatomical connections is lacking in mice. Therefore, we aimed to use fluorescent immunohistochemistry to quantify the density of KNDy synaptic input to KNDy soma in mouse brain slices. For the detection of KNDy-KNDy synapses, we performed fluorescent immunohistochemistry for synaptophysin (SYP), a marker of presynaptic input, and NKB on coronal brain slices from adult Kiss1-Cre/tdTomato mice. Z-stack images were taken with an FV3000 confocal microscope, with 10 KNDy cells imaged per animal. SYP-containing synaptic puncta with NKB and tdTomato (a marker for kisspeptin) that were closely apposed to TDT-containing soma were counted as KNDy-KNDy synapses. In addition, synaptic puncta containing either kisspeptin or NKB were identified to quantify input from non-KNDy populations containing these neuropeptides. Finally, SYP-containing puncta with no other label was quantified to assess input from other afferent neuronal populations. Our results show that the vast majority of synaptic inputs to KNDy soma (74.6% ± 9.8) originated from KNDy axons, while a smaller percentage of inputs originated from other populations, including NKB populations, kisspeptin populations, and populations not expressing the KNDy peptides. Together, these data support the hypothesis that synchronization between KNDy cells is mediated via direct synaptic communication. Ongoing studies using viral-mediated tract tracing techniques with Brainbow fluorescent inserts will further elucidate precise connectivity between individual KNDy cells and determine if KNDy inputs arise from neighboring cells or their own soma.

Kemal Ozdemirli*, Macit Emre Lacin, Shreya Guha, Kiersten Hawk, Caglar Oksuz, Murat Yildirim
Cleveland Clinic Lerner Research Institute, Neurosciences

High Throughput Analysis of Pupillometry Data Using Various Deep Learning Based Architectures.

Pupillometry is a non-invasive method for measuring autonomic function and cognitive effort. Pupil size changes have been demonstrated to be associated with pro-social behaviors, and decision making. However, pupillometry research has not been standardized across the labs. Thus, fast, high precision, and minimally supervised tools are required. In our experiments, we trained head-fixed mice on a reversal-learning task. We also utilized an infrared camera and a light source to monitor the pupil size during the task. Our goal was to develop a deep learning-based high throughput pupil analysis pipeline. We analyzed our pupil data using two different supervised deep learning packages called DeepLabCut (DLC) and Social LEAP Estimates Animal Poses (SLEAP). These are both well-known pose estimation tools and they do not require intensive computing power. To analyze pupil data, we employed our lab computer's single CPU and GPU. Both code packages output the x and y positions of the four points around the pupil, as well as the precision of their location. To train and assess their neural network (NN) architecture, we created and tested three distinct techniques. The first methodology is single-day analysis, in which we train a single neural network for each experiment. The second strategy involved generating an NN model for one animal by randomly extracting a set number of frames from each experiment. The final methodology was to create an NN model for all 10 animals by randomly taking a specified number of frames from each experiment. For DLC, we discovered that single-day analysis yields the most precise figures (Precision: 0.917 ± 0.044), but it takes over 3 working weeks to analyze the data using a CPU and 16 hours using a GPU. The single mouse concatenation had the second greatest mean precision value (0.881 ± 0.053), and it takes around 1 week to train and analyze the pupil data using a CPU, and 8 hours using a GPU. The mean precision value for the ten-mouse concatenation was the lowest (0.730 ± 0.065), while training and analysis took less than a week on a CPU and roughly 5 hours on a GPU. Interestingly, the mean precision value for the ten-mouse concatenation (0.953 ± 0.019) was higher than those with single mouse concatenation (0.884 ± 0.071) in SLEAP.

Karlee Patrick*, John Gunstad, Mary Beth Spitznagel

Department of Psychological Sciences, Kent State University

Pain in the Context of Virtual Neuropsychological Assessment of Older Adults.

Pain and cognitive impairment are prevalent and often co-occur in older adults. Neuropsychological evaluation is commonly employed in older adults with suspected cognitive impairment; however, prior work suggests pain may negatively impact cognitive test performance, and pain is often under-detected in this population. Alternative methods, such as video-based automatic coding of facial biomarkers of pain, might facilitate pain identification and enhance interpretation of neuropsychological evaluation results. The current study examined pain in the context of virtual neuropsychological assessment in 111 community-dwelling older adults, first seeking to validate the use of software developed to automatically code biomarkers of pain. Measures of pain, including self-report of acute and chronic pain and automatic coding of pain were compared while participants completed neuropsychological testing via Microsoft Teams. Self-reported pain was negatively associated with poorer performance on Oral Trail Making Test B (both acute [r = -0.25, p < .01] and chronic pain [r = -0.26, p < .01]) and the MoCA (acute pain only [r = -.23, p < .05]). Though automatic coding of pain was significantly associated with performance on neuropsychological tests, it did not predict self-report of pain or performance on neuropsychological measures after controlling for demographic factors and psychological symptoms. Despite past work suggesting that pain impacts cognitive assessment, the current study found limited evidence of an association between either self-reported or automatically coded pain biomarkers and neuropsychological test performance. However, the significant correlations that are present warrant further exploration to ensure that pain is not associated with test performance in other samples (e.g. individuals with higher levels of pain). Additionally, it is notable that all correlations between automatic coding of pain and neuropsychological test performance were in the expected negative direction, suggesting that automatic coding of pain may yet be useful for pain detection in this context. Future work should explore the utility of video-based automatic coding in samples with greater severity of pain and/or more significant cognitive impairment.

Madeline D. Porter*, James Liao, MD, David Escobar, Ph.D., Andre G. Machado, MD, Ph.D., Kenneth B. Baker, Ph.D.
Department of Neurosciences, Lerner Research Institute, Cleveland Clinic; Neurosciences, Biomedical Sciences, Kent State University

Functional Brain Network Changes in Patients Undergoing Deep Brain Stimulation for Essential Tremor.

Essential tremor (ET) is the most common movement disorder. It presents as a progressively severe 4-8 Hz kinetic tremor that predominantly affects the hands and arms. Up to 50% of patients do not respond to medication. Medication-refractory ET may be treated with deep brain stimulation (DBS) of ventral intermediate (Vim) thalamus, though it often loses efficacy as the disease progresses. Evidence indicates that ET arises as aberrant neural activity in the olivocerebellar circuit and spreads to muscles via the cerebebellothalamocorticomuscular (CTCM) pathway. The deep cerebellar nuclei (DCN) represent a potential new DBS target to interrupt tremor-related neural activity and disrupt this propagation. As a first step to evaluating this novel approach, we first must understand the role of the cerebellum in tremor propagation in the CTCM pathway. To that end, we will characterize tremor-related changes in 1) CTCM coherence in ET patients with DBS leads in Vim thalamus, and 2) neural activity across the CTCM network in response to therapeutic Vim DBS. We hypothesize that 1) tremor activity originates in the cerebellum, but only couples with thalamocorticomuscular circuits during movement; and 2) that DBS of the Vim decouples cerebellar activity from corticomuscular circuits independent of the active or resting state. Our study will enroll 15 patients with ET who have undergone Vim DBS prior to enrollment. We will evaluate their tremor and will collect simultaneous magnetoencephalography (MEG), electroencephalography (EEG), local field potential (LFP) from the DBS lead, electromyography (EMG), and accelerometry data during periods of rest and during motor tasks designed to elicit tremor. DBS will alternate between on and off. In our analyses, we will examine the magnitude, coupling, and direction of tremor-related neural and muscular activity. Our hypotheses will be satisfied if the data support that 1) tremor-related neural activity is present in the cerebellum regardless of activity or rest, 2) coherence across the CTCM pathway is increased during activity over rest, and 3) the cerebellum is the source of tremor pathology within the CTCM. These results will support the development of a phase I clinical trial to evaluate DCN as a DBS target for ET.

Sophia Pinti*, Richard Piet

Department of Biological Sciences, Kent State University

Noradrenaline has direct and indirect effects on the activity of kisspeptin neurons in the female RP3V.

In female rodents, kisspeptin neurons in the rostral periventricular area of the third ventricle (RP3VKiss) of the hypothalamus are responsible for driving a surge in the secretion of gonadotropin-releasing hormone (GnRH) and, subsequently, of luteinizing hormone (LH), triggering ovulation a few hours later. Noradrenaline (NA) release increases in the preoptic area of the hypothalamus, which includes the RP3V, prior to the surge in rodents. Prior research has shown that in vivo administration of α1 adrenergic receptor antagonist, prazosin, blunts or prevents the surge entirely. While NAergic signaling appears to contribute to generating the GnRH/LH surge, the mechanisms of this modulation remain unclear. We hypothesize that NA influences the surge by acting on RP3VKiss neurons. To investigate this, calcium imaging was performed on acute slices containing RP3VKiss cells. These slices were obtained from diestrus mice which express the genetically encoded fluorescent calcium indicator, GCaMP6f, in kisspeptin neurons. Increases in intracellular calcium-brought about by entry through calcium channels or by release from internal stores-result in increases in GCaMP6f cell fluorescence. GCaMP6f fluorescence is, thus, a proxy for cellular activity. Bath application of 100µM NA results in decreased cell activity in the majority of observed RP3VKiss cells, while a small proportion are excited or unaffected under these conditions. In the presence of 100µM prazosin, which prevents the surge in vivo, the inhibitory effect of NA on RP3VKiss neurons remains, with fewer observed excitations. We next tested whether the effect of NA on RP3VKiss neurons is direct by blocking electrical activity and synaptic transmission in the slice using a drug cocktail. In the presence of this cocktail, NA still produced a greater number of inhibitions than excitations, but a lesser proportion of cells were affected overall. This suggests that direct and indirect, excitatory, and inhibitory effects occur in RP3VKiss neurons in response to NA. Taken together, this reveals that NA at this concentration is primarily inhibitory, in contrast to our hypothesis. Additional work is needed to understand the pharmacology of these responses and how they converge to lend functionality to the LH surge.

Grant Ripley*, Emily Rabinowitz, Douglas Delahanty

Department of Psychological Sciences, Kent State University

Medical and Psychiatric Comorbidities in Patients with Chiari Malformation.

Introduction: Chiari Malformation (CM) is a chronic medical condition characterized by the descension of the cerebellar tonsils at least 5mm into the spinal canal. Despite affecting 1 in 1,000 Americans, little research has focused on its complex presentation and the extent to which CM presents with medical and psychiatric comorbidities. The present study described rates of medical and psychiatric comorbidities in a sample of adults with CM. Methods: A sample of adults with CM (N=116) were recruited through advertisement on a large non-profit website for involvement in a intervention for chronic pain due to CM. Participants completed surveys assessing demographics, self-report psychiatric, sleep, and medical diagnoses, surgical history, pain intensity using the visual analog scale (VAS), depression and anxiety using the Depression Anxiety Stress Scales (DASS-21), and posttraumatic stress disorder symptoms (PTSD) using the PTSD Checklist for DSM-5 (PCL-5). Results: Over 90% of the sample reported a diagnosis of CM type one and over half (63.79%) of participants reported undergoing at least one surgery to manage their CM. Participants reported moderate-severe pain interference (M=5.63, SD=1.86). Over 60% of participants reported taking pain medication, with 23% reporting opioid pain medication. The most common self-reported psychiatric diagnoses were major depressive disorder (16.38%), PTSD (14.66%), and attention-deficit hyperactivity disorder (10.34%). According to symptom indices, 12% of the sample had symptoms suggestive of moderate depression, 22% for anxiety, and 34% for PTSD. The most common comorbid medical conditions were scoliosis (19.83%), Ehlers-Danlos Syndrome (14.66%), and syringomyelia (13.79%). Participants also reported diagnoses of insomnia (28.28%), obstructive sleep apnea hypopnea (18.1%), and restless leg syndrome (10.34%). Discussion: The presence of psychiatric and medical comorbidities in CM highlights the complexity of the condition and emphasizes the need for integrative assessment and interventions to address comorbid conditions. Patients with CM may benefit from nonpharmacological interventions such as psychotherapy for persistent symptoms of pain, insomnia, and psychological distress.

Autumn Redd*, Thywill Ettey, Phoenix Green, Lique Coolen

Brain Health Research Institute, Department of Biological Sciences, Kent State University

Expression of Cholecystokinin in the Spinal Ejaculation Generator after Chronic Spinal Cord Injury in Male Rats.

Spinal cord injury (SCI) in men can lead to ejaculatory dysfunction. The spinal ejaculation generator (SEG) is the spinal center that coordinates the reflexes that control ejaculation. The SEG consists of a population of lumbar spinothalamic (LSt) cells. To trigger ejaculation, LSt cells receive sensory inputs and release four neuropeptides (galanin, enkephalin, cholecystokinin, and gastrin-releasing peptide) into autonomic and motor target areas within the lumbosacral spinal cord. Earlier research suggested that galanin, the general marker for LSt cells, and gastrin-releasing peptide are reduced following spinal contusion injury in male rats, while enkephalin is unaffected. The effects of SCI on cholecystokinin expression are unknown. In the present study, the expression of cholecystokinin mRNA within LSt cells was examined after mid-thoracic contusion injury in male rats using multiplex fluorescent in-situ hybridization (RNAScope). Spinal cords of male rats with sham or contusion injury at mid-thoracic spinal levels were collected at 4 weeks after injury, sectioned using cryostat, and collected on microscope slides. RNAscope was conducted using rat probes for galanin and cholecystokinin mRNA and images of transcript expression in LSt cells were captured using confocal microscopy. Analysis of transcript levels is currently in progress using Fiji/Image J. Preliminary observations have confirmed that 100% of LSt cells co-express galanin and cholecystokinin. This study will extend our knowledge of the impact of SCI on the molecular pathways within the SEG and may contribute to the development of treatment options.

Katlynd Reese*, Heather Caldwell
Department of Biological Sciences, Kent State University

Transient disruption of vasopressin 1a signaling on embryonic day 16.5 has subtle effects on social interactions in adult mice.

There is growing evidence that vasopressin (Avp), and the closely related nonapeptide oxytocin (Oxt), have roles in neural development. Previous work from our lab found that transient disruption of Oxt receptor (Oxtr) signaling on embryonic day (E) 16.5 results in sex-specific behavioral effects in adult mice. Given that Avp and Oxt are closely related, that crosstalk often occurs between their receptors, and that the Avp 1a receptor (Avpr1a) is present and functional during embryonic development, it is reasonable to speculate that disrupting Avpr1a signaling during embryonic development will affect adult behavior. Thus, we hypothesized that transient disruption of Avpr1a signaling at E16.5 would impact adult behavior. To test this hypothesis, 2µL of either an Avpr1a antagonist (Avpr1aA) or vehicle was injected into the lateral ventricle of each embryo and behavioral testing performed when the offspring were at least 2 months old. Transient Avpr1a disruption had no effect on anxiety-like or depressive-like behavior, as measured in open field, elevated plus, and forced swim tests. There was also no effect of Avpr1a disruption on social discrimination in males or females. However, Avpr1aA-treated males showed decreases in affiliative behavior on Day 3 of resident-intruder testing. Additionally, Avpr1aA treated animals had impairments in social novelty preference during a 3-chamber test. These data suggest that disruption of Avpr1a signaling at E16.5 has subtle, but measurable, impacts on the neural development of brain circuitry important to the regulation of social behavior. Given these promising results, future studies will continue to investigate the contribution of embryonic Avpr1a signaling to brain development and behavioral endpoints.

Aliyah J. Ross*, Allianna K. Hite, Anna Crites, Elayna M. Hallal, Grace A. Sonick, Lauren R. Scrimshaw, Jasmin N. Beaver, Lee T. Gilman

Department of Psychological Sciences, Kent State University

Evaluating how high salt diet levels affect active avoidance.

Past studies demonstrate excess salt intake in mice produces sex-specific effects on context fear behavior using classical conditioning. Thus far, however, studies have not assessed how high salt consumption affects operant behaviors. In our study, we used active avoidance - a form of aversive operant conditioning - to begin studying these effects of excess salt. We hypothesized a high salt diet would decrease female mice's retention and ability to learn because past studies found female mice display an increase in fear behavior while on a high salt diet. First, adult C57BL/6J mice were put on either a high salt (4.0% NaCl) or low salt (i.e., control; 0.4% NaCl) diet for two weeks. Next, we trained mice in an active avoidance task. Training lasted for five consecutive days. After a four-week interval from the first training day, mice went through final retention testing for three consecutive days. Contrary to our hypothesis, we detected no significant effect of salt, either on its own or in interaction with time. These findings indicate that excess salt does not impede learning and retention of behaviors that facilitate proactive avoidance of aversive outcomes in females. In the future, we plan to determine how stressor exposure between training and retention testing affects performance and run new cohorts with males to assess possible sex-related differences.

Ana Clara Campideli-Santana*, Raphael E. Szawka, Lique Coolen, Michael Lehman

Departamento de Fisiologia e Biofísica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brasil

Evaluation of the expression of endogenous opioid receptors in KNDy and GnRH neurons across the estrous cycle in female mice and rats.

Kisspeptin-neurokinin B-dynorphin (KNDy) neurons of the hypothalamus play a crucial role in the control of Gonadotropin-releasing hormone (GnRH) neurons, in a manner dependent on circulating levels of estradiol (E2). Evidence from pharmacological studies in rodents suggests that activation of opioids receptors can elicit different effects on the pulsatile GnRH secretion depending on the E2 milieu. The present study aimed to characterize, using RNAscope, the in-situ RNA expression of kappa-opioid (Oprk1), mu-opioid (Oprm1) and delta-opioid (Oprd1) receptors in KNDy and GnRH neurons of rodents under low and high E2 levels. Adult female Wistar rats and C57BL/6J mice were housed in controlled conditions of light and temperature. Vaginal cytology was evaluated daily and only animals displaying regular estrous cycles were used in the experiment. Rats and mice on proestrus and diestrus were deeply anesthetized using pentobarbital before transcardial perfusion with 4% paraformaldehyde (PFA). After the perfusion, brains were extracted and incubated in PFA for 24 hours, followed by incubation in sucrose solution until sinking. Brain sections of 12 µm were cryosectioned and placed onto slides. Sections were treated in order to evaluate the co-expression of Oprk1, Oprm1, Oprd1, and Gnrh1 or Oprk1, Oprm1, Oprd1, and Kiss1 genes in the same tissue sections. Briefly, sections were fixed in PFA and dehydrated in ethanol before treatment with protease reagent. Next, the RNA targets were simultaneously hybridized and amplified, followed by detection, imaging, and cleaving in groups of 3 targets. To achieve this, sections were incubated with 3-pooled MultiPlex probes and amplified. The cell nuclei were counterstained using Dapi and slides coverslipped. Images were obtained using a slide scanner (Olympus). Analysis is in progress, using ImageJ software for unbiased, automated quantification of mRNA co-expression in each tissue section. The number of cells expressing each mRNA will be quantified as the percentages of co-expression among the transcripts, and the average number of RNA transcripts overlaying kisspeptin and GnRH cells.

Coleman Sapp*, Emerson Egleston, Heather Caldwell

Biological Sciences Department, Kent State University

The Behavioral Function of Oxytocin Receptors in the Medial Amygdala.

The social behavior neural network (SBNN) is a network of important neural nodes, interconnected by glutamatergic and dopaminergic pathways. Importantly, each node in this network also expresses oxytocin receptors and this network is essential for expression of proper social behavior. Here, we wanted to explore how the presence or absence of oxytocin receptor signaling in the medial amygdala, an important node in this network, would affect behaviors related to social cognition. We hypothesized that mice lacking functional Oxt receptors (Oxtr) would have impaired social approach and social memory behaviors relative to behaviors observed in control mice. To test this hypothesis, adult male and female mice with loxP sites flanking oxytocin receptors were stereotaxically injected an adenovirus mediated Cre insertion to remove oxytocin receptors temporally (post primary development) and spatially (directly into the medial amygdala). Following a two-week period, mice were tested for anxiety-like behavior using open field and elevated plus maze tests, and social approach and social memory using a 3-chamber social cognition test. Male mice were also tested for aggressive behaviors through a 3-day resident-intruder test. This sequence of social behavior tests allows for an in-depth analysis of behaviors that are commonly associated with SBNN and oxytocin system functioning.

Lauren Scrimshaw*, Elayna Hallal, Allianna Hite, Aliyah Ross, Anna Crites, Grace Sonick, Cameron Russell, Lee Gilman

Department of Psychological Sciences, Kent State University

Reduced Function of Plasma Membrane Monoamine Transporters (PMAT) in Mice Augments Learning to Work for a Positive Reinforcer.

Plasma membrane monoamine transporter (PMAT) is a low affinity, high-capacity monoamine neurotransmitter transporter of dopamine and serotonin. Around 30% of humans in the population have a PMAT polymorphism that has been associated with reduced PMAT function. How reduced PMAT function affects behavior remains largely unknown, particularly because there are no drugs that selectively inhibit PMAT. Thus, the objective of this study was to use genetically modified PMAT-deficient mice in an appetitive operant conditioning study, to investigate how reduced PMAT function impacts behaviors required to obtain an outcome. In this study, we used two different cohorts: totaling 24 females, and 8 PMAT males with various genotypes. The three genotypes used in our study: wildtypes (WT); having full 100% functional PMAT, heterozygous (T); having reduced PMAT function (best for studying and comparing human PMAT polymorphisms), and knockouts (KO); having 0% functional PMAT. During appetitive operant, the mice were trained on a fixed ratio (FR)-1 schedule, then progressed to FR-3 and FR-5 schedules, followed by a progressive ratio (PR) schedule, swim stress 8 weeks later, then placed back into PR until criterion was met. We hypothesized PMAT knockout mice would perform better in operant behavior, completing FR schedules before their wildtype and heterozygous counterparts. We observed that all the female KO mice completed FR schedules within the first 40 days, as opposed to their female WT counterparts still actively running after 80+ days. While the male cohort is still being run, and not all female mice have gone through swim stress, the interactions of genes x environment x sex will be assessed in the near future. These findings, so far, suggest that normal PMAT function impedes acquisition of behavioral procedures that result in appetitive outcomes in female mice. Given we have observed sex specific of PMAT in other dimensions of behavior, we expect to observe a sex difference in terms of these operant behaviors too.

Marwan Shalih Maraicar*, Katherine Knies, Misha Psenicka, Jessica Williams, Robert Clements, Jennifer McDonough

Biological Sciences Department, Kent State University

Validation of neuroprotective effects of Betaine against MS using Coherent Anti-stokes Raman Scattering Microscopy.

Multiple sclerosis (MS) is a debilitating demyelinating disease characterized by inflammation, neurodegeneration, and the failure of oligodendrocyte progenitor cells (OPCs) to differentiate and remyelinate axons. This study explores a novel therapeutic strategy aimed at overcoming the differentiation block in OPCs and enhancing myelin repair in MS. MS pathogenesis involves complex interplay between immunological, environmental, and genetic factors. Dysregulation of methionine metabolism has been implicated in MS, leading to decreased levels of key methyl donors such as S-adenosylmethionine (SAM). Betaine homocysteine methyltransferase (BHMT) has emerged as a key player in restoring SAM levels and facilitating DNA and histone methylation crucial for OPC survival and differentiation. We hypothesized that augmenting the BHMT-betaine methylation pathway would improve motor disability and neuronal survival in the experimental autoimmune encephalomyelitis (EAE) mouse model, a model for MS. In the EAE mouse model, we administered betaine intraperitoneally. Our results demonstrated a significant improvement in motor ability in the betaine-treated mice during the chronic stages of the disease. Additionally, we employed coherent anti-Stokes Raman scattering (CARS) microscopy, a novel cutting-edge technique to assess myelin sheath composition around axons in the spinal cord without staining. Quantification of CARS intensity profiles revealed a substantial improvement in lipid content in the betaine-treated mouse models, validating our hypothesis. Our study highlights the potential of betaine-mediated epigenetic modification as a promising therapeutic avenue for MS. Future investigations will delve into the molecular mechanisms underlying betaine's neuroprotective roles in oligodendrocytes, offering insights into its application against MS. Furthermore, the use of CARS spectral analysis across different Raman frequencies opens doors to quantifying macromolecular differences between healthy and diseased brain and spinal cord tissues without the need for staining, promising a more cost-effective and unbiased approach. In conclusion, this research paves the way for innovative strategies in MS treatment, addressing the pressing need for myelin repair and OPCs differentiation.

Lara Shigo*, Brittany Smith, Angela Ridgel

Exercise Physiology Department, Kent State University

Factors Associated with Physical Activity in Individuals with Parkinson's Disease.

BACKGROUND: Parkinson's disease (PD) is a progressive neurodegenerative disorder most often affecting older adults. Symptoms include bradykinesia, tremor, rigidity, reductions in balance and mood disturbances. Physical activity (PA) may slow the progression of PD and improve motor symptoms. PURPOSE: The purpose of this study was to evaluate factors associated with PA in a large sample of people with PD. Researchers hypothesized that as duration of PD diagnosis increases, PASE scores decrease independent of age. METHODS: The Michael J Fox (MJF) Foundation manages a large (n>50,000) deidentified data set. The majority of survey respondents have been diagnosed with PD and a subset of respondents serve as healthy controls. 8,058 individuals with PD completed the Physical Activity Scale for the Elderly (PASE). Higher PASE scores indicate more daily PA. Additional surveys assessed demographics, PD-specific experiences, and health history. Permission to access data was obtained from MJF. Hypothesis testing (independent samples t-tests, ANOVA, ANCOVA) evaluated relationships between PASE score, duration of PD diagnosis (controlling for age), sex, income, depression, and anxiety. RESULTS: ANCOVA revealed significance among PASE score and time diagnosed with PD with age as a covariate (diagnosis <3 years: 123.68, 3-10 years: 121.95, 10+ years: 110.06, p<.001). Independent samples t-tests revealed significant differences in PASE scores between males (127.15) and females (113.71) with PD (p<.001) and between individuals with PD diagnosed with depression (107.92) and those not diagnosed with depression (136.76, p<.001). One-way ANOVA revealed significant differences in PASE scores across stratified income levels (p<.001). No significant difference in PASE score was found between individuals with PD diagnosed with anxiety (114.09) and those not diagnosed with anxiety (130.94, p=.006). DISCUSSION: Data indicates that PD contributes to increased inactivity independent of age. Individuals with PD who have concurrently been diagnosed with depression, who are female, or who have lower income levels may be at an elevated risk for inactivity. Health care providers may use this data to proactively encourage physical activity in individuals with PD.

Michael Skowrons*, Andreas Schander, Björn Lüssem

Physics Department, Kent State University

Neural Probes using Vertical Organic Electrochemical Transistors.

Organic electrochemical transistors (OECTs) are known for their high transconductance and biocompatibility, making for excellent biosensors. They have previously been used to record electrophysiological signals on the surface of the brain [1], and as a sensor for a variety of neurotransmitters [2]. Their high transconductance allows for local amplification of signals in situ, improving the signal-to-noise ratio when compared to traditional electrodes. A recent innovation in OECT design [3], where the channel is defined vertically by a passivation layer, reduces the channel length of the OECT to the sub-micron regime and significantly increases the transconductance. By combining vertical OECTs with a previously developed needle-shaped neural probe design [4], an improved electrophysiological sensor is developed. These sensors are currently undergoing in vivo measurement in rodents. 1. Khodagholy, D., Doublet, T., Quilichini, P. et al. In vivo recordings of brain activity using organic transistors. Nat Commun 4, 1575 (2013). 2. Marks, A., Griggs, S., Gasparini, N., Moser, M., Organic Electrochemical Transistors: An Emerging Technology for Biosensing. Adv. Mater. Interfaces 2022, 9, 2102039. 3. Koutsouras, D. A., Torricelli, F., Blom, P. W., Submicron Vertical Channel Organic Electrochemical Transistors with Ultrahigh Transconductance. Adv. Electron. Mater. 2023, 9, 2200868. 4. A. Schander, H. Stemmann, K. C. Claussen, A. K. Kreiter and W. Lang, "In-Vitro and In-Vivo Longevity Evaluation of Free-Floating Intracortical Silicon-Stiffened Neural Probes," 2019 9th International IEEE/EMBS Conference on Neural Engineering (NER), San Francisco, CA, USA, 2019, pp. 799-802

Mandalynn Slupek*, Sydney Severt, Devin Mueller

Biology Department, Kent State University

β-ADRENERGIC RECEPTOR BLOCKADE PERSISTENTLY DISRUPTS RETRIEVAL OF FENTANYL-ASSOCIATED MEMORY

Retrieval of drug-associated memories triggers craving and relapse in substance users, hindering treatment and cessation of use. Disrupting retrieval of drug-associated memories could significantly reduce or eliminate seeking and relapse rates in addicts. Previous work from our lab has shown that systemic β-adrenergic receptor blockade persistently impairs retrieval of cocaine-associated memories (Otis et al., 2011), an effect that could be isolated to the prelimbic medial prefrontal cortex (mPFC; Otis et al., 2013) and dorsal hippocampus (Otis et al., 2014). These findings suggest a retrieval network critical for the maintenance of substance abuse, but whether this serves as a common network for retrieval following any drug of abuse is unknown. Here we assessed whether retrieval of opioid-associated memory using the potent synthetic opioid, fentanyl, could be disrupted by β-adrenergic receptor blockade. We established a fentanyl conditioned place preference (CPP) following conditioning over 8 days in which rats (N=16) associated fentanyl with one of two distinct chambers. At the test, rats had free access between chambers and time in each was recorded. We found that propranolol (10 mg/kg, i.p.), a β-adrenergic receptor antagonist, administered prior to the first retrieval test persistently impaired fentanyl CPP compared to saline (p<0.005). Next, we will determine if the prelimbic mPFC is a common node in the retrieval network for drug-associated memories. Following conditioning, rats will be bilaterally infused with propranolol (10 ug/ul at a volume of 0.3 ul per side) or saline into the prelimbic mPFC. These investigations are underway to determine the specific brain regions common to addiction-retrieval pathways across drug classes. Overall, these findings suggest a widespread therapeutic use for propranolol in the treatment of addiction through prevention of drug-associated memory retrieval to reduce cue-elicited drug seeking and relapse.

Brittany Smith*, Younguk Kim, Lara Boman, Robert Melczak, Angela Ridgel

Exercise Science and Exercise Physiology, Kent State University

Patient-Specific Adaptive Dynamic Cycling is Associated with Improvement in PD Motor Symptoms.

BACKGROUND: High-cadence dynamic cycling is an effective therapy for improving motor symptoms in individuals with Parkinson's Disease (PD), as measured by the Unified Disease Parkinson's Rating Scale-Motor III (UPDRS-III). However, there is significant variability in individual responses to this therapy. Our lab developed a patient-specific adaptive dynamic cycling (PSADC) paradigm aimed to optimize entropy of cadence which may lead to effective exercise prescriptions for individuals at various stages of disease progression. PURPOSE: To evaluate the effectiveness of 12 sessions of PSADC on motor symptom improvement (UPDRS-III score) in individuals with PD. METHODS: Twenty-three individuals with idiopathic PD (Hoehn and Yahr Stage 1-5) were randomized into two groups: PSADC (n=13) or active control (n=10). All individuals completed 12 sessions of dynamic cycling on a SMART (Speed Manipulated Adaptive Rehabilitation Therapy) bicycle. Each session consisted of a 5-minute warm-up at 60 revolutions per minute (rpm), 30-minute exercise session (80 rpm), and 5-minute cool-down (60 rpm). Individuals in the PSADC group followed an adaptive exercise prescription in which resistance was optimized weekly, based on the individual's entropy of cadence, and cycling effort. The individuals in the active control group remained at a constant resistance for the entirety of the intervention. UPDRS-III was assessed in all participants prior to and following the intervention. Two-way analysis of variance (ANOVA) and paired samples t-tests were performed to detect differences in UPDRS-III score between the groups. RESULTS: There was a significant group by time interaction (F= 18.746, p <0.001). The PSADC group showed a significant reduction (improvement) in UPDRS-III score (Pre: 32.8, Post: 27.5; p<0.001), while the active control group showed no significant change in UPDRS-III score (Pre: 28.2, Post: 32.4, p=0.08). CONCLUSION: 12 sessions of PSADC significantly improved UPDRS-III score, compared to non-adaptive high-cadence dynamic cycling. These results suggest that optimizing entropy of cadence is valuable for motor symptom improvement. Future studies will develop machine learning algorithms designed to predict appropriate clinical exercise prescriptions for individuals with PD.

Matthew J. Sunthimer*, Yi Ye, James Tyler Moore*, Ethan White, Kelly J. Snyder, Jeffrey Mellott, Merri J. Rosen
School of Biomedical Sciences, Kent State University, Northeast Ohio Medical University

Interactions of Developmental Stress and Hearing Loss on Gap Detection and the Auditory Cortex.

Early conductive hearing loss (eCHL) is linked with lasting speech perception deficits. These can be attributed in part to compromised processing of temporally varying sounds such as short gaps, linked to reduced inhibition throughout the auditory system, particularly the auditory cortex (ACx). Early-life stress (ELS), which is a risk factor for poorer outcomes with eCHL, affects inhibition in non-sensory regions of the brain. However, we have previously demonstrated that ELS worsens perceptual and ACx sensitivity to gaps in sound, suggesting that inhibition in ACx may be susceptible to ELS. Maturation of parvalbumin (PV) inhibitory neurons plays a critical role in the development of cortical response properties, in part via activity-dependent accumulation of perineuronal nets (PNNs). eCHL and ELS have separately been shown to alter expression of PV neurons and PNNs in a region dependent manner, suggesting that the dual impact of these two developmental disruptions may be visible in ACx. Gerbils were divided into 4 groups: Controls, ELS, eCHL, and eCHL+ELS. ELS was induced via ten two-hour sessions of maternal separation and restraint during postnatal (P) days P9-24. Reversible CHL was induced using earplugs from ear-opening P11 to P24. Animals were either tested on perceptual tasks or used for immunohistochemistry. Behaviorally, detection of short gaps in ongoing sound were collected at P33, 36, and 39 (adolescence) and P83, 86, and 89 (adulthood) using gap-inhibition of the acoustic startle response. For immunohistochemistry, animals were perfused at either P35-40 or P85-95 and stained to visualize PV and PNNs at 20x in ACx. Measured during adolescence, ELS, eCHL, and their combination all impaired behavioral gap detection, but with no difference in severity. By adulthood, ELS and eCHL deficits were reduced but still present. However, the interaction of ELS and eCHL impaired thresholds much more than either individual insult. In ELS adolescent animals, ACx had reduced densities of PV cells, PNNs, and PV cells with PNNs. The reduced PV cell density continued into adulthood. For animals raised with eCHL or ELS+eCHL, forthcoming labeling data will be presented.

Mahtab Tehrani*, Sharad Shanbhag, Rahi Patel, Diana Kazimierski, Jeffrey Wenstrup
NEOMED Department of Anatomy and Neurobiology, Hearing Research Group

Representation of Social Vocalizations in the Mouse Inferior Colliculus.

We analyzed neuronal responses of the inferior colliculus (IC) to mouse social vocalizations. We asked: 1) how frequency tuning properties of neurons affect call responsiveness, 2) how responses might differ across the three main IC subdivisions, and 3) whether IC neurons in males and females respond differently to vocalizations. In both sexes, the distribution of characteristic frequencies (CF) was centered near 16 kHz, with a secondary peak near 50 kHz. This secondary peak was larger in males, mostly within the central subdivision (CIC). A subset of male CIC units, but no female units, had CFs of 64 kHz. Widths of tuning curves varied widely among low-CF units. Dorsal IC units had on average broader tuning than units in CIC or external nucleus. Non-ultrasonic vocalizations elicited responses in approximately 75% of IC units: in all subdivisions and across the tonotopic range. Among ultrasonic vocalizations (USVs, fundamental frequencies ≥20 kHz), stepped calls with harmonic elements as low as 30 kHz evoked responses in about 25% of IC units across subdivisions. In contrast, responses to tonal calls (all energy above 60 kHz) were much more limited (<10%). For males and females, overall responsiveness to social calls was similar. In general, USV responses among low-CF units could be of two types, with potentially different mechanisms underlying their excitation. Most commonly, broadly tuned low-CF units responded as long as USV bandwidth overlapped with their 60-dB SPL tuning bandwidth. Much less frequently, USV responses occurred in low-CF units without overlap between their tuning and the bandwidth of the USVs. These responses may result from low frequency cochlear distortion products, as proposed by Portfors and colleagues. Several conclusions result from these findings. First, responses to USVs are more restricted in the IC. Second, neurons in both low and high frequency representations of IC carry information about all vocal types. Third, each IC subdivision carries information about every vocal type to the auditory thalamus. This suggests that subsequent vocal processing centers in the auditory cortex and amygdala perform complex analyses based on inputs from a broad range of mouse social vocalizations.

Huimei Wang*, Yong Lu

Northeast Ohio Medical University (Neurobiology and Anatomy) and Kent State University (Biomedical Science)

Mechanisms underlying reduction of cellular excitability by high extracellular calcium concentrations in mouse MNTB neurons.

Calcium, a universal intracellular signaling molecule, plays essential roles in neural functions. Historically, in most in vitro brain slice electrophysiology studies, the calcium concentrations in artificial cerebrospinal fluid (ACSF) are of a wide range and typically much higher than the physiological value. At higher extracellular calcium concentrations, synaptic transmission is generally enhanced. However, the effects and the underlying mechanisms of calcium on intrinsic neuronal properties are diverse. Using whole-cell patch clamp in acute brainstem slices obtained from mice of either sex, we investigated the effects and the underlying mechanisms of higher extracellular calcium concentrations on intrinsic neuronal properties of neurons in the medial nucleus of the trapezoid body (MNTB), a key component in the sound localization circuitry. Compared to the physiological extracellular calcium concentration (1.2 mM), higher calcium concentrations (1.8 and 2.4 mM) significantly reduced the cellular excitability of MNTB neurons, resulting in decreased spike firing rate, increased spike threshold, and decreased ability to follow high frequency inputs. Higher magnesium concentrations produced subtle effects, perhaps due to surface charge screening. Strong expression of calcium-sensing receptor was detected in MNTB neurons. Mechanistically, higher calcium concentrations reduced voltage-gated sodium channel currents and shifted the activation curve rightward. These results call for careful considerations of the intrinsic neuronal properties from previous studies performed under conditions using higher-than-physiological extracellular calcium concentrations, and a transition of using physiological calcium concentration in brain slice experiments. Supported by NIH R01DC016054.

Keywords: calcium; excitability; medial nucleus of the trapezoid body.