UNIVERSITY OF MICHIGAN

NEUROSCIENCE CONFERENCE 2024

Poster Session Presentations

Abstracts, authors, and locations

DAY 1 - MONDAY, MAY 13

North Campus Research Complex
Building 18 Dining Hall

DAY 2 - TUESDAY, MAY 14

Biomedical Science Research Building
Atrium

MICHIGAN NEUROSCIENCE INSTITUTE
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MEDICAL SCHOOL NEUROSCIENCE GRADUATE PROGRAM
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NEUROSCIENCE
CONFERENCE 2024

DAY 1

Abstracts, authors, and locations

Monday, May 13 - 5:00 p.m.

North Campus Research Complex
Building 18 Dining Hall

Bowen Wang
Undergraduate Student

How Rat Hippocampal Oscillation Patterns Correlate with Ultrasonic Vocalizations During Trace Fear-Conditioning

Bowen Wang, Nat Kinsky, and Kamran Diba

This investigation delves into the nuanced interplay between rat ultrasonic vocalizations (USVs) and hippocampal oscillations within a fear-conditioning framework. The study illuminates the complex dynamics of 50kHz and 22kHz USVs—markers of diverse emotional states—and their intricate association with conditioned stimuli and shock events, challenging traditional notions of their emotional specificity. A key finding is the absence of significant coupling between USV emissions and theta oscillations, revealing minimal overlap and suggesting these occur at mutually exclusive time points. Phase analysis of theta oscillations at USV onset/offset offers exciting insights, suggesting a correlation with specific cognitive functions, such as encoding and retrieval processes. This is further supported by spectrogram analyses of theta and SWR oscillations at USV onsets and offsets, providing a more granular view of the oscillatory changes associated with vocalization events. Moreover, sharp wave ripples (SWRs) demonstrate a robust coupling with USVs, particularly with 22kHz calls, hinting at a significant role in memory processing during vocalization. This coupling, alongside the correlogram and power observations, underscores the intricate relationship between vocal behaviors, emotional states, and hippocampal activity. This comprehensive analysis not only advances our understanding of the neural dynamics underpinning rat vocal behavior in response to fear-inducing stimuli but also hints at broader implications for understanding emotional expression and cognitive processing in mammals. The findings questioned some of the older understandings and suggested a more nuanced interpretation of USV emissions in relation to hippocampal oscillations, providing a foundation for future research in neural dynamics and emotional cognition.

Chloe Rybicki-Kler
Graduate Student

Cortical acetylcholine levels increase during spatial navigation

Cholinergic muscarinic activation induces persistent firing of cortical principal neurons, providing a key cellular basis for theories of spatial working memory and path integration. The granular retrosplenial cortex (RSG) is important for successful spatial navigation and contains multiple subtypes of principal cells, including low-rheobase (LR) and regular spiking (RS). We have previously demonstrated that these two cell types participate in distinct, parallel circuits to process navigationally relevant inputs to the RSG. Here, we show that the transcriptomically, morphologically and biophysically distinct LR cell-type has a very different expression profile of cholinergic muscarinic receptors when compared to its RS neighbors. To investigate the cell type-specific response to activation of muscarinic receptors, we performed whole-cell recordings of RS (n=57) and LR (n=22) cells and characterized their response to muscarinic agonists. Consistent with our transcriptomic results, LR neurons did not show any cholinergically-evoked persistent firing, in stark contrast to all other principal neuronal subtypes examined within the RSG and across the frontoparietal cortex. These results were similar for both male and female mice. Using an integrate-and-fire model of the LR neuron, we show that this lack of persistence allows LR cells to rapidly compute angular head velocity, independent of cholinergic changes across brain states. Thus, the LR neuron is a unique cell type, optimized for rapid state-independent computations needed to encode angular head velocity, a critical component of spatial navigation.

Elie Huez
Graduate Student

Cholinergic Excitation and Inhibition of NPY Neurons in the Inferior Colliculus

Elie Huez, Marina Silveira, and Michael Roberts

The inferior colliculus (IC), a major integration hub in the central auditory pathway, receives dense cholinergic projections from the pedunculopontine tegmental nucleus (PPT), a brain region involved in arousal and attention. However, how cholinergic signaling modulates inhibitory neurons in the IC remains largely unknown. We recently identified Neuropeptide Y (NPY) as a marker for a class of GABAergic IC neurons. Here, we show that the excitability of NPY neurons can be enhanced or inhibited by acetylcholine (ACh). Using electrophysiology and pharmacology, we are testing the hypothesis that this bidirectional regulation of NPY neuron excitability is due to the differential expression of muscarinic (mAChRs) and nicotinic ACh receptors (nAChRs) in subpopulations of NPY neurons. To selectively target recordings to NPY neurons we utilized NPY-IRES2-FlpO x Ai65F mice in which NPY neurons express tdTomato. We used brain slice electrophysiology in acutely prepared IC slices to record the changes in membrane potential of NPY neurons during puff applications of 1 mM ACh. After recording control responses to ACh, we perfused various muscarinic and nicotinic receptor antagonists for 10 minutes and assessed how responses to ACh puffs changed. We found that 1mM ACh puffs onto NPY neurons induced depolarizing and hyperpolarizing responses. We are sequentially applying selective mAChR and nAChR antagonists on IC brain slices and assessing their ability to block responses to ACh puffs. Our preliminary data suggest that individual NPY neurons can express mAChRs, or nAChRs. We find that ACh elicits depolarizing and hyperpolarizing responses in subpopulations of NPY neurons. Our results to date suggest that nAChR and mAChR expression regulate the depolarizing and hyperpolarizing effects, respectively, of ACh on NPY neuron excitability. We, therefore, propose that cholinergic signaling in the IC may use selective and differential modulation of inhibitory circuits to enhance certain IC computations while inhibiting others.

Gunnar Quass, Ph.D.
Postdoctoral Fellow

Task-Dependent Activity in the Shell Inferior Colliculus

Gunnar L Quass, Meike M Rogalla, Alexander N Ford, Pierre F Apostolides

Active listening requires not only correctly identifying primary sound features, but also learning their associated behavioral relevance. Behaviorally relevant representations are abundant in auditory cortex and thalamus, but whether similar activity is present earlier in the auditory pathway is unclear. The non-lemniscal nuclei of the inferior colliculus (shell IC) receive a variety of acoustic, multi-sensory and neuromodulatory signals, suggesting in integratory role in perceptual learning. Indeed, many of the non-lemniscal IC's targets - higher order regions of the medial geniculate - famously code for both sounds and behavioral outcomes, and constitute major components of goal-oriented behavior. However, whether this joint coding of acoustic and behavioral information arises locally, or is already present in upstream IC neurons, remains unknown. We used multiphoton Ca2+-imaging, machine learning, and a reward-based discrimination task in mice to test if behaviorally relevant signals are present in shell IC neurons.

In line with previous results, we found a strong task-modulation of sound responses in a significant number of neurons. We further observed stark trial-outcome-dependent differences in population processing during sound presentation of equal sounds. This activity consists of separate sound- and movement-related parts that a SVM classifier can use to decode and even predict an animal's behavior already before a task-relevant action is taken. Remarkably, the processing of non-auditory, task-related variables continues after the sound and even after the reward period, suggesting that the shell IC integrates learned associations with sound stimuli on a scale of seconds. Thus, shell IC activity contains all the building blocks necessary for sound-behavior association – sound encoding, movement encoding, and retrospective processing, thus implying a role for the shell IC in processing stimulus-reward associations and task-driven behavior outside of cortico-thalamic networks.

Supported by NIH R01DC019090, The Whitehall Foundation, and the Hearing Health Foundation.

Hannah Oberle
Graduate Student

Differential Cortical Modulation of Inferior Colliculus Sub-Circuits

Hannah M. Oberle, Esther J. Choi, Clara Martinez-Voigt, Pierre F. Apostolides

The auditory cortex sends excitatory feedback (corticofugal) projections to the inferior colliculus (IC), a midbrain hub involved in complex sound coding. Corticofugal axons primarily target higher-order dorso-medial and lateral "shell" IC sub-nuclei. We previously characterized corticofugal transmission onto dorsomedial IC neurons, revealing single-cell and network mechanisms enabling non-linear computations in distinct IC cell classes (Oberle et al., 2022; 2023). However, whether corticofugal synaptic activity has similar or divergent effects in the lateral IC is unknown. We combined transgenic mouse lines, optogenetics, and patch-clamp electrophysiology in acute IC brain slices to measure corticofugal transmission in the lateral IC (n=55) and compared the results with dorsomedial IC recordings (n=25). We crossed VGAT-ires-cre and Ai14 fl/fl mice to record from GABAergic (VGAT+) and presumptive glutamatergic (VGAT-) neurons. In the auditory cortex we expressed the excitatory opsin Chronos to optogenetically activate auditory corticofugal axons with trains of light flashes. Nearly all dorsomedial IC neurons tested (22/24) exhibited EPSPs during optogenetic stimulation of corticofugal axons; surprisingly fewer lateral IC neurons in the same slices (20/55) showed EPSPs with the same stimulation. Moreover, corticofugal train EPSPs were significantly smaller in lateral compared to dorsomedial IC neurons, suggesting sparser convergence of corticofugal axons onto lateral IC neurons. Our prior study (Oberle et al., 2023) showed corticofugal signals drive polysynaptic excitation in dorsomedial VGAT+ neurons but not VGAT- neurons. However, preliminary data suggests this circuit motif is absent in the lateral IC: Majority of VGAT- and VGAT+ corticofugal EPSPs have onset latencies between 2 and 6 ms. We these experiments, we identified surprising intricacies of the auditory cortico-collicular pathway's impact on distinct IC sub-regions and how it shapes signaling in the auditory midbrain.

Xingyu Li, Ph.D.
Postdoctoral Fellow

The Mechanism of REM Sleep Breathing

Xingyu Li, Peng Li

Breathing is closely regulated by different states of wakefulness and sleep. During rapid eye movement (REM) sleep, when skeletal muscles undergo paralysis, respiratory muscles continue to function, ensuring normal breathing. However, the specific neural mechanisms underlying this phenomenon remain elusive. Utilizing in vivo fiber photometry, we observed increased neuronal activity in the neurons within the preBötzinger complex (preBötC), the medullary center responsible for inspiratory rhythm generation, particularly during apneic episodes in REM sleep compared to non-REM sleep (NREM) and wakefulness. Ablation of these neurons in the preBötC led to exacerbated apneas exclusively during REM sleep in mice, characterized by increased frequency and duration of apnea events. Conversely, selective activating these neurons induced apneas only during REM sleep. Through retrograde tracing and functional manipulation, we found an indirect connection between the dorsal part of the subcoeruleus nucleus (SubCD), a region implicated in REM sleep generation, and the preBötC, which regulates breathing during REM sleep. Therefore, facilitating regular respiration during REM sleep. In summary, our findings suggest that overactivation of the preBötC neurons contributes to apnea episodes during REM sleep, offering valuable insights into the underlying mechanisms of respiratory control in this sleep state.

Mekhala Kumar
Graduate Student

Auditory cortical mechanisms of self-generated sound-guided navigation

Mekhala Kumar, Gideon Rothschild

The ability to rapidly and efficiently process sensory stimuli, and in particular sounds, during locomotion is critical for survival and adaptive behaviour. Some incoming sounds during locomotion originate from external sources (such as the sound of a passing car) while others are self-generated (such as the sound of our own footsteps). Self-generated sounds may be predictable and uninformative in some cases, but in other situations, they carry rich behaviourally-relevant information, such as the substrate we are walking on, our locomotion speed, and our location, especially in situations where we cannot rely on visual cues, like walking around in the dark. Indeed, human studies show that self-generated sounds can influence behaviour in an ongoing manner. However, the neural mechanisms underlying the encoding and usage of self-generated sounds are poorly understood. To address this gap, I designed a novel experimental setup wherein freely-moving rats learn to navigate a track guided by their own footstep sounds. When rats were tested on their performance in this navigation task after learning, we found that they successfully use their footstep sounds to navigate in the darkness i.e. in the absence of visual cues, but not when visual cues are present. A key candidate region that could encode these footstep sounds and facilitate such sound-guided navigation is the auditory cortex. Inactivation of the auditory cortex of rats being tested in the darkness impaired their performance on this task. Additionally, we recorded neural activity from the auditory cortex during learning which showed robust responses to footstep sounds. Overall, our findings suggest that self-generated footstep sounds that are relevant for guiding behaviour are encoded by the auditory cortex.

Meike M Rogalla, Ph.D.
Postdoctoral Fellow

Probing neuronal circuits of spatial hearing during sound-localization behavior in head-fixed mice

Meike M Rogalla, Gunnar L Quass, Clara Martinez-Voigt, Harry Yardley, Kiran R Lahiri, Pierre F Apostolides

Humans and animals can re-learn to localize sounds following monaural hearing loss, even when binaural cues are absent. It is assumed that the re-learning relies on context-dependent plasticity mechanisms that 're-calibrate' the representation of auditory space in sound localization circuits. However, the exact processes underlying this learning-dependent plasticity remain unknown. This gap in knowledge is mainly derived from methodological difficulties in chronically recording the same neurons over extended periods and multiple acoustical conditions. To overcome this drawback of standard physiological recordings, high-resolution Ca2+ imaging could be used to track neuronal activity over multiple weeks. Using this approach however requires behavioral paradigms for head fixed animals.

We implemented a reward-based two-alternative forced choice behavioral paradigm for the investigation of sound localization while imaging neuronal activity in the auditory midbrain. We developed a setup to present broadband noise stimuli from distinct spatial positions (n = 12) within the horizontal frontal field by moving a speaker around the animals' head using a servo motor. The mouse had to discriminate right from left sound presentations by licking either a right or a left waterspout.

Our paradigm provides an approach for the determination of murine sound localization thresholds, and it can be applied for the investigation of neuronal space representation within the central auditory pathway using high resolution Ca2+ imaging. Thus, this setup – paradigm combination enables the long-term evaluation of auditory space representation in the same neurons of the central auditory pathway while mice engage in a sound localization task.

Swapnil Gavade
Staff

Calcium Activity Patterns of Mouse Dorsal CA1 Hippocampal Neurons during Object Exploration

Swapnil Gavade, Shany Yang, and Joanna-Spencer Segal

Object recognition is an important test used to study memory processes in laboratory rodents. Prior work has shown that the dorsal CA1 of the hippocampus in rodents is important for object recognition. Yet, very little is known about how dorsal CA1 neurons encode the attributes of objects, such as object identity and novelty, that are important for this task. To study this, we recorded calcium activity in these neurons using a miniature microscope in a freely moving mouse interacting with familiar or novel objects over several days in the same, familiar arena. Three different objects were used with multiple presentations. We identified an average number of 408 neurons per recording session; 160 individual neurons could be followed across all trials. Dorsal CA1 neurons exhibited increased synchrony of calcium activity (correlated activity) during object exploration. We identified distinct groups of neurons active during object exploration on individual days, "object cells," by comparing the observed activity to a shuffled data distribution. We found that on average, 21.32% of neurons were object cells. The specific neurons in the object cell population changed considerably across days regardless of object identity or familiarity. We employed UMAP (Uniform Manifold Approximation and Projection), a technique for dimensionality reduction, to facilitate unsupervised clustering. This technique did not reveal any clear neural patterns distinguishing between novelty or object novelty or identity. Finally, we used a support vector machine-based neural decoding approach trained on neural activity to attempt to decode object novelty and identity directly from the neural activity. This technique exhibited poor discrimination of object identity or novelty, though this could be enhanced by using a preselected population of object cells. Overall, our findings indicate that while an increase in synchronized neural activity was observed in the dorsal CA1 region during periods of object exploration, we could not identify information about object identity or novelty based on the neural activity alone. These findings suggest that while dorsal CA1 neurons encode information about object exploratory behavior, their activity may not play a direct role in object discrimination or novelty detection.

Lezio Bueno Jr., Ph.D.
Faculty

Learning and arousal dynamics from motoric and neurophysiologic metrics during a somatosensory task in mice

Lezio S. Bueno-Junior, Anjesh Ghimire, Mingxin Ding, and Brendon O. Watson

Learning in rodents can be analyzed on two timescales: task acquisition over training sessions and arousal fluctuations within training sessions. How do variations in motoric and neurophysiologic activity relate to behavioral performance over these timescales? We examined this in mice performing a whisker-based sensory discrimination task. Mice were trained for 12-14 daily sessions, each lasting approximately one hour to capture spontaneous performance fluctuations. We simultaneously tracked response and reward rates, alongside wheel running, pupil size, eyelid aperture and sensory cortical activity – here termed "non-performance variables". Non-performance variables were predictive of "impulsive", "disengaged", or "attentive" states, which had been defined a priori based on response and reward rates. When parsing these arousal states by task responses (hits, false alarms, correct rejections and misses), we detected uninstructed changes in wheel, eye and brain activity. Thus, learning and arousal fluctuations form a continuum evidenced by changes in behavioral and physiologic variables not directly controlled by task contingencies, even during periods of suboptimal performance. These findings improve our understanding of performance variations and implicit task acquisition in rodents, in addition to contributing an analytical framework for multidimensional monitoring of task performance, including in humans.

Rachel Wahlberg
Graduate Student

Hippocampal representations of episodic memory event boundaries

Rachel Wahlberg, Kavya Chandra, and Kamran Diba

While experience is continuous, we often recall the past in distinct 'chunks', often separated by location, time, and content - these chunks we refer to as episodic memory. However, the manner in which these memories are separated into distinct episodes is not well understood. Event boundaries, specific changes in location, time, or other stimuli, are thought to initiate transitions from one episodic memory to the next. Thus we hypothesize that the hippocampus, a region central to episodic memory consolidation and retrieval, contains neural representations of event boundaries. Specifically, I expect theta (~7Hz) oscillations, place cell sequences, and sharp wave ripple replays to tile the space between putative event boundaries, and for these to shift to represent new physical locations when a new pair of event boundaries is introduced. This study analyzes rodent behavior on a behavioral task on a track containing three putative event boundaries – changes in track color, track texture, or task motivation – paired with simultaneous hippocampal electrophysiology recordings to determine hippocampal markers of event boundaries. Today we outline behavioral methods and results for two rodents as well as preliminary electrophysiology results for a third rodent.

Emma Huels
Graduate Student

Effect of intravenous salvinorin A, the primary psychoactive molecule in Salvia divinorum, on brain network dynamics in rat

Emma R. Huels, Lucy Wang, Tiecheng Liu, George A. Mashour, and Dinesh Pal

Salvinorin A, a kappa-opioid agonist and the primary psychoactive molecule in Salvia divinorum, has received little attention amid the recentpsychedelic renaissance, which could be due to the virtual lack of preclinical brain dynamics data from animal studies to inform translational or clinicalstudies. Therefore, we determined the effect of intravenous administration of salvinorin A (2mg/kg) on brain dynamics in adult Sprague Dawley rats(n= 2 male, 1 female) using nondirectional (weighted phase lag index-wPLI) and directional (normalized symbolic transfer entropy-NSTE) measures offunctional brain connectivity. The rats were instrumented to record high-density (30 channel) electroencephalogram (EEG) across the cortex andreceive an intravenous bolus (5 minutes) infusion of salvinorin A or the vehicle control (14:26:60 DMSO:PEG400:saline). After 7-10 days of postsurgicalrecovery, EEG data were collected (0-300 Hz, sampling rate at 1 kHz) before, during, and after salvinorin A/vehicle infusion. EEG analyses wereperformed on artifact-free 1-5 minute EEG epochs before and immediately after salvinorin A or vehicle administration. The data are presented aspercent change (mean + SD) from the pre-salvinorin A/vehicle epoch. The most salient effects of salvinorin A occurred in the medium (65-125Hz) andhigh (125-155Hz) gamma bands. Salvinorin A increased wPLI in the medium gamma (24.55% ± 17.84%) and high gamma (61.11% ± 25.54%) rangeswhile the vehicle showed relatively small changes (medium gamma: 4.06% ± 15.64%; high gamma: -1.58% ± 4.25%). Salvinorin A also alteredfeedforward (parietal-to-frontal) and feedback (frontal-to-parietal) connectivity (NSTE), with decreases occurring in the medium gamma band(feedforward: -27.76 ± 4.96%; feedback: -25.43% ± 4.93) and increases in the high gamma band (feedforward: 50.62% ± 15.52%; feedback: 54.45% ±17.49%). Vehicle administration did not produce similar changes in medium gamma (feedforward: -2.31% ± 2.61%; feedback: 4.24% ± 8.54%) or highgamma (feedforward: -3.4% ± 2.97%; feedback: -2.4% ± 6.12%) directed connectivity. Salvinorin A also led to decreased theta directed connectivity(feedforward: -39.67% ± 5.55%; feedback: -46.5% ± 7.45%), with much smaller decreases occurring after vehicle infusion (feedforward: -14.64% ±26.42%; feedback: -26.54% ± 11.62%). To our knowledge, this is the first study to characterize the effects of salvinorin A on brain network dynamics ina rat model and will help inform future mechanistic and translational studies.

Jennifer Murray
Graduate Student

Mapping c-Fos in context fear conditioning: Evidence for alternative neural circuits after manipulation of affective information and between the sexes

Jennifer A. Murray, Megan Vaandrager, Katie Alltop, and Natalie C. Tronson,

Pavlovian fear conditioning has been extensively used to study the neurobiology and psychology of anxiety disorders and post-traumatic stress disorder (PTSD). In this study, we aim to understand what is learned and recalled in an associative fear response. We used context fear conditioning and varied predictability with high- vs low-prediction training and varied US valence with high vs moderate shock intensity (0.8mA vs 0.4mA). We assessed blocking as an index of prediction. We observed significant blocking of the new tone-shock association in continuous reinforcement-trained mice (CRft) at both 0.8mA and 0.4mA shock levels, driven by male animals. We also observed strong context generalization in partial reinforcement-trained mice (PRft), an effect that was stronger at 0.8mA compared with 0.4mA shock levels. Differences in context generalization between CRft and PRft and between 0.8mA and 0.4mA shock levels suggests that additional non-specific/non-predictive processes are more evident after PRft training and at higher shock intensities. To better understand the neurobiological mechanisms involved, we began to look at the neural circuits activated in recall of an aversive association. Studies have indicated that specific regions of the brain including the hippocampus (contextual representations) and amygdala (cue-shock associations) are involved in fear learning. Using immunohistochemistry and cFos imaging, we began to map the neural circuits involved in retrieval of fear memories after aversive conditioning. Primary goat serum antibodies were used against c-Fos. DAB staining was visualized using a light microscope. Nuclear proteins in the CA1 region of the hippocampus were analyzed with cell counts using ImageJ. Preliminary findings indicate that there are differences in the neural circuits activated in fear memory retrieval after manipulation of affective/motivational information and between the sexes. Understanding the role of additional information in the development of fear responses may have implications in understanding the poor prediction indicated in anxiety-like disorders and PTSD.
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Aishwarya Ramaswami
Undergraduate Student

The role of central amygdala corticotropin-releasing factor in positive incentive motivation

Aishwarya Ramaswami, Katie Emery, and Kent C. Berridge

Corticotropin-releasing factor (CRF) from central amygdala (CeA) neurons have traditionally been posited to generate distress that motivates reward seeking and relapse in addiction as a form of hedonic self-medication. However, new evidence suggests that CRF is alternatively involved in promoting incentive motivation to increase reward pursuit without requiring an aversive stress state. For example, optogenetic laser stimulation of CeA CRF-containing neurons of Crh-Cre rats can enhance motivation and intensify reward pursuit for laser-paired sucrose or cocaine rewards over an equal reward earned without laser stimulation. Animals will also optogenetically self-stimulate for CeA CRF neuron activation, indicating positive valence. However, it remains unclear whether these CeA CRF neurons are generating incentive effects via CRF release or other co-released neurotransmitters. It is also unclear what CRF circuitry underlies this incentive motivation. To test the role of CRF peptide, we administered intraventricular microinjections of a nonspecific CRF antagonist or of vehicle prior to two-choice tasks where rats could earn either laser-paired sucrose rewards or sucrose alone. Intraventricular microinjections were also administered prior to laser self-stimulation sessions to assess valence of CRF neuron stimulation. We found that optogenetic stimulation of CeA CRF neurons drives incentive motivation and is eliminated by global CRF antagonism, indicating that CRF is necessary for this motivational effect. Furthermore, to begin identifying the CeA CRF circuitry that underlies this incentive motivational effect, we optogenetically activated CeA CRF projections to the lateral hypothalamus (LH) and repeated two-choice and laser self-stimulation tasks. We show preliminary data that suggests the projection of CeA CRF neurons to the LH generates aversive motivation. Together, this work provides a role for CRF in driving incentive and aversive motivation that may contribute to drug seeking and relapse.

Christen Snyder
Graduate Student

A novel mouse model of the glucocorticoid withdrawal syndrome

Christen N. Snyder, Lana L. Haddad, Oliver Rubin, Chih-Lin Chang, Joanna Spencer-Segal

Glucocorticoids are among the most-prescribed chronic medications in the United States. It is estimated that 1-3% of the general population is currently prescribed synthetic glucocorticoid treatment, with the estimated average duration greater than 4 years. This therapy comes at a huge cost, as glucocorticoids cause serious and even life-threatening side effects. But cessation of chronic glucocorticoid treatment is hampered by a difficult withdrawal syndrome whose main manifestations include generalized pain, asthenia, and a loss of interest in normally pleasurable activities. Despite the central role of the withdrawal syndrome in prolonging excess glucocorticoid exposure, its mechanisms have never been studied. The goal of this work was to develop an animal model of glucocorticoid withdrawal that we can use to study its neural basis. We developed a novel mouse model involving chronic prednisolone administration in the drinking water followed by withdrawal to physiologic levels to elicit a withdrawal syndrome without frank adrenal insufficiency. Preliminary data using both male and female mice show behavioral changes during glucocorticoid withdrawal that resemble the key components of the human withdrawal syndrome. Mice in withdrawal showed an increase in nociceptive behaviors in the von Frey test. Females also showed decreased interaction with a novel conspecific despite no change in classic affective behaviors, which could suggest decreased interest in social contact that mice would usually find naturally rewarding. This mouse model can be used to understand the neural mechanisms underlying the glucocorticoid withdrawal syndrome, and eventually, to develop treatments that enable millions of patients to transition off long-term glucocorticoids.

Katherine Furman
Graduate Student

Melanin concentrating hormone efferents in the NAc mediate the reward value of food and do not induce REM sleep

Katherine L. Furman, Hannah C. Lyons, Jack R. Evans, Timothy Cha, Jayeeta Manna, Limei Zhu, Joanna Mattis, Christian R. Burgess

Animals must make informed decisions about what and how much to eat in order to maintain energy balance. Yet homeostatic need is not the sole factor in the decision to eat. Non-homeostatic motivators to eat are common, such as craving of sugary or fatty foods even when sated. Melanin-concentrating hormone (MCH) neurons of the lateral hypothalamus (LH) and zona incerta are a relevant neural target for both homeostatic and non-homeostatic motivators to eat. MCH neurons project to many brain areas including the nucleus accumbens (NAc), and have a role in numerous behaviors including feeding, sleep, learning, and reward.

We hypothesize that MCH projections to the NAc promote hedonic motivations to consume food but do not have a role in sleep-wake regulation. To address this hypothesis, we instrumented MCH-ChR2 mice with EEG/EMG headcaps and optic fibers placed either over the MCH neurons in the LH or their terminals in NAc, and investigated how optogenetic stimulation affected feeding and sleep behavior in different behavioral contexts. When optogenetic stimulation was delivered continuously, we observed that mice with stimulation of MCH neurons in the LH spent more time in REM sleep, while mice with terminal stimulation in the NAc did not show a sleep effect. However, when given the opportunity to choose between a port which delivers both food and acute optogenetic stimulation or a port which delivers stimulation alone, mice with terminal stimulation in the NAc had a significant preference for food paired with stimulation ($p<0.0001$), while mice with cell body stimulation of all MCH neurons in the LH did not. These findings begin to elucidate a mechanism by which MCH neurons differentially regulate feeding and arousal as a function of downstream projection area. Specifically, these findings suggest that MCH –> NAc stimulation is involved in promoting non-homeostatic motivation to consume food.

Rachel Rucker
Graduate Student

Dissecting the neural mechanisms by which hedonic hunger modulates aging

Rachel A Rucker, Kristina J Weaver, Sonakshi Raju, Tuhin Chakraborty, Robert A Holt, and Scott D Pletcher

Healthy aging may be a case of mind over matter. Experiments in systems ranging from worms to mice have established that neural states, which humans often associate with the feelings and motivations behind our behaviors, may be as influential as physical experiences in promoting a long and healthy life. One major motivational drive for animals is hunger, which promotes feeding. Feeding can be generated by the physiological need to consume nutrients as well as the hedonic properties of food. While brain circuits and mechanisms that regulate feeding have been described, it is unclear which of these contribute to the generation of motive forces that drive feeding. Based on visually identified and quantified behaviors exhibited by hungry flies, we have found that flies exhibit distinct and measurable hunger drives that can be homeostatic (i.e., need-based) or hedonic (i.e., pleasure-based), the latter of which is characterized by an increased feeding duration. We have implicated the ?'/?' mushroom body lobes in hedonic feeding and have demonstrated that homeostatic and hedonic drives interact, where increased homeostatic need can modulate hedonic feeding. We also utilize the detailed functional map of the fly brain and genetic tools to manipulate neural circuits and pathways rapidly, dynamically, and reversibly to modulate these feeding circuits and interrogate their effects on health and lifespan. Further dissection of the hedonic feeding circuit and direct manipulation of this circuit over a lifespan will provide us with a better understanding of how neural states can affect healthy aging.

Hanna Carmon
Graduate Student

Neuroimmune activity contributes to cholinergic dysfunction in sign-tracking rats.

Hanna Carmon, Evan Haley, Vinay Parikh, Martin Sarter, and Natalie C. Tronson

Some rats (sign-trackers; STs) are prone to attribute incentive salience to reward cues, which can manifest as a propensity to approach and contact Pavlovian cues, and for addiction-like behavior. STs also exhibit poor attentional performance, relative to their counterparts, the goal-trackers (GTs), mediated by attenuated cholinergic activity. In STs, increases in neuronal activity fail to translocate choline transporters (CHTs) into synaptosomal plasma membrane, thereby limiting the capacity of cholinergic terminals to sustain cholinergic activity. Here we investigated post-translational modifications responsible for disrupted CHT trafficking in STs, and the hypothesis that attenuated cholinergic activity in STs causes exaggerated (neuro)immune responses. We previously demonstrated that intracellular CHTs, but not plasma membrane CHTs, are highly ubiquitinated in sign-tracking rats compared to GTs. Activation of the innate immune system by systemic administration of lipopolysaccharide (LPS) increased ubiquitination levels of cortical and striatal CHTs in GTs, but not STs. STs and GTs also showed differences in neuroimmune states. Prior to an immune challenge, cytokine levels in cortex and striatum are elevated in STs compared to GTs. For most all cytokines measured, only GTs showed increased levels after LPS. In the frontal cortex at baseline, STs further demonstrate reduced SALL1 expression, a key transcriptional regulator of microglia identity and function, in the frontal cortex. Consequently, STs also express elevated general microglia levels with limited change in expression levels following immune activation. As LPS effects were largely restricted to GTs, increasing microglia expression, and reducing SALL1 expression, the available evidence is consistent with the view that ST and GTs differ in neuroimmune processes, including cytokine levels, microglia activity, and neuronal states, both at baseline and stimulated states. This finding opens the possibility that individual differences in neuroimmune states contribute to cholinergic function, behavioral phenotypes, and vulnerability to addiction.

Katherine Najor
Undergraduate Student

Extended Access to Cocaine Flips Affective Valence of Motivation Driven by Extended Amygdala Corticotropin Releasing Factor Neurons

Katie Emery, Katherine Najor, Maya Sheth, Xiaoya Huang, and Kent Berridge

Corticotropin-releasing factor (CRF) is generally known as the body's master stress hormone, associated with negative affect and discomfort. Traditional neuroscience opponent process theories of addiction posit that CRF release in the extended amygdala mediates the distress associated with withdrawal symptoms; however, recent literature has found CRF to facilitate incentive motivation. Specifically, CRF has been found to generate enhanced motivation for reward-seeking behavior in extended amygdala, specifically the central nucleus of the amygdala (CeA). These findings, however, were obtained from drug naive animals, and thus we are unable to determine how CRF may function differently after extended drug use. It is possible that although CRF is able to facilitate incentive motivation in rodents without prior drug exposure, extended access to drugs may flip the affective valence of CRF to a more typical aversive role. To test this hypothesis, Crh-Cre+ rats self-administered cocaine on an extended access schedule. We then assessed the affective valence of CeA CRF neurons via optogenetic stimulation during behavior tasks that evaluate motivation. To determine whether CRF function flips with extended access, we measured this valence before drug access, during acute withdrawal, and in prolonged abstinence. We found that during acute withdrawal, behavior is substantially suppressed and there is no incentive value to CRF; however, this suppression is alleviated in prolonged abstinence. This finding gives us more insight into the role of CRF in driving relapse and drug seeking.

Tyler Kudlak
Graduate Student

Neurophysiological and behavioral synchronization in group-living and sleeping mice

Tyler Kudlak, Maria I. Sotelo, Chelsea Markunas, Chani Kohtz, Alexei L. Vyssotski, Gideon Rothschild, and Ada Eban-Rothschild

While the impacts of social interactions on animal development, physiology, and behavior are well documented, very little is known about how social context affects sleep, as animals are predominantly isolated during laboratory sleep studies. In this study, we employed wireless neurophysiological devices to characterize sleep behavior and neurophysiology in multiple freely moving mice living together under varying social conditions. In addition, as many animal species sleep while in close physical proximity to conspecifics, including humans, we investigated whether mice are motivated to huddle during sleep. We employed video recording alongside simultaneous EEG/EMG recording from multiple mice within a group over 24 hours and designed a novel behavioral apparatus that allows mice to choose between huddling with a conspecific or sleeping alone, under different experimental conditions. Furthermore, we employed a deep-learning-based approach to classify huddling behavior from video recording data. Finally, we characterized sleep architecture under different social conditions to examine how sleep is modulated by social context. We found that mice seek physical contact before initiating sleep and sleep in close proximity to one another. Our findings further suggest that huddling during sleep is a motivated behavior as mice are willing to give up their preferred sleeping location, even when heated, to have social contact during sleep. We also found that social sleeping fragments NREMS but synchronizes different neurophysiological features during sleep. Notably, we revealed that co-sleeping male siblings display a significant synchronization in REMS timing, although this was not present in female or unfamiliar mice. Our findings provide novel insights into the motivation for physical contact and social modulation of sleep. Considering the prevalence of sleep disturbances and social isolation, understanding how social factors impact sleep health is essential. Further insights into these behavioral and neurophysiological interactions can yield strategies for combatting social stress-related sleep disturbances.

Duan Li, Ph.D.
Faculty

Anesthesia Alters Complexity of Stimulus-induced Neuronal Firing Patterns in Rat Visual Cortex

Duan Li, Anthony G. Hudetz

Complexity of neuronal firing patterns is an important index of sensory information processing that may be directly affected in altered states of consciousness. Recent investigations found that general anesthesia disrupts visual cortex neuronal responses to flash stimuli up to hundreds of milliseconds post-stimulus and decreases the complexity of the early (0-200 ms) post-stimulus response. How anesthesia alters complexity of the late (>200 ms) response component remains unclear. The latter is important as it likely reflects recurrent processing necessary for conscious vision. Additionally, there is growing evidence for spontaneous shifts in large-scale brain states at constant anesthetic agent concentration, suggesting a partial dissociation between neuronal state and anesthetic level. Whether spontaneous state transitions at fixed anesthetic concentration occur also during post-stimulus activity and complexity is unclear. Here we aimed to investigate these questions in rats at different levels of anesthesia produced by the inhalational agent desflurane. The results suggest the presence of multiple neuronal states that represent distinct visual stimulus-evoked spiking patterns on the time scale of tens of seconds at constant anesthetic concentrations. The complexity of post-stimulus responses (1-500 ms) decreases in neuronal states at increasing depth of anesthesia, which may reflect the disruption of sensory information processing.

Princess Felix
Graduate Student

Elucidating the Role of Glucocorticoid Receptors in the Inhibitory Control of Cue-Motivated Behaviors

Princess Felix, Alexandra Turfe, Stephen E. Chang, Jaydin Adams, Elena Cooper, James P. Herman, and Shelly B. Flagel

The glucocorticoid receptor (GR) has been implicated in the pathophysiology of several psychiatric disorders including impulse control and substance use disorders. How GR impacts behaviors relevant to psychiatric disorders in the absence of stress is not known. Here we used GR-CRISPR transgenic rats to assess the effects of GR knockdown in a top-down cortico-striatal circuit on inhibitory control. Specifically, we selectively knocked-down GR in glutamatergic afferents projecting from the prelimbic cortex (PrL) to the nucleus accumbens core (NAcC). We assessed the effect of this manipulation on the propensity to attribute incentive salience to a reward-associated cue using a Pavlovian conditioned approach paradigm. Prior studies have revealed that rats with a propensity to attribute incentive salience to reward-cues (i.e., sign-trackers) show increased impulsive action, have attentional deficits, and are more likely to exhibit cue-induced reinstatement of drug-seeking behavior relative to rats that primarily attribute predictive value to reward-cues (i.e., goal-trackers). We found that GR knockdown within the PrL-NAcC pathway results in an increased propensity to sign-track regardless of sex. During the first session, approach behavior did not differ between groups; upon training, rats without GR had greater sign-tracking behavior relative to wildtype controls. These findings suggest that GR function in a top-down corticostriatal circuit plays a role in incentive motivation.

Rajer (Jung-Chien) Hsieh
Graduate Student

Higher cortical inputs for brain stem trigeminal sensory nuclei

Rajer(Jung-Chien) Hsieh

Top-down projection from higher cortical area to spinal cord and brainstem are traditionally seen as primary motor pathways. Some evidence from cortical-spinal tract neurons suggest its direct regulation of sensory inflow by targeting dorsal horn circuit(Liu et al, 2018). This long-range projection may indicate direct cognitive influence on perception but not just pathway. Trigeminal nerve nuclei(TGNs) in brainstem, including principal trigeminal nucleus(PrV), spinal trigeminal nucleus oral(SpVo), spinal trigeminal nucleus interpolar(SpVi), spinal trigeminal nucleus caudal(SpVc), serves as the secondary hub relaying the input from trigeminal ganglia(TG) to thalamus. Given its early sensory processing role in CNS, we hypothesize the top-down innervation from higher cortical area may also exist for sensory gating in addition to motor output, analogous to the circuit from cortical-spinal tract neurons. However, the neocortex-> TGNs circuit has not been described in detail due to lack of synaptic resolution of tracers previously used. Here we used two strategies to confirm the circuit: 1)S1/S2 anterograde tracing in a synaptophysin-reporter animal and 2) TGN monosynaptic retrograde rabies virus tracing.

Intensive projection from S1/S2 to TGNs subnuclei was observed. Interestingly, the "core" and "shell" projection pattern in SpVi and SpVc may suggest its sensory gating role in addition to motor function. "core" TGN is close to the local motor reflex circuit (motor nucleus of trigeminal and parvicellular reticular nucleus) and "shell" TGN receives the synapses from trigeminal ganglia. Based on the monosynaptic retrograde rabies tracing, the majority of rabies signal were found in hindbrain reticular formation, which indicated the intensive connection with the local motor circuit. Despite the small fraction of cortical inputs to TGN(2~3% in each animal), rabies signals can often be found in contra-lateral S1/S2 layer 5/6 neurons. Surprisingly, a preferential circuit of S2-caudal TGNs (SpVi/SpVc) and S1-rostral TGN(Pr5/SpVo) was observed, which may indicate a parallel gating pathway for different modality. A systemic CNS inputs to TGN were described, including contra-lateral visceral cortex, primary auditory cortex(A1), red nucleus(RN); Ipsilateral paraventricular hypothalamus(PVN), parasubthalamic nucleus(PSTN), parabrachial nucleus(PB), nucleus of solitary tract(NTS); bilateral primary motor cortex(M1), superior colliculus motor(SCm). Future work such as behavior assay of conditional ablation mice and single cell TGN transcriptomics will be included to decipher the role of top-down innervation and TGN cell type.

Stephanie Desrochers, Ph.D.
Postdoctoral Fellow

Tracing top-down and bottom-up pathways through the paraventricular thalamus in the sign-tracker/goal-tracker model

Stephanie S. Desrochers, Shelly B. Flagel

Cues in our environment usually guide appropriate behavior, however, cues can also gain inordinate control over our actions. Individual differences in the attribution of incentive salience to reward predictive cues may be of relevance to psychiatric disorders. For example, the sight of drug-related imagery may lead to craving and relapse in some individuals with substance use disorders. We are able to study these individual differences in cue-responding in a preclinical rodent model using a Pavlovian Conditioned Approach paradigm (PavCA), where a lever-cue predicts a food reward outcome. Rats deemed sign-trackers preferentially interact with the lever-cue, while goal-trackers tend to interact with the food magazine. Previous work in this model has demonstrated that sign-trackers more strongly engage subcortical neural circuitry, while goal-trackers engage more cortical neural circuitry. These bottom-up and top-down signaling mechanisms integrate at the level of the paraventricular thalamus (PVT), which then sends output to the nucleus accumbens (NAc). PVT projections modulate dopamine release in the NAc, which is integral to the expression of sign-tracking behavior. However, we do not yet know how all of these projections interact anatomically to modulate sign- and goal-tracking behavior. Here, we developed a method to trace multisynaptic input/output pathways through the PVT, and then will examine how these pathways are differentially activated in response to cue exposure in sign- and goal-trackers. High titers of AAV1 serotype viruses can anterogradely travel over synapses, which combined with retrograde viruses, allows us to label cells in the PVT which receive input from both/either the lateral hypothalamus and the prelimbic cortex, and then output to the NAc Shell. Ongoing work is using this method in combination with PavCA testing to determine whether these specific pathways are anatomically or functionally different in sign- versus goal-trackers.

Shany Yang
Graduate Student

Uncovering the role of persistent lipocalin-2 in sepsis survivors

Shany E. Yang, Jennifer Meng, Holland H. Hubert, and Joanna L. Spencer-Segal

Sepsis survivors often suffer from lingering neuropsychiatric symptoms including anxiety. In mice, cecal ligation and puncture (CLP) is one method used to induce acute infection that can be treated to recovery. CLP survivors experience persistent anxiety-like behaviors and inflammation, including systemic upregulation of the cytokine, lipocalin-2 (LCN2). We previously identified lcn2 as the top upregulated transcript in the hippocampus 3 weeks after CLP (24.6-fold increase relative to SHAM). This study aimed to test the hypothesis that LCN2 mediates the persistent neuroinflammation and anxiety-like behavior observed in CLP survivors. Adult lcn2loxP/Cre-ERT2 and Cre-negative mice underwent SHAM or CLP surgery (n=8-11 per group including males and females). After recovery, they received vehicle or 75mg/kg of tamoxifen for 5 consecutive days to induce Cre recombination and subsequent lcn2 knockout. Anxiety-like behaviors were examined 2 weeks after surgery using an open field and an elevated zero maze (EZM). Using RT-qPCR, we assessed brain mRNA expression of leucine-rich alpha-2-glycoprotein 1 (lrg1), p-selectin (selp), and S100 calcium-binding protein A8 (s100a8), which we previously demonstrated to be upregulated in CLP survivors. Our findings showed that lcn2-/- mice exhibited locomotor deficits and anxiety-like behavior. However, in Cre- mice, tamoxifen also independently decreased locomotion. Finally, brain mRNA levels of lrg1 and selp were elevated after CLP and positively correlated with brain lcn2 mRNA levels. Overall, tamoxifen treatment induced long-lasting behavioral deficits, which prevented any conclusion about the effect of lcn2 knockout on anxiety-like behavior. This requires consideration in the future when designing behavioral studies using inducible knockouts. Nonetheless, our RT-qPCR results suggest a potential role for brain lcn2 in persistent inflammation following CLP.

Amanda Gregolynskyj
Graduate Student

αA-crystallin impacts expression of homeostasis-influencing molecules in Müller glial cells

Amanda Gregolynskyj, Patrice E. Fort

Müller glial cells (MGCs) are promising therapeutic targets for diabetic retinopathy due to their central role in retinal homeostasis and the retinal immune response. We previously showed that the small heat shock protein αA-crystallin (CRYAA) dampens MGC-mediated inflammatory cytokine induction in culture and decreases expression of fibroblast growth factor (Fgf2) in whole retina. This study aimed to fully characterize how CRYAA impacts MGC expression of molecules that influence homeostasis, basally and during diabetic-like stress. Basal gene expression was measured in wild-type (WT) and Cryaa knockout (AKO) C57BL/6 mice. Selective expression of green fluorescent protein (GFP) in MGCs was achieved by injecting mouse pups (postnatal day 3-5) with an MGC-specific viral vector, ShH10-CMV-GFP. At 8-10 weeks, retinas were dissociated and GFP+ MGCs were collected by fluorescence-assisted cell sorting, followed by gene expression analysis via qPCR. CRYAA regulation of MGC expression profiles was further investigated during diabetic-like stress. Primary MGCs were cultured from AKO mouse pup retinas (P 10-14). Cells were then transfected with WT CRYAA or an empty vector (EV) under control (5mM glucose) or diabetic-like (25 mM glucose + 100 ng/mL) conditions. Gene expression was assessed by qPCR. Fgf2 and inflammatory cytokine expression was significantly increased in AKO MGCs isolated by FACS compared to WTs. In primary MGCs under control conditions, overexpressing WT CRYAA decreased Fgf2 transcript levels compared to cells transfected with the EV. However, under diabetic-like stress, CRYAA significantly upregulated Fgf2 expression. Our findings support that CRYAA regulates expression of homeostasis-influencing molecules in MGCs. Interestingly, CRYAA downregulated Fgf2 expression in primary MGCs under control conditions, but upregulated it during diabetic-like stress, suggesting CRYAA may increase trophic support provided by MGCs during stress.

Astrid van Irsen
Graduate Student

Characterization of D1-type medium spiny neurons that project to the lateral hypothalamic area, and the role of this pathway in systemic glucose metabolism

Astrid A. S. van Irsen, Tess Kool, Andries Kalsbeek, Carol F. Elias, Carrie R. Ferrario, Susanne E. la Fleur

The nucleus accumbens (NAc) plays a critical role in reward and food-motivated behavior, and is comprised of core and shell sub-regions. Medium spiny neurons (MSNs) comprise 95% of all NAc neurons, with the majority of these expressing either D1 (Drd1) or D2 (Drd2) type dopamine receptors. The Drd1-MSNs mainly project directly to target areas, while Drd2-MSNs project indirectly via the ventral pallidum. In rats, deep brain stimulation of the NAc medial shell (mshNAc) increases blood glucose and plasma glucagon concentrations compared to sham or NAc core stimulation. Moreover, infusion of vanoxerine, a dopamine reuptake inhibitor, into the mshNAc of rats decreases hepatic glucose production, blood glucose and plasma glucagon concentrations compared to vehicle. These seemingly contradictory effects could potentially be explained by opposing activity in Drd1- vs Drd2-MSNs. Using male transgenic Drd1-Cre rats, our initial results show that mshNAc Drd1-MSNs mainly project to the lateral hypothalamic area (LHA). In addition, chemogenetic activation of this mshNAc Drd1-MSN to LHA pathway plays a role in glucose tolerance. We compared overall activation of Drd1-MSNs in the mshNAc to specific activation of the mshNAc Drd1-MSN to LHA connection, on glucose tolerance. Activation of the mshNAc Drd1-MSN to LHA connection improved glucose tolerance (drug effect $p<0.05$ and interaction effect drug x time $p<0.01$, $n=9$), whereas overall mshNAc Drd1-MSN activation did not result in a significant effect. However, little is known about other receptors or neuropeptides localized in this population of mshNAc Drd1-MSNs that project to the LHA. Therefore, here we will characterize the chemical phenotypes of this cell population in the NAc using fluorescent in situ hybridization. Overall, our findings will increase the understanding of neural circuitry underlying the central control of systemic glucose metabolism.

Alisha Spoelman
Staff

A Structural Dissection of Sensory Innervation Within the Mouse Airway

Alisha Spoelman, Xingyu Li, and Peng Li

Interoception is a vital process where the brain receives and processes sensory information from inside the body, including sensations like hunger, heart rate, and the urge to breathe. Despite the crucial function breathing plays in sustaining life, surprisingly little is known about how breathing is controlled by the interoceptive signals from the respiratory system. Research in this field is further complicated by the fact that the vagus nerve (cranial nerve X), which carries ascending sensory information from the internal organs, shows significant structural differences between rodent species. Specifically, the nodose and jugular ganglia comprising the vagal ganglia complex have distinct developmental origins and organization that differs between rats, mice, and guinea pigs. In this work, we mapped the neurons associated with spatial respiratory regions in mice to better understand respiratory sensation and establish a foundation for future functional manipulations. We developed surgical methods for targeting neurons in three regions of the respiratory system: the proximal trachea, distal trachea, and distal lung, then used cholera toxin B (CTB) and adeno-associated viruses (AAVs) to fluorescently label the associated neurons. Our tracing experiments revealed that while the majority of fluorescently labeled cell bodies were located within the nodose ganglia, a significant number of labeled neurons were also found in the jugular ganglia. Furthermore, co-localized labeling was observed across the entire vagal ganglia for all conditions in which different fluorescent channels of CTB were injected into two respiratory regions. These results provide insight into potential mechanisms for spatial resolution of stimuli within the airways and establish a crucial foundation to enable future functional manipulations. Additionally, this structural dissection of mouse airway innervation will enable investigations leading to a better understanding of respiratory diseases.

Deanna Cannizzaro
Graduate Student

Trigeminal Innervation of the Submandibular Salivary Glands

Deanna Cannizzaro, Akash Gandhi, Brian Constantinescu, Joshua Emrick

The major salivary glands are essential, actively secreting saliva full of water, proteins, and immunoglobulins necessary for oral health. Hyposalivation and dysregulated saliva composition affect 10-30% of the general population, greatly diminishing quality of life from difficulty of speaking and swallowing, enamel deterioration, and increased periodontal disease. It is well known that salivary gland outputs are controlled by the autonomic nervous system (i.e., sympathetic and parasympathetic innervation), though therapies remain limited. Our preliminary data suggest that the major salivary glands also receive direct neuronal innervation by the trigeminal ganglia (TG), which detects and transmits sensory information from the mouth, head, and face to the brain. By cannulating Wharton's ducts, we gain access to one of the major salivary glands (i.e., submandibular). In these experiments, in situ hybridization and in vivo calcium imaging allowed us to visualize neuronal activity of the TG sensory neurons innervating the submandibular gland and classify the type of genes they express. Future work will explore how TG neurons contribute to the modulation of the salivary glands that may present a new avenue of therapeutic targets to alleviate altered saliva production or composition. Furthermore, our studies may uncover sensory neuronal networks involved in the innervation of glands and other deep tissues.

Karin Harumi Uchima Koecklin, Ph.D.
Postdoctoral Fellow

Neural Pathways Involved in Bruxism-like Behavior in Mice

Uchima Koecklin, KH, Peng, L

The orofacial functions require coordinated jaw movements performed by the masticatory muscles. However, disturbances in these functions, such as bruxism, could lead to severe consequences in the individual's organism. Although the clinical consequences of these disturbances have been well studied, the neural pathways responsible in the control of the masticatory muscles during both normal and abnormal function remain incompletely understood. The masticatory muscles are innervated by the trigeminal nerve, which comprises four distinct motor and sensory nuclei in the brainstem. Among these nuclei, the mesencephalic nucleus of the trigeminal nerve (Vmes) stands out as a unique sensory nucleus for masseter muscle proprioception. To elucidate the role of Vmes in the control of jaw movements, we genetically targeted these neurons to identify their neuronal connectivity and manipulate their function. Through anterograde tracing, we found that Vmes neurons project to the motor nucleus of the trigeminal nerve and its premotor areas. Furthermore, optogenetic activation of Vmes neurons induced bruxism-like behavior in mice, while chemogenetic activation of Vmes neurons increased the activity of the masticatory muscles. These findings suggest that Vmes neurons play a significant role in the development of brux-like behaviors. Our research on the neural pathways controlling the jaw movements will help improve the clinical management of disturbances in orofacial functions.

Ling-Yu Liu
Staff

Determining tooth-brainstem circuits mediating reflex and pain

Ling-Yu Liu, Josh Emrick

Somatosensory neurons are responsible for detecting physical components of the environment to enable appropriate behavioral responses toward survival. A subgroup of these specialized cells, termed nociceptors, respond to damaging i.e., noxious stimuli. Activation of nociceptors is known to produce reflex responses and/or perceptual outputs including pain. For example, prior work indicates that pain is an expected output from activation of tooth-innervating neurons; however we have also determined that the activation of these sensory neurons triggers a jaw-opening reflex. We sought to determine the distinct brainstem circuitry that enables these diverse outputs from a single nociceptive input. To begin we applied optogenetic stimulation to activate tooth-innervating neurons and their brainstem targets. Following stimulation, we evaluated c-Fos expression as a proxy for neuronal activation. As previously described, we identified a population of neurons within the spinal trigeminal nucleus, caudal part (Sp5C) that expressed c-Fos. However, optogenetic activation also stimulated previously undescribed populations in both the trigeminal transition zone (5Tr) and the trigeminosolitary zone (5Sol). Ongoing work will determine the molecular identity of target neurons in these three distinct brainstem nuclei. Future work will be aimed at mapping the connectivity of these neurons and determining their distinct or shared outputs related to jaw-opening and pain.

Marie Anderson
Staff

Interaction Between Noise Exposure and Aging on Rat Vestibular Sensory Epithelia

Marie Anderson, David Bauer, Ariane Kanicki, Richard Altschuler, W. Michael King, and Courtney Stewart

Previous work has highlighted the short-term consequences of noise exposure on calyx only afferent terminals on vestibular sensory epithelia. Although some changes in the vestibular periphery have been documented, changes in the number of calyx-only afferent terminals at key timepoints in the rat's lifespan have not been shown, and the effect that noise exposure may have on the aging vestibular periphery is unclear. We hypothesize that a decline in calretinin immunolabeling of calyx-only afferent terminals will occur with age, and that this effect will be greater in rats that have been exposed to 120dB SPL 1.5 kHz 3-octave band noise (3OBN). Rats participating in longitudinal studies of vestibular-dependent behaviors and vestibular evoked potentials were exposed to 120dB SPL 1.5kHz 3-octave band noise continuously for up to 6-hours. Upon completion of experiments ears were either immediately collected or rats were maintained in the vivarium until a target age was reached. At this time, rats' ears were collected and immunostained with antibodies against combinations of antibodies including calretinin, myosin7a, beta-3 tubulin, and neurofilament. Whole vestibular sensory epithelia were then mounted on slides and imaged using a confocal microscope. The focus of these analyses was on a previously characterized 100x200 micron region of interest at the bend of the striola in the saccule and the center of the striolar zone in the utricle. Calyceal terminals, including calyx-only afferents were quantified for all rats and categorized by age and noise exposure history. Counts across age groups for noise-exposed and control rats were compared. Results: Overall, noise exposure was related to lower calyx-only afferent terminal counts regardless of age. There was also a decrease in mean calyx-only afferent terminals due to age in control rats in the saccule and utricle, but a greater difference was observed between older rats and younger rats with the same noise exposure history. The combined effect of noise and aging was greater in the saccule than the utricle. These data suggest that while there is a small decrease in calyx-only afferent terminals related to natural aging, noise exposure decreases the number of calyx-only afferent terminals in the saccule and utricle to a greater extent. Although the dose of noise used to demonstrate this effect is significant, it demonstrates the potential impact of extreme noise exposure on a population of afferents with particular importance for rapid responses that may stabilize the body during an abrupt perturbation.

Andrew Ouellette
Graduate Student

Dendritic spines as a mediator of memory in DO Mice

Andrew Ouellette, Jeremy Herskowitz, Kelsey Greathouse, Audrey Weber, Niran Hadad, and Catherine Kaczorowski

The intersection of genetic diversity, memory, and synaptic function is a critical component in better understanding age-related cognitive decline. Diversity Outbred mice offer the opportunity to investigate cognitive aging across a genetically diverse population in a controlled lab environment. DO mice exhibit an appreciable amount of variance in Contextual Fear Memory and Acquisition (CFM, CFA), which can be linked to individual differences in genetic background (Ouellette A. et al, 2022, Cell Reports). Here, we investigate the role of dendritic spines as a mediator of individual age-related changes in memory. While there was not a decline in CFM or CFA between 8 and 18mo, we observed expectedly wide range in individual memory outcomes. Thin spine density significantly decreased between 8mo and 18mo, and stubby spine density increased. Spine volume across all spine types increased with age. Apical thin spine density explained 61% of the variance in CFM outcomes, while thin spine volume explained 79% and 43% of variance CFA in apical and basal dendrites respectively. Thin spine density, however, did not associate with CFA outcomes.

We have linked dendritic spine density and morphology as a potential mediator of individual outcomes across a genetically diverse population. Our results suggest that there may be an age-related conversion of thin to stubby spine types, and that mice with fewer thin spines are more likely to have better CFM outcomes, coinciding with reports of thin spines as dynamic "learning spines" rather than "memory storage" spines (Hayashi, Y. 2005, Neuron). We also show that the size of these "learning" thin spines may be more important memory acquisition than their total density.

Mariah Berchulski
Staff

A heritable change in action potential half width that correlates with cognitive resilience

Mariah R Berchulski, Sophie LA Martin, Shannon Moore, Kristen MS O'Connell, and Catherine C Kaczorowski

Resilience to non-pathological aging is characterized by successful maintenance of cognitive performance well into late adulthood and old age. Identification of factors governing resilience may lead to novel therapeutic interventions to enhance health span of the population as lifespan continues to lengthen. Analysis of cognitive performance in a contextual fear memory task for the B6-BXD genetic reference panel (Neuner et al., 2019) stratifies the population into resilient and susceptible strains. We seek to examine neuronal functional changes within the hippocampus that may underly resilience to cognitive aging.

We adapted the Patch-Seq method (Cadwell et al., 2017) to investigate cell type specific functional, morphological, and transcriptional changes associated with non-pathological aging using the B6-BXD reference panel. Whole cell patch clamp was used to evaluate intrinsic excitability of excitatory neurons within the Dentate Gyrus (DG) and CA1 of 14 month old mice from resilient and susceptible strains. The cells were filled and stained for downstream analysis of neuronal morphology and cellular contents, including the nucleus, were collected after recording to characterize the transcriptome.

Comparison between all four strains revealed multiple differences in intrinsic properties, but when treating cognitive function as a continuous factor across mice in our resilient and susceptible strains, we excitingly found that performance correlated with a single intrinsic property (action potential halfwidth in the DG), which was also highly heritable. Moving forward, we will use an integrated analysis of excitability, transcriptome, behavior, and morphology to nominate mechanisms underlying resilience to non-pathological aging.

Liz Litkowski, Ph.D.
Postdoctoral Fellow

Poor Glucose Metabolism Explains Cognitive Decline in Normal Aging

Elizabeth M Litkowski, Amy R Dunn, Miko Dai, Dave Bridges, Kristen MS O'Connell, and Catherine C Kaczorowski

Diabetes and dementia have an unexpectedly high rate of co-morbidity but the healthcare burden of these diseases differs by race/ethnicity backgrounds and by biological sex. Given this diversity, we aimed to determine how glucose metabolism modifies normal aging-related cognitive decline in a genetically diverse mouse panel: AD-BXD (Fig.1). During the AD-BXD breeding process, about half of the progeny will be non-transgenic for AD mutations. We utilized the non-transgenic mice to study the normal aging process while on standard chow.

We found that, for non-transgenic males on normal chow diets, cognitive function declined between 6 and 14 months as a function of poorer glucose metabolism at 14 months (Fig. 2). Genetic diversity explained 20% of the variation in this relationship. In contrast, males with AD mutations on a chow diet did not experience cognitive decline with worse glucose metabolism at 14 months (Fig.3). In a sexually dimorphic response for non-transgenic (Fig.4) and AD (Fig.5) females on a chow diet, there was no relationship between glucose metabolism at 14 months and change in cognition.

In the normal aging process poorer glucose metabolism is associated with cognitive decline in males on a chow diet. These same findings were not exhibited in AD males and females or non-transgenic females. The use of the AD-BXD mouse panel illuminated the fact that decline differs across diverse genetic background strains independent of AD mutations. It also illustrates differing strain results by sex. These findings using a genetically diverse mouse panel are proving to be invaluable in developing precision medicine practices tailored to individuals. Methods: The data were collected longitudinally from 516 non-transgenic AD-BXD mice (39 unique strains). Glucose metabolism was assessed through an intraperitoneal glucose tolerance test (IPGTT). Contextual fear memory (CFM) was the measure of cognitive function.

Hannah Lyons
Staff

Utilizing the novel Q111-BXD Mouse Panel to investigate cognitive performance in Huntington's Disease

Hannah Lyons, Gaurav Kaul, Lauren Benovich, Stephanie Boas, Ada Eban-Rothschild, and Catherine Kaczorowski

Huntington's disease (HD) is a dominantly inherited neurodegenerative disorder caused by a trinucleotide CAG repeat expansion in the huntingtin gene (HTT). HD diagnosis typically follows motor symptom onset; however, cognitive dysfunction also impacts patient quality of life, and is often overlooked when it precedes motor onset. Previous studies report substantial variability in clinical presentation of HD non-motor features, even between individuals with comparable CAG repeat length. This variation could be due to individual genetic differences beyond HTT expansion. To investigate the role of genetic background on HD cognitive decline, we generated a novel mouse panel to evaluate differential effects of HTT expansion across diverse genetic backgrounds.

Experiments shown here are part of a larger phenotypic characterization study of 40 recombinant-inbred BXD strains to assess motor, cognitive, and neuropsychiatric HD-relevant traits at multiple ages. Cognitive performance was assessed in female HttQ111 (Q111) carriers and non-transgenic (Ntg) littermates from 23 BXD strains using a two-day passive avoidance paradigm. This is a long-term memory task where on Day 1, animals are shocked in the dark side of a light-dark chamber. On Day 2 the animal's latency-to-cross to the dark chamber, and total time spent in each side are recorded. Better memory is indicated by a longer latency-to-cross and less time spent in the dark chamber on Day 2. Data were analyzed using the animal tracking software Detect Any Mouse Model.

Here, we show data from 3-month Q111- and Ntg-BXD mice (n = 130) from 23 BXD strains. Even at this early timepoint, the contribution of genetic background to cognitive performance is evident. We predict that cognitive performance will show strain-specific decline in older Q111-BXD mice. The genetic diversity of this mouse panel allows for quantitative trait loci mapping. Genetic modifiers identified in this study may inform novel therapeutic strategies for cognitive decline in HD.

Kevin Charland
Graduate Student

Genetic Background Modifies Neuronal Electrophysiological Responses to A?1-42 In Vitro

Kevin Charland, Verena Wu, Evan Carey, Shannon Moore, Bethany MacDonald, and Catherine Kaczorowski

Alzheimer's disease (AD) is the most common form of dementia and is characterized by cognitive impairments (particularly in learning and memory). These impairments are thought to be driven by neuropathological features including beta-amyloid (A?) plaques and changes in neuronal excitability. However, little is known how genetic background influences the effects of A? on neuronal activity. Here, we addressed this gap by measuring neuronal activity after A?1-42 treatment using Microelectrode Array (MEA) plates with cortical and hippocampal mixed cultures isolated from a genetically diverse mouse panel. Interestingly, treatment with 2.5?M A?1-42 initially reduced neuronal activity in all conditions. However, B6-BXD39 males tended to recover neuronal activity earlier with A?1-42 treatment while males from the strain B6-BXD124 maintained reduced activity. These data demonstrate that genetic background is a critical determinant of the reduced neuronal excitability in the presence of A?1-42. We plan to extend our investigation in several important ways including examining region-specific (cortical vs. hippocampal) and sex-specific differences in Abeta's effect on neuronal excitability, as well as investigating molecular pathways and signals that may further modulate this effect. For example, treatment with ?-estradiol (E2) has been shown to reduce A? accumulation in both preclinical and clinical studies. Furthermore, E2 can be produced by neurons and astrocytes in both males and females, and modulates many cellular functions including neurogenesis, synaptic plasticity, mitochondrial functioning, and cellular transcription. Additional investigation into the influences of E2 on neuronal activity in a genetically diverse model system can help elucidate the role E2 plays in AD and help identify novel therapeutic targets.

Lauren Fish, Ph.D.
Postdoctoral Fellow

A cognitive resilience gene expression signature in excitatory intratelencephalic cortical neurons

Lauren A. Fish, Maria A. Telpoukhovskaia, Jonathan Algoo, Niran Hadad, Brianna Gurdon, Yanchao Dai, Andrew R. Ouellette, Sarah M. Neuner, Amy R. Dunn, Jon A. L. Willcox, Yiyang Wu, Logan Dumitrescu, Orhan Bellur, Ji-Gang Zhang, Kristen M.S. O'Connell, Eric B. Dammer, Nicholas T. Seyfried, Sukalp Muzumdar, Jesse Gillis, Paul Robson, Matthias Arnold, Timothy J. Hohman, Vivek M. Philip, Vilas Menon, and Catherine C. Kaczorowski

Alzheimer's disease (AD) is a devastating form of dementia, and its prevalence is rising as human lifespan increases. Our lab created the AD-BXD mouse model, which expresses AD mutations across a genetically diverse reference panel (BXD), to identify factors that confer resilience to cognitive decline in AD. This model mimics key characteristics of human AD including variation in age of onset and severity of cognitive decline. To facilitate discovery of conserved mechanisms of resilience to AD, we generated a cross-species single-nuclei transcriptomic dataset from normal and AD human and AD-BXD mouse brains. We found the strongest gene expression signature associated with resilience arises from excitatory layer 4/5 (eL4/5) cortical intratelencephalic neurons. Further, we used a hierarchical mapping algorithm to show that the eL4/5 neurons expressing this resilience gene signature are distributed throughout the frontal cortex. This resilience signature includes genes involved in synaptic plasticity, vesicle transport, and axonal and dendritic development. Using a human reference validation and drug nomination pipeline, we found that 27 of the 61 genes in the signature are druggable and identified several candidate drugs for further investigation (Telpoukhovskaia et al., 2022). Ongoing projects in the lab aim to evaluate the efficacy of nominated drugs and profile the learning-specific proteomes of eL4/5 neurons in resilient and susceptible AD-BXD strains. When integrated with existing genetic, behavioral, and pathological data, our work will elucidate the cellular, molecular, and genetic mechanisms that contribute to cognitive resilience in face of neurodegenerative disease pathology.

Lauren Benovich
Staff

Utilizing the novel Q111-BXD mouse panel to explore neuropsychiatric traits in Huntington's Disease

Lauren Benovich, Augustine Vanlianuk, Hannah Lyons, Stephanie Boas, and Catherine Kaczorowski

Huntington's disease (HD) is a dominantly-inherited disorder caused by CAG repeat expansion in the huntingtin (HTT) gene. HD is neurodegenerative in nature, and symptoms comprise motor, cognitive, and neuropsychiatric features.

Generally, neuropsychiatric symptoms, such as depression and anxiety, appear before hallmark motor symptoms; they also are reported to be the most debilitating of HD symptoms and result in the greatest decrease in quality of life of HD patients.

Generally, individuals harboring longer CAG repeats exhibit more severe and earlier onset of symptoms; however, there is substantial variation even between patients with identical CAG repeat lengths. This variation could potentially be explained by additional genetic variants which modify the HD symptom severity. We have generated a novel HD mouse model on a genetically diverse background in order to predict the heritability of HD phenotypes and identify genetic modifiers of HD relevant traits.

Here, we utilized the Q111 knock-in HD mouse model and crossed it to 40 genetically segregated BXD strains (HD-BXD). This allows for quantitative trait loci mapping to identify regions of the genome which contribute to HD-relevant neuropsychiatric phenotypes. To assess depressive-like behavior of female Q111 carriers (n = 41) and non-transgenic (Ntg; n=30) littermate controls across 11 BXD strains, we utilize the tail suspension test. For this assay, mice are suspended by their tail. Increased depressive-like behavior is indicated by increased time spent immobile while suspended. At 3 months of age, time-spent-immobile was calculated to be highly heritable. Using quantitative trait loci (QTL) mapping, we identified a novel significant QTL peak in Chr. 16 in only Q111-BXD strains. We will next utilize mediation analysis to evaluate genes within this peak for future validation experiments. Genetic modifiers identified in this study will be cross-referenced with human HD data to inform novel therapeutic strategies for neuropsychiatric symptoms in HD.

Augustine Vanlianuk
Staff

Quantitative Trait Loci Mapping of Motor Function at 3 and 6 Months of Age in Genetically Diverse Mouse Model of Huntington's Disease

Augustine Vanlianuk, Stephanie Boas, Catherine Kaczorowski

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by abnormal CAG repeat expansion in the huntingtin (HTT) gene. In HD, motor symptoms serve as the hallmark diagnostic criterion. While previous studies have shown that CAG repeat length is generally predictive of age of motor symptom onset and severity, substantial variation is observed between patients, even those with identical CAG repeat lengths. Within this variation, some of it is indicated by genetic factors. Hence, we investigated these factors by generating a genetically diverse HD mouse model by crossing the Q111 knock-in HD mouse model to several strains from the BXD mouse panel. The motor function of Q111 carriers and non-transgenic (Ntg) littermates was assessed using an accelerating rotarod. Mice were tested longitudinally at three and six months of age. Rotarod performance at both ages was calculated to be heritable, demonstrating the contribution of genetic background to variation in motor function. Quantitative trait loci (QTL) mapping of rotarod performance identified several suggestive genomic regions where variation between BXD strains was associated with variation in rotarod performance. While several peaks were relatively conserved between Ntg- and Q111-BXDs, genotype-specific peaks were also identified at both ages. These data indicate that genetic modulation of motor performance differs depending on the context of HD genotype and age. We will continue to age animals within this panel and investigate identified genes within suggestive and significant QTL peaks as potential modifiers of HD-relevant motor function. Genetic modifiers identified in this study will be cross-referenced with human HD data to inform novel therapeutic strategies for HD motor impairment.

Yu Chen, Ph.D.
Postdoctoral Fellow

Data-independent acquisition proteomic approach reveals calcium-activated potassium channels and ADP-ribosylation factors are linked to cognitive resilience to Alzheimer's disease

Yu Chen, Lauren A. Fish, Tamara K. Stevenson, Yiding Cao, Michael C. Saul, Niran Hadad, Amy R. Dunn, Jon A.L. Willcox, Julia E. Robbins, Vivek M. Philip, Gennifer E. Merrihew, Jea Park, Saranya Canchi, Michael J. MacCoss, and Catherine C. Kaczorowski

An individual's genetic makeup plays a significant role in determining the resilience/susceptibility to Alzheimer's disease (AD). Although advances in genetics have significantly enhanced our understanding of inheritable risk factors for AD, the ultimate biological effectors of AD genetic and environmental risk are often proteins and metabolic pathways they modulate. Identification of these effector proteins will provide new insights into mechanisms contributing to the variability in susceptibility to impaired cognitive function in AD. To this end, we took an unbiased data-independent acquisition (DIA) liquid chromatography mass spectrometry (LC-MS) proteomics approach to measure in-depth coverage of protein abundance at the whole proteome level of prefrontal cortex on a genetically diverse AD mouse population (AD-BXD), a translationally relevant panel that models the extensive variability in human cognitive decline progression. We used a novel quantitative metric for determining cognitive resilience along a continuum. A linear regression analysis of contextual fear memory performance in AD-BXD strains compared to their Ntg-BXD counterparts was performed. A residual, or the numerical deviation from linear regression line of best fit for a given strain, was then calculated; we defined this residual as "resilience trait". Correlation analysis between protein abundance and this quantitative resilience trait has identified several candidate proteins associated with early onset AD resilience (KCNN1 & 2) and late-stage AD (ADP-ribosylation factors). Additionally, weighted protein co-expression network analysis (WPCNA) was performed to assess whether there are any co-expressed protein modules were significantly associated with the resilience trait. We discovered a cluster of co-expressed vascular-and blood-related proteins that regulate protease and hydrolase activity to be negatively associated with the resilience trait at early onset stages of AD, and a group of keratin proteins to be positively associated with the resilience trait at later stages of AD. In summary, our work reveals novel proteomic disease-related changes associated with cognitive resilience to AD that has not been observed at transcriptomic level.

Alexa Putka
Graduate Student

Cerebellar lipids are dysregulated in Spinocerebellar ataxia type 3 mice and post-mortem patients

Alexandra F. Putka, Varshasnata Mohanty, Vikram O. Sundararajan, Stephanie M. Cologna, and Hayley S. McLoughlin

Our lab recently uncovered robust oligodendrocyte maturation and myelination deficits in Spinocerebellar ataxia type 3 (SCA3), the most common dominantly inherited ataxia in the world. This adds to the growing body of work demonstrating oligodendrocyte (OL) abnormalities in neurodegenerative diseases. SCA3 is a uniformly fatal disorder caused by a polyglutamine (polyQ) repeat expansion in the ATXN3 protein, resulting in gray and white matter loss in vulnerable regions including the cerebellum. As a monogenic disease amongst a host of polygenic neurodegenerative disorders, SCA3 is ideally situated for paradigmatic investigations into the mechanism underlying OL impairments. To understand the mechanism(s) underlying the SCA3 OL maturation deficit, we focused on the top gene ontology biological process disrupted in a disease-vulnerable brain region of symptomatic SCA3 mice compared to WT controls: cholesterol/sterol biosynthetic processes. Lipids (including cholesterol) are understudied in SCA3 and represent a potential contributor to impaired OL maturation. Indeed, myelin is enriched for lipids compared to other biological membranes, and disrupted lipid levels lead to deficient OL maturation/myelination. To investigate lipid alterations in SCA3, we conducted liquid chromatography-mass spectrometry (LC-MS)-based lipidomics on the disease-vulnerable cerebellum of symptomatic SCA3 mice and post-mortem patients. We found striking differences in the lipid profile between SCA3 and control samples, including a three-fold reduction in cholesterol. Among the lipid pathways dysregulated in disease is the "membrane component," which could indicate impaired myelin sheath composition. To our knowledge, this is the first unbiased study of lipids within SCA3 mouse and patient brains. This study establishes lipid dysregulation as an undescribed feature of SCA3 and sets the stage for future investigations of how lipid dysregulation contributes to OL maturation/myelination deficits. Such work is expected to increase our understanding of SCA3 pathogenesis and identify potential therapeutic targets across the neurodegenerative disease field.

Jorge Contreras
Graduate Student

Impact of Scn2a Haploinsufficiency on Auditory Processing Abnormalities in a ASD Mouse Model

Jorge Contreras, Han Gyu Bae, and Jun Hee Kim

Auditory processing abnormalities, including auditory hypersensitivity and diminished auditory spatial attention, are notable and frequent characteristics of autism spectrum disorder (ASD). The Scn2a gene, which encodes the sodium channel NaV1.2, is recognized as a significant genetic determinant for ASD. In this study, we investigate the influence of Scn2a haploinsufficiency on the cellular, synaptic, and behavioral functions within the auditory brainstem circuitry, using conventional Scn2a heterozygous mice (Scn2a+/?) that have a loss-of-function mutation in Scn2a.

Through in vivo auditory function tests, Scn2a+/? mice demonstrated normal responses in distortion product otoacoustic emissions (DPOAE) and auditory brainstem responses (ABRs). However, a noteworthy observation was the pronounced startle response that Scn2a+/? mice displayed to sudden, loud auditory stimuli in the acoustic startle response (ASR) tests. This response is indicative of heightened sensory perception. Furthermore, whole-cell recordings of medial nucleus of the trapezoid body (MNTB) principal neurons show significant differences in the number of action potentials, pre-hearing onset, between the Scn2a+/? mice and wild-type mice during auditory brainstem development.

These data suggest that while Scn2a haploinsufficiency does not appear to affect auditory transmission (as evidenced by normal ABRs), it does impair sensory gating, resulting in increased ASR. Firing pattern disruption at the cellular level prior to hearing onset could have implications into early adulthood as observed by the in vivo auditory tests. The findings offer valuable insights into the role of Scn2a in auditory processing abnormalities associated with ASD.

Megan Dykstra
Graduate Student

Regulation and Toxicity of TDP43 Splice Variants in ALS and FTD

Megan Dykstra, Kaitlin Weskamp, Nico Gomez, Xingli Li, Elizabeth Tank, Emile Pinarbasi, Corey Stewart, Becky Glineburg, Peter Todd, and Sami Barmada

Nuclear exclusion and cytoplasmic aggregation of TDP43 is the pathological signature of ALS and FTD. However, we understand little about the underlying reasons for TDP43 mislocalization or the relative impacts of its corresponding gain- and loss- of function effects on neuronal survival.

Our previous studies suggested that TDP43 mislocalization may be due to the production of TDP43 splice variants that are prone to aggregation and actively exported from the nucleus. These 'short' (s)TDP43 isoforms are evolutionarily conserved, but their regulation and function remain fundamentally unclear.

Here, we show that sTDP43 is produced by the same negative feedback loop that regulates TDP43 levels in all cells, wherein TDP43 binds to its own RNA, resulting in alternatively spliced transcripts that are destabilized by nonsense mediated RNA decay (NMD). We found that the alternative isoforms generated in this process are those that encode sTDP43. Consistent with this, TDP43 overexpression and NMD inhibition increase sTDP43 production at both the protein and RNA levels. Using optical pulse labeling, a non-invasive method for measuring protein turnover, we noted that sTDP43 is rapidly cleared by macroautophagy, further contributing to low steady-state levels in healthy cells.

Bypassing these regulatory mechanisms through overexpression of sTDP43 recapitulates TDP43 mislocalization and neurodegeneration. By introducing mutations that interfere with the functional domains of sTDP43, we found that sTDP43-dependent toxicity requires RNA binding as well as dimerization with full length TDP43, suggesting both gain- and loss-of-function mechanisms contributing to toxicity. RNA sequencing experiments in HEK293T cells confirmed this hypothesis, demonstrating effects consistent with TDP43 deposition as well as knockdown.

In ongoing studies, we are exploring the impact of sTDP43 accumulation on endogenous TDP43 function in iPSC-derived neurons. Together, these investigations may prove essential for elucidating the function of sTDP43, as well as the consequences of sTDP43 accumulation in ALS and FTD.

Emily Peirent
Graduate Student

ASXL3: Connecting chromatin biology to neurodevelopmental disorders

Emily Peirent, Yao-Chang Tsan, Samantha Regan, Charles Ryan, and Stephanie Bielas

Genetic studies of neurodevelopmental disorders highlight chromatin's importance in corticogenesis, with pathogenic variants enriched in its regulatory networks. Dynamic regulation of histone modifications is critical for the transcriptional plasticity required during this cellular differentiation. One such modification is mono-ubiquitination of histone H2A (H2AUb1), a conserved, traditionally repressive histone mark reversed by the polycomb repressive deubiquitinase (PR-DUB) complex. We identified de novo dominant truncating variants in ASXL3, a key component of PR-DUB, as the genetic basis of both Bainbridge Ropers Syndrome (BRS) and autism spectrum disorder (ASD), characterized by failure to thrive, global developmental delay, feeding problems, hypotonia, profound speech deficits, and intellectual disability. Dysregulation of H2AUb1 is a key molecular pathology in primary cells derived from BRS patients. To investigate this pathology in early corticogenesis, we generated 3D human cortical organoids from CRISPR-edited and patient-derived hPSCs, where we observed ASXL3-dependent defects in neuronal differentiation. We utilized transcriptomic and epigenomic techniques to probe the role of ASXL3-dependent H2AUb1deubiquitinationin regulating transcriptional profiles critical to NPC fate decisions during corticogenesis. Together, our functional investigation of BRS- and ASD-associated genetic variants provides molecular insights towards elucidating the role of H2AUb1 in neural development.

Han-Gyu Bae, Ph.D.
Postdoctoral Fellow

Roles of oligodendroglial Scn2a in myelination, neural circuitry, and auditory hypersensitivity in ASD

Han-Gyu Bae, Wan-Chen Wu, Kaila Nip, and Elizabeth Gould

Autism spectrum disorder (ASD) is characterized by a complex etiology, with genetic determinants significantly influencing its manifestation. Among these, the Scn2a gene emerges as a pivotal player, crucially involved in oligodendrocyte (OL) function. The present study elucidates the underexplored roles of Scn2a in OL functionality, subsequently affecting myelination and auditory neural processes. The results reveal a nuanced interplay between OLs and axons, where Scn2a deletion causes alterations in OL differentiation and myelination. This disruption, in turn, instigates changes in axonal properties and neuronal activities at the single cell level. Furthermore, OL-specific Scn2a deletion compromises the integrity of neural circuitry within auditory pathways, leading auditory hypersensitivity—a common sensory abnormality observed in ASD. Through transcriptional profiling, we identified alterations in the expression of myelin-associated genes, highlighting the cellular consequences engendered by Scn2a deletion. In summary, the findings of this study provide unprecedented insights into the pathway from Scn2a deletion in OL to sensory abnormalities in ASD, underscoring the integral role of Scn2a-mediated OL myelination in auditory responses. This research thereby provides novel insights into the intricate tapestry of genetic and cellular interactions inherent in ASD.

Juan Mato
Graduate Student

SCA3 mouse models exhibit prominent ultrastructural and electrophysiological peripheral nerve abnormalities

Mato J, Hayes J, Naeem A, Feldman E, and McLoughlin HS

Despite being the most common dominantly inherited ataxia, there is currently no effective treatment for Spinocerebellar ataxia type 3 (SCA3). Historically, pre-clinical and clinical studies of SCA3 have focused on the role of central nervous system neuronal and glial populations in pathogenesis. However, more than half of patients with SCA3 suffer debilitating peripheral neuropathy symptoms. These patients manifest sensory abnormalities in electrophysiological recordings of peripheral nerve function as well as "stock and glove" burning sensations, implicating two major cell types: Schwann cells and dorsal root ganglia sensory neurons. Schwann cells, the myelinating glia of the peripheral nervous system, accumulate mutant ATXN3 and display reduced myelination of SCA3 patient sciatic nerves. This is in line with a demyelinating model of neuropathy in which myelination deficits inhibit effective sensory signal conduction in a distal-proximal gradient along nerves. Sensory neurons in SCA3 patient's dorsal root ganglia show neurodegenerative markers that when combined with loss of sensory signal amplitudes in peripheral nerves may reflect an axonopathy model of disease involving a uniform reduction of signal amplitude due to a loss of sensory fibers in nerves. Studies characterizing the onset and progression of peripheral neuropathy in SCA3 patients are essentially lacking, and pre-clinical attempts to understand this pathology have failed to probe progressive markers of disease across natural history. To fill in this gap, I have begun to utilize two SCA3 mouse models to investigate the behavioral, electrophysiological, and histological breadth of the peripheral neuropathy disease timeline. Findings from this work, will inform future therapeutic avenues for SCA3-related peripheral neuropathy and may offer broader applications to other polyglutamine repeat expansion diseases.

Bailey Masser
Graduate Student

Assessing miR-masking Antisense Oligonucleotides (MMOs) for the Treatment of Spinocerebellar Ataxia Type 15/16

Bailey E. Masser, Jacob P. Stein, Ping-Jung Lin, and Hayley S. McLoughlin

Spinocerebellar ataxias (SCAs) comprise a diverse group of dominantly inherited neurodegenerative ataxias, with over 50 subtypes and more than 40 genes identified to date. Despite this heterogeneity, neuronal calcium (Ca2+) signaling disruptions have been repeatedly reported in the pathogenesis of SCAs. Polyglutamine SCAs (SCA 1, 2, 3, 6, 7, and 17) are associated with cerebellar Ca2+ signaling dysfunction, linked primarily to the inositol 1,4,5-trisphosphate receptor, type 1 (IP3R1). IP3R1 is an endoplasmic reticulum membrane Ca2+ channel essential for cerebellar Purkinje neuron function and survival, with heterozygous deletions of its encoding gene (ITPR1) causing SCA15/16, a slowly progressive, pure cerebellar ataxia. Supplementation of IP3R1 as treatment for SCA15/16 is challenged by the large size of the channel (~10 kb coding sequence). IP3R1, like many ion channels, is a "Goldilocks" protein sensitive to both up- and down-regulation (causative of SCA29). To overcome this, we propose the use of antisense oligonucleotides (ASOs) to preclude microRNA (miRNA)-mediated silencing and increase the translational efficiency of the remaining allele. We hypothesize that these miR-masking ASOs (MMOs) will bind to miRNA recognition elements (MREs) within the ITPR1 3' untranslated region (UTR), preventing miRNA binding and restoring IP3R1 expression and function. Here, we identified and validated eleven ITPR1-miRNA interactions using an ITPR1 3' UTR reporter construct. Directed mutagenesis of the most active MREs prioritized the miR-25 family MRE and MMOs were designed complementary to this MRE. The top scoring MMO demonstrated target site protection in our reporter system and preliminary data supports protection of endogenous ITPR1 transcripts. Ongoing studies are assessing the safety and efficacy of MMOs both in vitro and in vivo. This approach holds promise not only for treatment of SCA15/16, but also for other channelopathies caused by haploinsufficiency, as it enables restoration rather than overexpression of channel expression and function.

Ryan Neff
Graduate Student

Normalization of DSCAM in the Ts65Dn mouse model of Down Syndrome ameliorates dendritic phenotypes in the dentate gyrus of the hippocampus.

R. Neff, T. Hergenreder, B. Ye, G. Murphy

Down Syndrome (DS), which results from trisomy of human chromosome 21 (HSA21), is characterized by physical and psychomotor impairments and neurodevelopmental delays. Determining the genetic substrates that underlie the impairments observed in DS will not only lead to viable treatments for DS individuals, but also provide insight into the role of those genes in normal cognition. The Down Syndrome Cell Adhesion Molecule (DSCAM) is located on HSA21 and has been shown to be crucial for mediating neurodevelopmental processes such as presynaptic growth. It remains unknown as to whether DSCAM contributes to cognitive phenotypes and neurodevelopmental changes associated with the hippocampus, a region critical for learning and memory. The Ts65Dn mouse model of DS replicates many of the human phenotypes, including abnormalities in neuronal structure within the hippocampus. Through genetic correction of DSCAM (i.e., normalization) in the Ts65Dn, we aim to determine the relative contribution of DSCAM overexpression to the cognitive phenotypes in DS. In this study, we utilized Golgi-Cox staining in fixed brain tissue from 5–6-month-old mice to assess the morphology of dendritic spines in the CA1 and dentate gyrus (DG) regions of the dorsal hippocampus. We observed regional differences in DS spine density, with an increase seen in CA1 pyramidal cells and a decrease in granule cells of the DG. Additionally, there was an enlargement of DS CA1 spines. Through DSCAM normalization, we saw a correction of the spine density decrease in the DG with no effect on CA1 dendritic phenotypes. Furthermore, we observed that neurogenesis in the DG was decreased in DS, and that DSCAM normalization rescued this deficit as well. Overall, our findings provide evidence for DSCAM overexpression as a central contributor to the abnormalities in neuronal structure within the DS brain and suggest a promising therapeutic target for DS research.

Mariana Sierra
Post-baccalaureate student (PREP)

Generation of Self-Organizing Single-Rosette Cortical Organoids (SOSR-COs) lacking ATXN3, the disease gene in Spinocerebellar Ataxia type 3

Mariana Sierra, Rachael Powers, Camellia Huang, Carmo Costa, Donald Seyfried, and Henry Paulson

Increasingly, researchers are employing brain organoids derived from induced pluripotent stem cells (iPSCs) or human embryonic stem cells (hESCs) to model neurodevelopmental and neurodegenerative disorders. Among these disorders are repeat expansion diseases, including Spinocerebellar Ataxia Type 3 (SCA3), an autosomal dominant neurodegenerative disease caused by a polyglutamine-encoding CAG repeat expansion in the ATXN3 gene, which encodes a deubiquitinating enzyme. To date, the use of organoids to model features of SCA3 or to investigate the normal role of the ATXN3 gene has been severely limited, and the models that have been generated derive from ESC spheres known as embryoid bodies (EB), which contain multiple neural rosette structures. This presence of multiple organizing centers results in structural heterogeneity across samples, limiting analysis of the neurodevelopmental and molecular consequences of normal or mutant ATXN3 gene expression. To overcome this limitation, we leveraged a newly developed protocol from the Tidball/Parent labs at the University of Michigan to generate brain-like organoids harboring a single neural rosette, termed self-organizing single-rosette cortical organoids (SOSR-COs). Given the reproducible nature of SOSR-COs and the absence of knowledge about the role of ATXN3 in neurodevelopment, we generated cortical SOSR-COs expressing or lacking ATXN3 to better study this disease-linked gene. We have developed and are aging SOSR-COs from a control hESC line (UM4-6), a control iPSC line (KOLF2.1J), and a CRISPR-generated ATXN3 Knockout (KO) KOLF2.1J line. Unlike wild type organoids, developing ATXN3 KO SOSR-COs at 11 days display abnormal microtubule network formation and multiple secondary rosettes; ongoing studies will assess ATXN3 KO SOSR-COs up to 5 months of age, a point at which control SOSR-COs demonstrate diverse CNS cell types. In ongoing studies, we will quantify changes over time in neuronal cytoarchitecture and compare transcriptional profiles in organoids lacking or expressing ATXN3.

Sarah Kargbo-Hill, Ph.D.
Faculty

A cast of mis-spliced genes act at the center of TDP-43-related neurodegeneration in ALS/FTD

Velina Kozareva, Sahba Seddighi, Yue A. Qi, Stanislav Tsitkov, Veronica Ryan, Dan Ramos, Anna-Leigh Brown, Caroline Esnault, Ryan Dale, Steven Coon, Pietro Fratta, Michael E. Ward, Ernest Frankel, and Sarah Kargbo-Hill

Loss of TDP-43 from the nucleus is an early pathogenic feature in neurodegenerative diseases including ALS/FTD. TDP-43 is a DNA/RNA binding protein associated with multiple axes of RNA metabolism including transcription, RNA splicing, RNA transport, and RNA stability. However, it is unclear in which processes TDP-43 acts directly or indirectly, and which aspects are most important for neuronal survival and function. One direct role of TDP-43 is to repress splicing of genomic regions, called cryptic exons, and prevent their inclusion into mature RNA transcripts. We previously found 100s of transcripts with cryptic exons. To investigate how inclusion of cryptic exons impacts interacting genes and pathways impacted by TDP-43 loss, we took a multi-omics approach. We assessed human iPSC-derived neurons with TDP-43 knockdown for changes in splicing, transcriptome, proteome, chromatin accessibility, RNA stability and neuron survival. We then performed an integrative network analysis that allowed us to cluster related genes and pathways. We found targets of cryptic splicing at the center of nodes related to mRNA processing, axon outgrowth, synaptic activity, and autophagy. These studies may reveal mechanisms behind the pleiotropic effects observed upon TDP-43 loss, and provide novel targets for therapeutic intervention.

Sarah Hocevar
Graduate Student

Early nanoparticle immunomodulation preserves motor function following cervical spinal cord injury

Sarah E Hocevar, Brian C Ross, Yinghao Wang, Cecelia R Crowther, and Lonnie D Shea

In addition to the initial physical trauma, spinal cord injury (SCI) triggers an immediate influx of immune cells that secrete pro-inflammatory cytokines and reactive oxygen species that cause secondary tissue damage. Ablation of myeloid cells, however, leads to worse functional outcomes due to their role in wound healing. Therefore, we aim to reprogram these immune cells towards a less inflammatory and more pro-regenerative phenotype. Our lab has previously shown that poly(lactide-co-glycolide) (PLG) nanoparticles (NPs) delivered intravenously within 2 hours post-injury (hpi) reduce immune cell infiltration into the spinal cord and enhance motor function. We sought to investigate the window in time which NP administration can successfully modulate the immune response and promote functional sparing. Glucocorticoid treatment is only effective if given within 8 hours of SCI, suggesting that there may also be a critical period for immunomodulation. We hypothesized that NPs must be administered prior to significant accumulation of monocytes and neutrophils to substantially preserve neural circuits and cells. A mouse C5 lateral hemisection was used and resected tissue was replaced with a microporous, biocompatible PLG scaffold. To study the dynamics of immune cell infiltration and secondary tissue damage, mice received 1 mg NPs or vehicle intravenously every 24 hours for 7 days following injury with the first injection starting at the following times: 2, 4, 12, or 24 hpi. While numbers of infiltrating immune cells in the spinal cord were similar between NP-treated and control mice at 2 days post-injury, by day 5 there were significantly fewer total myeloid cells and by day 7 there were fewer macrophages. Additionally, at day 7, macrophages were polarized more towards an anti-inflammatory M2 phenotype rather than a pro-inflammatory M1 phenotype. More neurons were spared on the contralateral side to the injury in NP-treated mice when compared to injured control mice, however, there were not significant differences based on NP administration timing and fewer neurons compared to uninjured controls. Neuromuscular junction (NMJ) innervation was used as a measure of neural circuitry sparing. In the ATZ muscle, which is directly innervated at the level of the injury site, NP treatment did not significantly increase sparing of NMJs. However, in the forearm flexor muscles, which are innervated immediately caudal to the injury, early NP treatment starting at 2 hpi was associated with a greater number of total and innervated motor endplates. The increased sparing of neurons and neural circuits in the 2 hpi NP group corresponds with increased motor function, as measured by the number of correct placements and mistakes on a horizontal ladder beam test. Motor performance for the 2 hpi group stayed consistent through 84 dpi, but motor performance for the other groups continued to improve up to 28 dpi before plateauing. Collectively, these results suggest that early intervention with NPs can modulate the inflammatory response and preserve motor function and circuits following SCI.

Emily Januck
Graduate Student

Integrated analysis reveals Warburg-like metabolic reprogramming in Niemann-Pick type C disease mouse brain

Emily C. Januck, Alice Townsend, Thaddeus J. Kunkel, Kyle A. Sullivan, Jean Merlet, Chandimal Pathmasiri, Stephanie Cologna, Daniel A. Jacobson, and Andrew P. Lieberman

To gain insights into the cell types and mechanisms underlying Niemann-Pick type C disease (NPC) neuropathology, we performed single-nucleus RNA sequencing (snRNAseq) on the forebrains of Npc1 null mice and littermate controls. We recently reported this unbiased analysis of mice at postnatal day 16, uncovering unexpected epigenetic dysregulation in oligodendrocyte lineage cells. Here, we report analysis of forebrains of 7 week old mice, an early timepoint where adult mutant mice exhibit detectable motor abnormalities in our colony. Approximately 42,000 nuclei were sequenced (~7000/mouse, 3 mice/genotype) at a depth of ~50,000 reads per cell. Twenty cell clusters were identified based on the expression of previously established marker genes. Thousands of differentially expressed genes were identified in neurons, glia, and endothelial cells, with a strong bias toward downregulated genes. GO term analysis of genes downregulated across all cell types revealed a significant enrichment for genes related to metabolism and mitochondrial function.

To validate these findings, we compared differentially expressed genes in our snRNAseq dataset with complimentary proteomics datasets completed on adult NPC mouse brains. To accomplish this, we applied a novel computational algorithm called Mechanistic Exploration of Networks for Team-based Omics Research (MENTOR) analysis to cluster this integrated list into related clades for further biological analysis. From this clustering, we identified 2 clades be composed almost entirely of metabolic genes.

To understand the consequence of these extensive changes in metabolic genes, we performed central carbon metabolite profiling on forebrains of an independent cohort of mice. These results revealed increased levels of glycolytic intermediates and an increased pyruvate: citric acid ratio in NPC brains, consistent with a shift from the TCA cycle to glycolysis. Moreover, broad downregulation of genes encoding components of the electron transport chain was associated with elevated levels of NADH. Our findings indicate that the NPC mouse brain is characterized by Warburg-like metabolic reprogramming resulting in an oxidative to glycolytic shift, opening new potential avenues for therapeutic intervention.

Luis Cassinotti, Ph.D.
Faculty

Exploring the Mechanisms of Age-Related Hearing Loss Using Transcriptomics

Luis R. Cassinotti, Gunseli Wallace, Beatriz C. Borges, Matthew Finneran, M. Charles Liberman, Roman J. Giger, and Gabriel Corfas

Age-related hearing loss (ARHL) results from progressive alterations of the auditory system. This pathology has psychological and medical comorbidities, including social isolation, frailty, depression, and cognitive decline. ARHL prevalence doubles every decade of life starting at around age 50. Whereas about 15% of middle-aged individuals (50 to 59 years old) are affected, the prevalence reaches 63.1% for those 70 and older. Despite being the most prevalent sensory deficit in older adults, and having enormous societal and economic impact, no therapies to prevent or slow ARHL exist. This is, in part, because the cellular and molecular mechanisms of ARHL remain poorly understood.

We are using mice to probe the molecular mechanisms of ARHL. As in humans, mice in many strains start showing ARHL in middle-age (around 1 year of age). To characterize the initial molecular changes in early-stage ARHL, we used bulk RNA sequencing to identify inner ear genes whose expression levels change between young adult (8-week-old) and middle age (62-week-old). For sequencing, total RNA was extracted from otic capsules from three animals at each age. Downstream analysis of transcriptomics data includes principal component analysis (PCA), heatmap analysis of distance matrix, differential expression of genes and Gene Ontology (GO) analysis.

Marc Padilla
Graduate Student

Single nucleus RNA-sequencing-mediated identification of NTS neuron populations that decrease feeding and body weight

Obesity promotes diabetes, heart disease, and other metabolic disorders. New medicines (such as incretin receptor agonists) that target the brainstem effectively reduce appetite and body weight but exhibit aversive effects (e.g., nausea and vomiting) that limit their therapeutic utility for some patients. To identify new targets for the therapy of obesity, we must comprehensively understand the brainstem circuits that can promote the long-term suppression of food intake and body weight and distinguish whether or not each of these circuits promotes aversive responses.

Our previous single nucleus RNA-sequencing (snRNA-seq) analysis of the dorsal vagal complex (DVC) defined nucleus tractus solitarius (NTS) neuron populations in an unbiased manner. In addition to identifying unique snRNA-seq populations marked by Calcr/Prlh and Lepr/Gcg (which were previously shown to mediate the non-aversive long-term suppression of food intake and body weight), integrating our snRNA-seq analysis with GWAS BMI data suggested roles for NTS Eya1 neurons in long-term energy balance. We also found that Cck, which was previously used to mark neurons that mediate the aversive suppression of food intake, maps to multiple snRNA-seq defined NTS populations, including non-aversive Lepr/Gcg neurons and two other populations (marked by Rxfp1 and Vglut3,) of unknown function.

We thus generated Eya1Cre and Rxfp1Cre mice and obtained vGlut3Cre mice with which to define the function of the appurtenant NTS neuron populations. To date, we have chemogenetically activated NTS Eya1 neurons, revealing their ability to mediate the suppression of food intake without provoking aversive effects. Upcoming studies will determine the function of NTS Rxfp1 and vGlut3 neuron populations, as well as defining potential roles for NTS Eya1 cells in other responses. Ultimately, these studies will define NTS neuron populations that contribute to the long-term control of energy balance and identify the NTS population(s) that mediate aversive responses, thereby revealing salient neuron populations to target for the non-aversive treatment of obesity.

Tamara Stevenson, Ph.D.
Postdoctoral Fellow

Dendritic spine morphological signatures of cognitive resilience to Alzheimer's disease

Stevenson TK, Berchulski M, Moore SJ, and Kaczorowski CC

While dendritic spine loss is an established phenotype of normal aging and Alzheimer's disease (AD) pathology, few studies have examined the relationship between spine loss and cognitive resilience in normal aging and AD. In one study, significant reductions in spine density were observed for patients with cognitive impairment and AD pathology, but spine density was similar between cognitively normal control patients and cognitively normal patients with AD pathology (patients with cognitive impairment but no AD pathology were not included in the study). These data provide evidence to support the hypothesis that resilience to dendritic spine loss protects against cognitive decline due to AD. The mechanisms that underlie this resilience, however, are unknown. Accordingly, we will use the genetically diverse, but fully isogenic, AD-BXD mouse panel to identify genetic variants that confer resilience to spine loss in cognitively resilient and susceptible AD-BXD strains. Four AD-BXD strains were chosen based on their cognitive performance at 14 months of age (cognitively resilient: AD-BXD99 and AD-BXD124; cognitively susceptible: AD-B6 and AD-B6xD2). Dentate granule (DG) cells from 14-month-old female AD-BXD and Ntg-BXD mice were filled with biocytin and stained with streptavidin-488. Images were acquired with a Leica Stellaris 5 confocal microscope (63x, 1.4 NA), and analyzed using Neurolucida360. Here we present DG dendritic spine density (#/10µm), morphology (thin/mushroom/stubby/filipodia), head diameter, and length data from AD-B6 and Ntg-B6 mice. Morphometry data are correlated with cognitive performance data. Taken together, these data will elucidate the contribution of changes in spine morphometry to cognitive decline in AD.

Vikram Sundararajan
Undergraduate Student

Characterizing cholesterol dyshomeostasis in spinocerebellar ataxia type 3

Vikram O. Sundararajan, Alexandra F. Putka, Varshasnata Mohanty, Isabel G. Wellik, Stephanie M. Cologna, Hayley S. McLoughlin

White matter alterations have recently been identified as a shared signature across many neurodegenerative diseases. Spinocerebellar ataxia type 3 (SCA3), the most common dominantly inherited ataxia in the world, is caused by a polyglutamine repeat expansion in the ATXN3 protein, resulting in neuron loss in disease-vulnerable brain regions. We recently uncovered a robust deficit in oligodendrocyte maturation and myelination in SCA3. The mechanism underlying this oligodendrocyte maturation impairment remains unknown. Taking an unbiased approach, RNA-sequencing revealed the top gene ontology biological pathway dysregulated in symptomatic SCA3 mice relative to WT controls to be "cholesterol biosynthetic processes." Cholesterol is an essential component of myelin, and inhibiting cholesterol biosynthesis hinders OL maturation. We aimed to characterize cholesterol metabolic pathway alterations in SCA3, establishing the foundation for future studies investigating the contribution of cholesterol dysfunction to impaired OL maturation. We found a dramatic reduction in cholesterol levels in the disease-vulnerable cerebellum of SCA3 mice and post-mortem patients compared to controls. Cholesterol is synthesized de novo in the brain; therefore, we investigated the biosynthetic pathway on a transcript and protein level. We found no change to cholesterol turnover enzymes in diseased mice. In contrast, cholesterol biosynthetic transcripts were reduced in the brainstem, but not the cerebellum, of SCA3 mice. This was intriguing given the decrease in cerebellar cholesterol levels. Toward possible mechanisms, we confirmed that a mutant ATXN3 toxic gain-of-function mechanism underlies the brainstem cholesterol biosynthesis transcriptional impairment. We also characterized levels of SREBP2, a master transcriptional regulator of cholesterol biosynthesis. Active SREBP2 expression was unchanged in the brainstem, but increased in the cerebellum, of SCA3 mice relative to controls. This suggests possible compensatory upregulation of SREBP2 in the cerebellum that normalizes biosynthesis transcripts but fails to rescue cholesterol levels. Our work establishes cholesterol dysregulation as an uncharacterized feature of SCA3 and potential therapeutic target.

Daniel Wahl, Ph.D.
Faculty

Rewiring of cortical glucose metabolism fuels human brain cancer growth

Andrew J. Scott, Anjali Mittal, Baharan Meghdadi, Sravya Palavalasa, Abhinav Achreja, Alexandra O'Brien, Ayesha U. Kothari, Weihua Zhou, Jie Xu, Angelica Lin, Kari Wilder-Romans, Donna M. Edwards, Zhe Wu, Jiane Feng, Anthony C. Andren, Li Zhang, Vijay Tarnal, Kimberly A. Redic, Nathan Qi, Joshua Fischer, Ethan Yang, Michael S. Regan, Sylwia A. Stopka, Gerard Baquer, Theodore S. Lawrence, Sriram Venneti, Nathalie Y. R. Agar, Costas A. Lyssiotis,Wajd N. Al-Holou, Deepak Nagrath, and Daniel R. Wahl

The brain avidly consumes glucose to fuel neurophysiology. Cancers of the brain, such as glioblastoma (GBM), lose aspects of normal biology and gain the ability to proliferate and invade healthy tissue. How brain cancers rewire glucose utilization to fuel these processes is poorly understood. Here we perform infusions of 13C-labeled glucose into patients and mice with brain cancer to define the metabolic fates of glucose-derived carbon in tumor and cortex. By combining these measurements with quantitative metabolic flux analysis, we find that human cortex funnels glucose-derived carbons towards physiologic processes including TCA cycle oxidation and neurotransmitter synthesis. In contrast, brain cancers downregulate these physiologic processes, scavenge alternative carbon sources from the environment, and instead use glucose-derived carbons to produce molecules needed for proliferation and invasion. Targeting this metabolic rewiring in mice through dietary modulation selectively alters GBM metabolism and slows tumor growth

Fiona Molloy, Ph.D.
Postdoctoral Fellow

Contributions of the Graphical Properties of the Functional Connectome to General Intelligence

Fiona Molloy, Aman Taxali, Mike Angstadt, and Chandra Sripada

Graph theory is a powerful tool for quantifying abstract organizational properties of complex systems from social networks to biological systems, including the human brain. In neuroscience, graph theory has been applied to structural and functional MRI, revealing key features of the complex interactions between brain regions, including small-worldness and modularity. This framework has promise in elucidating the neural bases supporting cognition and intelligence, but previous research presents conflicting evidence in the ability of these metrics to predict general cognitive ability (GCA). Here, to identify which topological properties meaningfully relate to GCA, we utilize resting state fMRI and behavioral data in two of the largest neuroimaging datasets to date. The datasets include the Adolescent Brain Cognitive Development study (ABCD), consisting of 11,875 9- and 10-year-olds recruited at 21 sites across the US, and the Human Connectome Project (HCP), which includes 1,200 young adults. Both datasets collected extensive neurocognitive batteries from which GCA is computed using bifactor modeling. We comprehensively assess graph theory metrics across different levels (node-, intrinsic connectivity network-, and whole-brain-levels), and multiple modeling/ processing choices. In the ABCD sample, we establish three main conclusions. First, whole-brain measures do not contribute meaningfully to GCA. Second, in contrast, node-level measures, particularly module degree, are significantly predictive of GCA. Third, this node-level information represents 60% of predictive ability afforded by the entire multivariate connectome, but the explained variance is strictly a subset, i.e., adding graph theoretical measures does not provide additional information than that contained in the connectome. Critically, we replicate these findings in the HCP sample. This study is the most definitive account of graph theory measures and GCA to date and reveals guidelines for maximizing predictive accuracy and indicates the importance of further research into the complex sub-network interactions to uncover the neural basis of general intelligence.

Hyunwoo Jang
Graduate Student

Measuring the dynamic balance of integration and segregation underlying conscious states

Hyunwoo Jang, George A. Mashour, Anthony G. Hudetz, and Zirui Huang

Integration and segregation (or differentiation) are considered two key features of neural systems thought to mediate consciousness. The balance of integration and segregation in brain networks is critical for optimal function and has been a focus of research for over a decade. However, a consensus on how, precisely, to measure this balance has proven elusive. In this study we aimed to formulate a metric that captures two major surrogates of integration-segregation balance, i.e., functional connectivity strength and network topology. Through application to dynamic functional connectivity in humans, we investigate the potential of this metric to reflect shifts in conscious states during the loss and recovery of responsiveness (LOR and ROR, respectively). Furthermore, at a more granular level, we examine whether a sequential pattern of subnetwork changes in integration-segregation balance exists during the transitions to LOR and ROR. Finally, we use machine learning to identify if the topo-connectivity can reliably predict different states of consciousness.

James Eckner, M.D., M.S.
Faculty

Influence of concussion and collision sport history on later self-reported mood outcomes: Results from the U-M Alumni Brain Health Study

Eckner JT, Lorincz MT, Giordani B, Seagly K, Almeida A, Millward D, Broglio SP, Varangis E, and Veliz PT

Exercise and sport participation carry a number of important health benefits. However, potential long-term effects of sport-related concussion and repetitive head impact exposure on athletes' long-term brain health represent a significant concern in the sports medicine community and society at large. The purpose of this study was to assess relationships between concussion history and collision sport participation with self-reported mood outcomes in a sample of former U-M male and female collegiate athletes and non-athletes.

This was a retrospective cohort study in 1,049 athlete and non-athlete U-M alumni (50.1±16.6 years old; 46.9% female), who last attended the university 10 or more years ago. Participants completed a 25-minute online survey. Independent variables included lifetime number of concussions and total seasons of collision sport participation. Dependent variables included history of mood/psychiatric diagnoses, PROMIS Emotional Distress- Anger/Irritability t-score, and Patient Health Questionnaire-8 (PHQ-8) score. Statistical analysis used multivariable logistic and linear regression models accounting for other relevant covariates.

In full models, mood/psychiatric diagnoses were neither associated with concussion history ($p=0.675$) nor seasons of collision sport ($p=0.769$); worse PROMIS Emotional Distress- Anger/Irritability t score was associated with number of concussions ($p=0.003$), but not seasons of collision sport ($p=0.399$); worse PHQ-8 score was associated with number of concussions ($p=0.046$), but not seasons of collision sport ($p=0.244$).

Worse self-reported mood outcomes later in life were more strongly associated with concussion history than participation in collision sport, although effects were small and did not meet thresholds for clinical significance. Additional studies with objective data are needed.

Julia Evanski
Graduate Student

White Matter Integrity as a Predictor of Post-Traumatic Pain: Insights into Structural Biomarkers and Pain Development

Julia M. Evanski, Chelsea M. Kaplan, Samuel A. McLean, and Steven E. Harte

Chronic pain conditions are estimated to impact 20% of adults worldwide. One common trigger of chronic widespread pain (CWP) is traumatic stress exposure. Approximately 1/3 of people who experience a traumatic event (e.g., motor vehicle accident) develop CWP. We leveraged the Advancing Understanding of Recovery After Trauma (AURORA) Study dataset to examine if white matter microstructure predicts development of CWP after a non-life-threatening traumatic event. Participants (n=163) who reported no pain at baseline underwent an MRI scan two-weeks after experiencing a traumatic event. Six months following the traumatic event, 42 participants developed CWP, while the remaining 121 participants did not. Using fractional anisotropy (FA) as a measure of white matter directionality, we completed an a priori analysis of white matter tracts previously reported to have altered microstructure in individuals with chronic pain compared to controls. The tracts examined include the corpus callosum (body, splenium, genu) and the following bilateral tracts: cingulum bundle, parahippocampal branch of the cingulum, and the inferior fronto-occipital fasciculus. With the inclusion of covariates (age, sex, race/ethnicity, and intracranial volume), the left cingulum bundle had significantly lower FA in those that developed CWP (OR=0.56, CI[0.37-0.83], p=0.006). This is consistent with prior reports where lower FA of the left cingulum bundle was found in those with chronic musculoskeletal pain. The cingulum bundle innervates fibers originating in the anterior cingulate cortex which is an important region implicated in emotion, pain sensitivity and chronic pain. Lower FA in the cingulum bundle may reflect differences in structural connectivity in pain processing centers in the anterior cingulate cortex and limbic regions, which may be a risk factor for the development of chronic pain.

Jiwon Park, Ph.D.
Postdoctoral Fellow

Hearing Loss Accelerates Alzheimer's Disease progress through Tau phosphorylation.

Jiwon Park, Jun Hee Kim

Hearing loss is one of the risk factors of Alzheimer's disease (AD). Although many research have shown this, the underlying mechanism linking these two diseases is lacking. In this study, we used 5x FAD mice, an Alzheimer's disease model, which are genetically engineered mice that overexpress amyloid precursor protein and its enzyme presenilin 1. To construct the conductive hearing loss model, ear plugs were inserted into both mouse ears for two months. After the ear plug was removed, the hearing ability was assessed with an Auditory Brainstem Response (ABR) test. There was no significant difference in hearing ability between the WT mice and WT with ear plug-inserted mice (WT+EP). However, 5x FAD mice with ear plug-inserted mice (5x FAD+EP) were impaired their hearing ability. Next, we observed amyloid beta (A?) and tau phosphorylation (p-Tau), pathological markers of Alzheimer's disease, in the mouse brain by immunofluorescence. The 5x FAD mice had increased A? expression in the cortex and hippocampus. But there were no changes through the EP. Increased expression of p-Tau was not presented in 5x FAD but was significantly increased in 5x FAD+EP compared to other groups. As these results show that hearing loss accelerates Alzheimer's disease pathology through tau phosphorylation.

Navyateja Korimerla, Ph.D.
Postdoctoral Fellow

Reciprocal links between methionine metabolism and DNA repair in brain tumors

Navyateja Korimerla, Kari Wilder-Romans, Jie Xu, Isra Haq, Peter Kalev, Nathan Qi, Charles Evans, Maureen Kachman, Weihua Zhou, Angelica Lin, Zitong Zhao, Andrew J Scott, Alexandra O'Brien, Ayesha Kothari, Sravya Palavalasa, Erik R Peterson, Marc L Hyer, Katya Marjon, Costas A Lyssiotis, Taryn Sleger, and Daniel R Wahl

Glioblastoma (GBM) is uniformly lethal due to intrinsic resistance to standard-of-care radiation (RT) and chemotherapy. Altered cellular metabolism is a key mediator of GBM RT resistance. Methionine uptake is drastically elevated in GBMs compared to normal cells but whether this impacts treatment resistance is uncertain, as is the metabolic fates of methionine once it enters GBM cells.

Here, we find that RT acutely increases the levels of methionine-related metabolites in a variety of RT-resistant GBM models. Stable isotope tracing studies further revealed that RT acutely activates methionine to S-adenosyl methionine (SAM) conversion through an active signaling event through DNA damage response. In vivo tumor SAM synthesis increases after radiation, while normal brain SAM production remains unchanged, indicating a tumor-specific metabolic alteration in response to RT conferring treatment resistance. Pharmacological and dietary strategies to block methionine to SAM conversion slowed the DNA damage repair and increased cell death following RT. These effects are selective to GBMs lacking the methionine salvage enzyme methylthioadenosine phosphorylase (MTAP). Pharmacological inhibition of SAM synthesis hindered tumor growth in flank and orthotopic in vivo GBM models when combined with RT.

In short, these results highlight a new signaling link between DNA damage and SAM synthesis and define the metabolic fates of methionine in GBM in vivo. Inhibiting the RT-induced SAM synthesis slows the DNA damage repair and augments RT efficacy in GBM. This therapeutic strategy provides scope for combining MAT2A inhibitors with RT in GBMs with impaired methionine salvage and spares the adjacent normal brain.

Mark Zuppichini, Ph.D.
Postdoctoral Fellow

GABA Levels are Significantly Lower in Mild Cognitive Impairment Patients

Mark D. Zuppichini, Abbey M. Hamlin, Quan Zhou, Esther Kim, Kayla Wyatt, Noah Reardon, and Thad A. Polk

Gamma-aminobutyric acid (GABA), the brain's major inhibitory neurotransmitter, has been identified as one factor that might contribute to the functional deterioration and cognitive decline observed in Alzheimer's disease (AD). Animal models of AD have established an important role for GABA and human functional neuroimaging studies have observed hyperexcitability in brain regions associated with cognitive and sensory function in both AD patients and in patients with mild cognitive impairment (MCI), consistent with a disruption of inhibitory GABAergic processing. Furthermore, studies utilizing magnetic resonance spectroscopy (MRS) have observed age-related reductions in GABA in several brain regions in healthy older adults. However, there has yet to be an MRS study comparing GABA levels in MCI patients compared to age-matched healthy older adults. In the present study, we utilized MRS to measure GABA levels in bilateral auditory, sensorimotor, and ventrovisual voxels of interest (VOI) in healthy older adults (n=30) and MCI patients (n=17). Additionally, we applied a tissue correction strategy to control for the dependency of GABA measurements on underlying VOI tissue composition. MCI patients exhibited significantly ($p < .001$) lower levels of GABA in all VOIs except for the left auditory VOI (MCI still exhibited lower levels in this region, but the effect was not significant).

Samantha Regan, Ph.D.
Postdoctoral Fellow

ASXL3 in cortical development: Molecular insights from rare genetic variants

Samantha L. Regan, Brain T. McGrath, Charles Ryan, and Stephanie L. Bielas

De novo dominant ASXL3 frameshift variants are the genetic basis of Bainbridge-Ropers Syndrome (BRS) and syndromic autism spectrum disorder (ASD). ASXL3 is a component of the Polycomb repressive deubiquitinase (PR-DUB) complex that is critical for Polycomb-mediated transcriptional repression. PR-DUB catalyzes the removal of ubiquitin from Histone 2A (H2AUb1), a repressive histone modification catalyzed by Polycomb repressive complex 1 (PRC1). The molecular mechanism of H2AUb1 and the cellular processes it regulates during normal development and disease remain largely unexplored. To investigate the role of ASXL3 in development we generated a mouse model that carries a clinically relevant Asxl3 frameshift variant (Asxl3fs). Genetic inactivation of Asxl3 leads to perinatal lethality, multi-organ developmental defects, and increased levels of H2AUb1. Within the developing cerebral cortex, loss of ASXL3 perturbs the composition of excitatory neurons and fidelity of cortical layer deposition. We carried out single-cell RNA sequencing at three developmental stages during neurogenesis to characterize the cellular composition and transcriptomic changes. The emerging pathogenic model based on analysis of multiple cell types, at sequential developmental timepoints, implicates overactivation of Notch signaling that alters NPCs proliferation and timing of differentiation. These early developmental defects lead to altered composition of excitatory neurons with aberrant expression of proneural genes responsible for layer specificity at later timepoints. Across cortical development, dysregulated genes were enriched for high confidence ASD risk genes, implicating a convergent pathological mechanism. Together our findings underscore the importance of ASXL3 in Polycomb transcriptional repression during development and provide insight into developmental mechanisms altered by ASD risk genes.

Isha Verma, Ph.D.
Postdoctoral Fellow

Human stem cell model to study the role of a pathogenic splice site variant in SCN1B gene in Dravet Syndrome

Isha Verma, Shreeya Bakshi, Yukun Yuan, Jack M. Parent, and Lori L. Isom

Dravet Syndrome (DS), a developmental and epileptic encephalopathy, has been linked to the variants in the gene encoding the β1 subunit of voltage-gated sodium channels, SCN1B. β1 has a variety of functions, including modulating sodium current and promoting cell-cell and cell-matrix adhesion. SCN1B encodes two isoforms, a transmembrane isoform, β1, and a soluble secreted isoform β1B. Recently, a new variant SCN1B c.449-2A>G was discovered in the 3' splice acceptor site of intron 3. Due to its location, this variant could result in aberrant splicing of SCN1B. To study this, we generated induced cortical excitatory neurons from iPSCs by doxycycline (dox)-inducible expression of the transcription factors NGN2. Stable dox-inducible cell lines were generated from SCN1B c.449-2A>G patient, unaffected father, and unrelated control iPSC lines using the PiggyBac transposon system. Preliminary RT-PCR data of patient with this variant revealed the presence of three different transcripts from SCN1B being produced, including (1) a product including exon 3 of β1B from the retention of intron 3, (2) SCN1B with exon 4 omitted, and (3) SCN1B with a portion of exon 4 missing. Based on the predicted amino acid sequences of these transcripts, we determined that none of these proteins contain the sequence that encodes the transmembrane domain of β1. Additionally, β1, the transmembrane isoform, is also not produced. We are currently performing electrophysiological recordings from these induced excitatory neurons. Our findings can potentially delineate pathogenic mechanisms associated with SCN1B c.449-2A>G variant in DS.

Farzanna Mohamed
Graduate Student

Enhancing activation of μ opioid and 5HT1B/D receptors by inhibition of Gβγ signaling produces antihyperalgesia

Farzanna A. Mohamed, Alan V. Smrcka, Emily M. Jutkiewicz

Although μ opioid receptor (MOR) agonists provide short-term pain relief, they also produce adverse effects and are frequently misused, increasing susceptibility for opioid use disorder (OUD). It is necessary to find alternative analgesics with low abuse liability. Previous findings have shown that an inhibitor of Gβγ protein subunits, gallein, potentiates the antinociceptive effects of morphine without altering its rewarding effects in vivo. Therefore, we sought to evaluate if gallein-mediated inhibition of Gβγ signaling can enhance antihyperalgesic effects produced by endogenous opioid-induced activation of MOR. We treated female and male C57BL6/N mice with the Gβγ inhibitor gallein (10 mg/kg, i.p.), morphine (10 mg/kg, i.p.), or the 5HT1B/D agonist sumatriptan (0.6 mg/kg, i.p.) 90 min after nitroglycerin (NTG, 10 mg/kg, i.p.) and measured tail withdrawal latencies from a 46oC water bath. NTG decreased tail withdrawal latencies as compared with baseline, indicating a hyperalgesic state. Gallein, like morphine and sumatriptan, reversed NTG-induced decreases in withdrawal latencies. The nonselective opioid antagonist naloxone (NLX, 3.2 mg/kg, i.p.) attenuated the antihyperalgesic effects of gallein and morphine, but not sumatriptan, suggesting that activity at opioid receptors mediate the antihyperalgesic effects of gallein. GR127935 (3 mg/kg, s.c.), a 5HT1B/D antagonist, blocked the antihyperalgesic effects of sumatriptan and gallein, but not morphine. Interestingly, these data suggest that gallein may potentiate antihyperalgesic activity of endogenous opioid peptides and serotonin acting through opioid and 5HT1B/D receptors, respectively. Future studies will evaluate the role of the other Gi/o-coupled receptors in gallein-mediated antihyperalgesia.

Anjelica Ferguson
Undergraduate Student

Perceptual and neural correlates of sensory and cognitive priming in chord sequences

Anjelica J. Ferguson, Jackson E. Graves, Barbara Tillmann, Anahita H. Mehta

Western music theory emphasizes the importance of tonal key centers, but investigating the neural correlates of perceived tonality requires controlling for low-level effects such as sensory priming. Past studies have shown behavioral and neural differences in response to sounds that deviate from expected musical contexts, with evidence for higher-level effects of cognitive priming. This study aims to determine the relative contributions of cognitive and sensory priming to harmonic expectation, measured using behavioral responses and event-related potentials (ERPs) using electroencephalography (EEG). We used seven-chord sequences divided into three conditions: tonally expected (final chord fits preceding context), unexpected (final chord is unlikely given the tonal context), and atonal (no strong tonal expectations). To reduce sensory priming, the context and final chord were filtered into mutually exclusive spectral regions. We also included a condition where the context chords shared no pitches with the final chord. These stimulus manipulations allowed us to separate sensory priming into frequency adaptation (controlled by filtering) and pitch priming (controlled by limiting shared pitches between the context chords and the final chord). The unexpected stimuli were composed by raising and lowering the context of the chord progressions by one semitone. Listeners rated the stimuli on a scale from 1 (unexpected) to 7 (expected) and listened to the same stimuli in a semi-passive EEG paradigm. Our behavioral data suggest that harmonic expectation ratings are consistent across filtering conditions and follow the intended pattern, with expected rated higher than atonal and unexpected rated lower. Our hypothesis is that ERPs from unexpected conditions will exhibit greater amplitude compared to expected conditions, while ERPs from the atonal conditions will be smaller due to the absence of a strong contextual cue. These findings will contribute to our understanding of the contributions of sensory and cognitive priming to the perception of tonal structure.

UNIVERSITY OF MICHIGAN
NEUROSCIENCE
CONFERENCE 2024

DAY 2

Abstracts, authors, and locations

Tuesday, May 14 - 9:00 a.m.

Biomedical Science Research Building

Hoi Kei (Amy) Choi
Staff

COVID-19-like immune challenge causes long-lasting memory deficits: Role of neuroimmune priming effects

COVID-19 has affected more than 770 million individuals worldwide, and up to 40% of survivors experience post-acute covid sequelae (PASC, "long COVID"). The symptoms of long COVID include "brain fog", cognitive impairments, and mood-related symptoms such as depression or anxiety. SARS-COV-2 virus only rarely infects the brain, suggesting that other effects of COVID-19 cause changes including memory impairments. Innate immune activation, or inflammation, during illnesses is known to modulate memory, cognition, and mood. Recent studies demonstrate that effects of a peripheral immune challenge on memory can last months after resolution of the inflammatory response (Tchessalova & Tronson 2019, 2020). Whereas much of the prior work has focused on Toll-like receptor (TLR) 4 and TLR3-mediated mechanisms, single stranded RNA (ssRNA) viruses like SARS-COV2 trigger innate immune activation via TLR7 and TLR8. Although less well-studied than its counterparts, TLR7 has previously been linked with cognitive impairments and neurodegenerative disorders including Alzheimer's Disease (AD). In this project we examined the hypothesis that TLR7-triggered immune challenge causes lasting changes in the brain that contribute to the cognitive impairments in long-COVID, and increase risk for age-related cognitive decline and dementias including AD. We used our two week subchronic immune challenge protocol to determine whether the TLR7 agonist R848 (400-1000?g/kg) causes emerging memory impairments or anxiety- and depression-like phenotypes. Further, we identified the time course of cytokine responses over the recovery period and determined the persistence of neuroimmune priming 8 weeks after immune challenge. We observed a clear dose-response of cytokine and chemokine elevations in the hippocampus of young and mid-aged male and female to an acute dose of R848. Eight weeks after immune challenge, we observed memory impairments that were more striking in male animals. These effects on memory were not due to ongoing elevations in neuroimmune activation during behavioral tasks. Nevertheless, persistent changes in immune priming suggest that changes in neuroimmune states or activity might modulate memory processes even in the absence of elevated cytokines. This work is an initial step towards understanding how persistent changes in neuroimmune processes after viral illnesses including COVID-19 might contribute to risks for cognitive deficits, age-related cognitive decline, and Alzheimer's Disease in the decades to come.

Julia Kravchenko
Graduate Student

Age and genetic background modulate the effect of Alzheimer's Disease on sleep

Julia A. Kravchenko, Surjeet Singh, Amy Dunn, Kristen O'Connell, Catherine C. Kaczorowski

Alzheimer's Disease (AD) is known to contribute towards changes to sleep, including decreased sleep duration and increased sleep fragmentation. While significant advances have been made in characterizing these changes, the underlying genetics are yet unknown. As such, we decided to use a forward genetic approach to identify genes controlling sleep in AD in a panel of genetically diverse mice (AD-BXDs).

Female mice from 47 strains in the AD-BXD panel carrying the 5xFAD transgene (n =214) and non-transgenic littermate controls (n = 216) completed sleep testing in the PiezoSleep Tracking System at 6 and 14 months of age. The percent of time spent sleeping was calculated over 4 testing days by automated sleep/wake scoring.

Non-transgenic (Ntg) mice sleep more over 24 hours than 5xFAD counterparts, and more in old age. These patterns are particularly enhanced in the dark (active) phase, where the difference in sleep quantity between Ntg and 5xFAD animals is magnified by increased sleep in Ntg animals with age and a significant decrease in sleep in 5xFAD carrying animals with age. Interestingly, during the light (inactive) phase, 5xFAD animals slept more than non-transgenic counterparts at both 6 and 14 months of age. Heritability estimates for percent time sleeping over 24 hours, the 12-hour light period, and 12-hour dark period at 6 and 14 months of age were between 0.64-0.76 for both non-transgenic and 5xFAD animals, suggesting that genetic background may largely explain the observed changes.

Genetic background modulates the effect of AD and age on sleep in the AD-BXD panel. Since sleep is vital for memory consolidation, future work will aim to examine cognitive performance of these strains and map the genes causing changes to sleep in AD.

Ruonan Li, Ph.D.
Postdoctoral Fellow

CytoFLARE: A genetically encoded tool for reporting and manipulating neurons activated during cognition and behavior

Need Authors

Advancements in genetically encoded tools have facilitated the recording and manipulation of neuronal ensembles associated with specific cognitive processes and behavior. However, significant technological gaps remain in genetically accessing and controlling neuronal ensembles based on their physiological activities in vivo. While immediate early gene (IEG)-based reporters have been developed for monitoring and manipulating neural activity in mammals, they have limitations in sensitivity, temporal resolution, and applicability to different brain regions and stimuli. FLARE, a light- and calcium-gated transcriptional reporting system that labels neurons activated during specific time windows, was previously developed to address these limitations. FLARE offers improved temporal resolution and broader usability compared to existing IEG-based reporters. However, a major limitation of FLARE is its low sensitivity to physiological neuronal activities. Here we present a new generation of FLARE technology, termed cytoFLARE, which has enhanced sensitivity than FLARE. We demonstrate the implementation of cytoFLARE in Drosophila larvae. By expressing the cytoFLARE system in specific groups of neurons in larval nociceptive pathway, we show its ability of reporting neural activity elicited by sensory stimulations with a defined time window. The successful development of cytoFLARE provides a valuable tool for studying and manipulating sparse subsets of neurons activated within specific periods during cognition and behavior, addressing a critical need in neuroscience research.

Michaela Broadnax
Staff

Developing a Face-Name Task for Inclusive Representation and fMRI Compatibility

Michaela Broadnax, Eleanna Varangis

As individuals age, remembering faces and names can pose challenges, particularly with the cross-race effect (CRE) potentially exacerbating difficulties in recalling faces from racial/ethnic groups different from one's own. Our study aimed to develop and validate a new cognitive task for functional magnetic resonance imaging (fMRI) research in diverse samples of older adults. The study included two parts: (1) dementia screening and demographic questionnaires, and (2) the novel cognitive task. We assessed 34 participants (age 55-79) using a novel face-name task featuring older male and female faces representing individuals who identify as Caucasian, Asian, Black, South Asian, Latino/a, or Multi-Ethnic. During encoding, participants viewed blocks of 4 face-name pairs. During recall, participants engaged in a forced-choice recall task, selecting the first letter of the face's name from three options. Half of the participants (n=17) saw the faces appear in a random order during recall, while the other half (n=17) saw the faces appear in the same order at both encoding and recall. Participants completed 12 blocks of 4 faces each. We hypothesized that performance would be negatively correlated with age and that recall would be better in the same order condition compared to randomized recall. Additionally, we expect the CRE to result in better accuracy for same-race faces. Results show that performance is negatively related to participant age, and that randomized recall was associated with poorer performance than same-order recall. Further, while we were under-powered to sufficiently test the CRE, we will present results summarized by participant and stimulus race.

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Occludin carboxy terminus is a dynein adaptor required for mouse embryonic development

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Previous studies suggest occludin Ser490 to alanine (S490A) mutation reduce or prevent vascular endothelial growth factor (VEGF)-induced vascular permeability and angiogenesis in cell culture and transgenic animals. However, its specific mechanism of action remains unclear. In the current study, we further demonstrated that occludin knockout mice expressing S490A dramatically reduced collateral angiogenesis after branch retinal vein occlusion (BRVO) and middle cerebral artery occlusion (MCAO) angiogenesis. Recent data reveals that the occludin knockout mice still express a splice variant from an internal ribosome entry site at exon 4 (isoform 4) that includes 14 amino acids in the transmembrane region and the carboxy-terminal tail with the Ser490 phosphorylation site. Here, we provide data to support the hypothesis that the occludin carboxy terminus acts a dynein adaptor and deletion is embryonic lethal. We demonstrated that isoform 4 occludin was necessary and sufficient to localize to centrosomes in U2OS cells and expression of an S490A of isoform 4 inhibited VEGF-induced proliferation and permeability in primary bovine retinal endothelial cells (BREC). Capture assays of dynein light intermediate chain 2 (LIC2) with occludin mutants in U2OS cells that were co-transfected both GFP-tagged occludin mutants with and HA-tagged LIC2 identified a region of occludin necessary for interaction with LIC2 that possessed homology with known dynein adapters. Germline deletion for gene deletion of full-length occludin and isoform 4 by Occfl5/fl5: EllaCre+ was embryonic lethal due to vascular hemorrhage and led to gastroschisis in some animals. These results reveal a novel role for occludin interaction with dynein LIC2 and to allow trafficking from the cell border and to centrosomes in a phosphorylation dependent manner contributing to both endothelial permeability and proliferation control in response to VEGF. Deletion of occludin, including isoform 4, leads to embryonic lethality, demonstrating the importance of the occludin carboxy terminus.

Eleanor Mills
Undergraduate Student

Understanding genetic contributors of microcephaly: a polycomb perspective

Eleanor Mills, Charles Ryan

Congenital microcephaly is a cause of shortened lifespan and significant morbidity in young children. The increasing accessibility of whole exome sequencing has brought to light pathogenic variants in polycomb group proteins that underlie defects in neural development, such as mutations in the paralogues RING1 and RNF2, both of which facilitate the monoubiquitination of histone 2A (H2AUb1) in Polycomb Repressive Complex 1 (PRC1). Despite the identification of genetic contributors, mechanistic understanding of how these variants lead to disease remains poor. PRC1-dependent H2AUb1 is thought to play a significant role in DNA damage repair. To investigate whether disruption of this PRC1 function contributes to human microcephaly, we assessed the effect of pathogenic variants in RING1 and RNF2 on DNA damage repair in human neural progenitor cells (NPCs). We found that NPCs harboring RING1 and RNF2 pathogenic variants displayed delayed double strand DNA break repair. In light of this, we propose that RING1 and RNF2 play key roles in ubiquitination and DNA damage repair during neurogenesis, a capacity that is necessary for the production of a normal sized brain.

Eric Ji
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A genome wide screen for modifiers of CGG repeat-associated neurodegeneration in Drosophila

Eric Ji, Evrim Yildirim, Peter Todd

Fragile X-associated tremor/ataxia syndrome (FXTAS) is a neurodegenerative disease caused by a transcribed CGG repeat expansion in the 5'UTR of FMR1 gene. CGG repeats induce neurodegeneration as RNA by interacting with and sequestering RNA binding proteins while also triggering repeat-associated non-AUG initiated (RAN) translation of toxic peptides. To better understand how these repeats induce neurodegeneration, we used a Drosophila Melanogaster model of FXTAS where we expressed CGG repeats in master pacemaker neurons. PDF cell expression triggered accumulation of RAN translated peptides in these neurons and age-dependent loss of circadian rhythmicity. We took advantage of the extensive genetic variance among the fully sequenced inbred lines of Drosophila Genetic Reference Panel (DGRP) to identify potential modifiers of CGG repeat toxicity. Expression of CGG repeats in pacemakers of DGRP lines decreased the circadian rhythm strength with different extent in each line. We are conducting a genome wide association study (GWAS) to identify genes responsible for the differential response to the challenge with CGG repeats. Future research will assess specific genes identified through this GWAS approach to better understand the mechanism of disease pathology as well as potential therapeutic approaches for FXTAS.

Sumegha Ponnaluri
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Effect of Inactivation of Prefrontal Cortex on Sleep-Wake States in Rat

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There is a large body of literature to support subcortical regulation of sleep-wake states, but there have been limited studies to investigate the role of cortex in sleep-wake regulation. Previously, we have demonstrated that cholinergic stimulation of prefrontal cortex can reverse general anesthesia and promote wakefulness in unanesthetized rats, and there is emerging evidence to suggest that the prefrontal cortex might be a critical node in arousal state control. To better understand the direct role of prefrontal cortex in the regulation of sleep-wake states, we quantified the effect of prefrontal cortex inactivation, via local tetrodotoxin (156 μM) infusion, on sleep architecture. Under surgical isoflurane anesthesia, adult Sprague Dawley rats (n=7 male) were instrumented with electrodes to record electroencephalogram (EEG) from frontal and parietal cortex, and electromyogram (EMG) from dorsal nuchal muscles. In addition, a bilateral guide cannula was implanted aimed at the medial prefrontal cortex for tetrodotoxin infusion. After at least a week of post-surgical recovery and conditioning to the recording chambers, rats received bilateral microinjection (500 nL) of either 156 μM tetrodotoxin or 0.9% saline (vehicle control). The tetrodotoxin and 0.9% saline infusions were performed 30-minutes before the start of lights-OFF period (8:00 pm) after which EEG and EMG data were recorded for 24h across dark and light cycles. The EEG and EMG data were manually scored (SleepSign, Kissei Comtec Inc.) in 4-second intervals into wakefulness, slow-wave sleep, and rapid eye movement sleep, and then averaged in 3h bins across 24h recording period. A linear mixed model was used to compare the changes in percent time spent in each state, mean duration per episode for each state, and mean number of episodes for each state, between the vehicle control and tetrodotoxin-infusion sessions. Inactivation of prefrontal cortex via tetrodotoxin infusion produced long lasting statistically significant increase in slow-wave sleep ($p<0.05$ for 0-3h, 4-6h, 7-9h, 10-12h, 13-15, 16-18, 22-24) and decrease in wakefulness ($p<0.05$ for 0-3h, 4-6h, 10-12h) and rapid eye movement sleep ($p<0.05$ for 7-9h, 10-12h, 13-15h). These data further support a causal contribution of prefrontal cortex in regulating arousal states.

Valentina Knapp
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The role of extended amygdala CRF in incentive motivation and addiction

Valentina Knapp, Katie Emery, Lucas Tittle, Kent Berridge

Corticotropin-releasing factor (CRF) neurons are traditionally assumed to generate aversive stress states (George et al., 2012). However, other evidence shows that CRF neurons in nucleus accumbens (NAc) can generate positively-valenced incentive motivation to pursue and consume rewards (Lemos et al., 2012; Peciña et al., 2006). For example, optogenetic laser stimulation of CRF neurons in the NAc of crh-Cre rats intensifies and focuses pursuit of a laser-paired sucrose or cocaine reward over an equal reward without laser stimulation, and also supports laser self-stimulation of CRF neurons indicating positive valence of CRF neuronal excitation in NAc (Baumgartner et al., 2021, 2022). However, several major issues remain. First, CRF neurons co-release other neurotransmitters. Thus, it is unknown whether CRF itself versus other neurotransmitters mediate the positively-valenced motivation. To specifically test the role of CRF peptide, we administered i.c.v. microinjections of a nonspecific CRF antagonist or of vehicle prior to NAc laser self-stimulation by crh-Cre rats, or prior to 2-choice tasks in which rats could choose to earn either laser-paired sucrose reward or identical sucrose reward without laser. Preliminary results suggest that CRF receptor blockade reduces incentive motivation effects from optogenetic stimulation of CRF neurons in NAc, indicating a positively-valenced role for CRF neurotransmitter. A major issue remaining is that the motivational valence effects of CRF in extended amygdala have been posited to become more negative after chronic drug exposure, growing as an aversive b-process to produce withdrawal, distress, and relapse in addiction (Koob, 2010). A potential CRF switch in motivational valence from positive to negative is consistent with reports that severe stress experiences reverse the motivational valence of CRF signaling in NAc (Lemos et al., 2012). This work would clarify the multiple roles for CRF neurons in NAc in driving motivation that could contribute to addictive relapse and drug seeking.

Raymond Li
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Different outcome values influence the vigor of sign-tracking behavior

Raymond Li, Stephanie S. Desrochers, Shelly B. Flagel

In our day-to-day lives, cues in the environment guide appropriate behavior. Cues such as fast-food signs alert us to the availability of tasty food; when we see them, we think of that food and stop to eat. Sometimes, however, cues can gain inordinate control over an individual's actions, leading to maladaptive behavior. During Pavlovian conditioning, some rats exhibit an approach response that includes orienting and interaction directed towards a reward-predictive cue, a phenomenon known as "sign-tracking". Rats that exhibit this behavior, known as "sign-trackers", attribute incentive motivational value to reward-associated cues and have a greater propensity for impulsive behavior, attentional deficits, and cue-induced drug-seeking behavior. However, it is not known if different outcome values affect the vigor of sign-tracking behavior. For example, would you approach a shop sign for a higher value food (like ice-cream) faster with more excitement than one for a lower value food (like salad)? Here, we will explore how the value of reward associated with a cue affects the degree of responding. Specifically, a standard Pavlovian conditioned approach procedure consists of trials in which a lever-cue is presented for 8 seconds, and, upon its retraction, food reward is delivered in an adjacent food cup. In this experiment, two lever-cues are randomly presented one at a time with the lever-cue on one side of the chamber predicting a large reward (3 pellets) and the lever-cue on the other side predicting a smaller reward (1 pellet). We expect sign-trackers to exhibit more interaction with the lever-cue associated with the large reward compared to that associated with the smaller reward. This study will offer insights into identifying additional characteristics of sign-tracking behavior and guide future work in understanding how cues associated reward affect motivated behaviors relevant to psychiatric disorders such as addiction.

Sian Tian
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Synaptic Loss in the Inner Plexiform Layer of the Retina in a Preclinical Mouse Model of Multiple Sclerosis

Sian Tian, Gabrielle Mey, Jackson McGrath, Sebastian Werneburg

There is an unmet clinical need for understanding the cellular and molecular mechanisms driving the progressive neurodegeneration and disruption of brain networks that result in permanent disability in Multiple Sclerosis (MS) patients. Using the retinogeniculate system, a visual circuit frequently affected in MS, the Werneburg lab's previous work identified microglia-mediated retinal ganglion cell (RGC) synapse elimination in the lateral geniculate nucleus (LGN). Notably, this synapse loss occurred before other hallmark pathologies, disrupted circuit connectivity, and interfered with proper visual function. More recently, we also identified thinning of the inner plexiform layer (IPL), a synapse-dense region in the retina that can be clinically monitored by non-invasive optical coherence tomography imaging. This thinning occurred before patients with a relapsing-remitting MS transitioned to a secondary progressive disease course suggesting that tracking synaptic changes could be a useful diagnostic marker that precedes disability worsening. Although clinically relevant, the mechanism underlying IPL thinning and the loss of synapses remains unknown. Thus, our ongoing work focuses on assessing potential causes underlying synapse loss in the retina, including microglial elimination of synaptic terminals, as well as neuronal cell death and axonal degeneration. This work will better describe synaptic alterations not only within the IPL, but also within the visual system as a whole, and provide valuable insights into the role of synaptic dysfunction in the progressive disassembly of neuronal circuits that contribute to functional decline in MS.

John Shultz
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Degenerative Pathologies in the Superior Colliculus in the Preclinical Multiple Sclerosis Animal Model of Cuprizone-Induced Demyelination

John Shultz, Gabrielle Mey, Jackson McGrath, Sebastian Werneburg

Multiple Sclerosis (MS) is an inflammatory demyelinating disease that is characterized by progressive neurodegeneration and the disassembly of neural circuits. Similar to other neurodegenerative diseases, degeneration in MS has unclear etiology but results in permanent disability in patients. Previous research has been largely devoted to studying de- and remyelination, as well as axon degeneration and neuronal cell death. More recent work from our lab identified retinal ganglion cell (RGC) synapse loss and disruption of synaptic circuits through engulfment by reactive microglia in the lateral geniculate nucleus (LGN). Early on, this synaptic elimination caused functional visual impairments independent of other hallmark pathologies in multiple MS-relevant animal models. Although vision impairment is highly prevalent in MS patients, if and how synapses are altered in other parts of the visual pathway remain unclear. Here, we use functional approaches, such as photoacoustic imaging, that allow for the simultaneous assessment of multiple brain regions within the visual pathway, and combine these with postmortem tissue analysis to determine the extent and severity of key pathologies across the visual system. Thus, our ongoing work focuses on the comparative assessment of myelin, axon, neuron, and synapse degeneration as well as local neuroinflammation across different visual areas including the superior colliculus, the LGN, and the visual cortex in an MS-relevant preclinical mouse model. Assessing these pathologies across the visual pathway will not only better describe the extent of MS-relevant key pathologies, but may also provide new insights into factors contributing to the vulnerability or resistance of neural circuits in this devastating neurological disorder