2019 MiSfN Annual Meeting Abstracts

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2. Then last name

Abstract order

Last name	Theme (SFN criteria)
Barea	Cognition
Burson	Cognition
Hardy	Cognition
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Pruitt	Cognition
Sadik	Cognition
Waara	Cognition
Babcock	Development
Blinkiewicz	Development
Bonefas	Development
Doherty	Development
Ketchum	Development
Medendorp	Development
Morgan	Development
Nelson	Development
Paulisin	Development
Phaneuf	Development
Sheltz-Kempf	Development
Stoner	Development
Timmerman	Development
Beekly	Integrative Physiology and Behavior; Motivation and Emotion
Bosse	Integrative Physiology and Behavior; Motivation and Emotion
Burroughs	Integrative Physiology and Behavior; Motivation and Emotion
Cargile	Integrative Physiology and Behavior; Motivation and Emotion
Chinnusamy	Integrative Physiology and Behavior; Motivation and Emotion
Davidson	Integrative Physiology and Behavior; Motivation and Emotion
Doyle	Integrative Physiology and Behavior; Motivation and Emotion
Durga	Integrative Physiology and Behavior; Motivation and Emotion
Durrett	Integrative Physiology and Behavior; Motivation and Emotion
Fry	Integrative Physiology and Behavior; Motivation and Emotion

Last name	Theme (SFN criteria)
Gheidi	Integrative Physiology and Behavior; Motivation and Emotion
Grasser	Integrative Physiology and Behavior; Motivation and Emotion
Karavidha	Integrative Physiology and Behavior; Motivation and Emotion
Kohler	Integrative Physiology and Behavior; Motivation and Emotion
Manning	Integrative Physiology and Behavior; Motivation and Emotion
McLocklin	Integrative Physiology and Behavior; Motivation and Emotion
Parikh	Integrative Physiology and Behavior; Motivation and Emotion
Pence	Integrative Physiology and Behavior; Motivation and Emotion
Rajeshkumar	Integrative Physiology and Behavior; Motivation and Emotion
Raycraft	Integrative Physiology and Behavior; Motivation and Emotion
Reppucci	Integrative Physiology and Behavior; Motivation and Emotion
Risca	Integrative Physiology and Behavior; Motivation and Emotion
Rodriguez	Integrative Physiology and Behavior; Motivation and Emotion
Showers	Integrative Physiology and Behavior; Motivation and Emotion
Stark	Integrative Physiology and Behavior; Motivation and Emotion
Thomas	Integrative Physiology and Behavior; Motivation and Emotion
Thompson	Integrative Physiology and Behavior; Motivation and Emotion
Tyan	Integrative Physiology and Behavior; Motivation and Emotion
White	Integrative Physiology and Behavior; Motivation and Emotion
Almeida Alves	Neural Excitability, Synapses, and Glia
Caballero-Floran	Neural Excitability, Synapses, and Glia
Catalfano	Neural Excitability, Synapses, and Glia
Chaby	Neural Excitability, Synapses, and Glia
Chen	Neural Excitability, Synapses, and Glia
France	Neural Excitability, Synapses, and Glia
Garay	Neural Excitability, Synapses, and Glia
Gregory	Neural Excitability, Synapses, and Glia
Hughes	Neural Excitability, Synapses, and Glia
Nelson	Neural Excitability, Synapses, and Glia
Scheib	Neural Excitability, Synapses, and Glia
Stanchfield	Neural Excitability, Synapses, and Glia
Tsukahara	Neural Excitability, Synapses, and Glia

Last name	Theme (SFN criteria)
Var	Neural Excitability, Synapses, and Glia
Ali	Neurodegenerative Disorders and Injury
Anderson	Neurodegenerative Disorders and Injury
Calvo-Ochoa	Neurodegenerative Disorders and Injury
Combs	Neurodegenerative Disorders and Injury
Eppler	Neurodegenerative Disorders and Injury
Gall	Neurodegenerative Disorders and Injury
Gezer	Neurodegenerative Disorders and Injury
Jaster	Neurodegenerative Disorders and Injury
Kendziorski	Neurodegenerative Disorders and Injury
Kochmanski	Neurodegenerative Disorders and Injury
Koneru	Neurodegenerative Disorders and Injury
Lynch	Neurodegenerative Disorders and Injury
Mecklenburg	Neurodegenerative Disorders and Injury
Miller	Neurodegenerative Disorders and Injury
Munro	Neurodegenerative Disorders and Injury
Nath	Neurodegenerative Disorders and Injury
Paris	Neurodegenerative Disorders and Injury
Phadte	Neurodegenerative Disorders and Injury
Ray	Neurodegenerative Disorders and Injury
Sluzala	Neurodegenerative Disorders and Injury
Stoll	Neurodegenerative Disorders and Injury
Suresh	Neurodegenerative Disorders and Injury
Tien	Neurodegenerative Disorders and Injury
Webster	Neurodegenerative Disorders and Injury
Wright	Neurodegenerative Disorders and Injury
Bonekamp	Sensory and Motor Systems
Cintrón-Colón	Sensory and Motor Systems
Edwards	Sensory and Motor Systems
Groves	Sensory and Motor Systems
Harding	Sensory and Motor Systems
Maser	Sensory and Motor Systems

Last name	Theme (SFN criteria)
Pardo-Garcia	Sensory and Motor Systems
Railing	Sensory and Motor Systems
Shafau	Sensory and Motor Systems
Spivey	Sensory and Motor Systems
Watral	Sensory and Motor Systems
Crespo	Techniques
Matchynski	Techniques
Pal	Techniques
Schumaker	Techniques
Srinageshwar	Techniques
Woznicki	Techniques

Cognition

Title: Associations between white matter microstructure and verbal learning retest effects differ in normal aging, MCI and Alzheimer's disease

Authors: Mikayla Barea & Andrew R. Bender
Institution: Michigan State University, College of Human Medicine

Compromised verbal learning is an established marker of decline in mild cognitive impairment (MCI) and Alzheimer's disease (AD). Applying novel mathematical models for estimating verbal learning curves provides additional potential markers of disease incidence or progression. In addition, AD is associated with changes in white matter (WM) microstructure which may be linked with learning decrements. We sought to investigate associations between novel learning parameters and WM diffusion properties in older adults diagnosed with MCI and AD and in normal controls.

The sample included 126 participants (39.68% female; mean age at screening=72.93; SD=7.19 years) from Alzheimer's Disease Neuroimaging Initiative (ADNI) GO or ADNI 2 study data. All participants had baseline diffusion tensor imaging (DTI) data, and Rey auditory verbal learning test (RAVLT) data at baseline and 12-month follow-up when administration of the RAVLT baseline list was repeated. Participant categories were based on diagnosis at 24-month follow-up: cognitively normal (CN; n=41), MCI (n=60), and AD (n=25). Applying a mathematical model to the five RAVLT learning trials produced measures of initial level, rate of learning, and peak performance, separately for both occasions. We calculated residualized gain scores to estimate 12-month change for each measure. We evaluated bootstrapped zero-order correlations between baseline DTI measures of fractional anisotropy (FA) and mean diffusivity (MD) and the three learning parameters and their gain scores, separately by diagnosis.

Results showed associations between WM and learning parameters differ by diagnosis. Fornix FA was associated with concurrent initial level in CN, but with 12-month initial level in MCI and with peak performance at baseline in AD participants. Retest benefits for CN and MCI were greater than for AD patients. Although AD patients did not initially benefit from prior exposure to test materials, learning following a 1-year delay was still manifest in higher peak performance. In addition, lower MD in uncinate fasciculus and parahippocampal cingulum bundle was associated with greater longitudinal improvement in initial level, independent of disease status.

In conclusion, relating DTI parameters of WM with measures of learning within and between occasions of measurement reveals different WM correlates of learning at different levels across the Alzheimer's disease spectrum. In particular, relationships are noted at the fornix, uncinate fasciculus, and cingulum, highlighting their potential as targets of intervention and as markers of decline.

Effects of repeated variable stress in adolescence on adult response to traumatic stress and brain catecholamines in rats

Nicole Burson[1], Lauren E. Chaby[1], Nareen Sadik[1], Israel Liberzon[2], Shane A. Perrine[1]

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[2]Texas A&M College of Medicine, Department of Psychiatry

Adolescent stress exposure can have lasting effects on vulnerability to adverse effects of traumatic stress in adulthood. This study investigated how repeated variable stress in adolescence shapes effects of trauma-like stress in adulthood on cognition and catecholamine levels in brain regions mediating fear learning. To do this, male Sprague Dawley rats were exposed to repeated variable stress in mid-adolescence and then single-prolonged stress (SPS) in early adulthood, followed by fear learning, extinction, and extinction retention testing. A control group was not exposed to stress and went through fear learning testing alone. In brain regions mediating fear learning, that were collected immediately after fear extinction-retention testing, we determined norepinephrine, serotonin, and dopamine concentrations. We found that in the prelimbic cortex, exposure to the combination of adolescent-chronic and adult-traumatic stress increased levels of norepinephrine and serotonin, but adult-traumatic stress alone did not affect either catecholamine. In the striatum, adult-traumatic stress elevated norepinephrine, but this effect was reversed by exposure to repeated variable stress in mid-adolescence. In the hippocampus, exposure to adult-traumatic stress decreased norepinephrine levels. Exposure to repeated variable stress in adolescence buffered adverse cognitive effects of traumatic stress. Adult catecholamine levels in brain regions mediating this cognitive effect were also shaped by adolescent-stress exposure, suggesting that stress exposure in adolescence has diffuse, region-specific effects on responsivity to traumatic stress in adulthood.

Martial arts based meditative techniques reduce cortico-limbic response to distress in children with cancer

Natalie Hardy, Allesandra Iadipaolo, Cindy Cohen, Elimelech Goldberg, Jeffrey Taub, Felicity Harper, Kristopher Dulay, Rebecca Cramer, Autumm Heeter, Shelley Paulisin, Martin H. Bluth, Craig Peters, Farrah Elrahal, Christine Rabinak, Hilary A. Marusak

Background

Neuroimaging studies in adults demonstrate that meditation modulates activity in cortico-limbic regions associated with pain, emotion reactivity, and arousal. Although recent studies indicate that meditation is also effective for reducing pain and emotional distress in children and adolescents, no studies have examined the functional neural correlates of meditation in a pediatric sample. To address this, we used functional magnetic resonance imaging (fMRI) to examine the effects of martial arts-based (MAB) meditative and non-meditative emotion regulation techniques on cortico-limbic response in a sample of childhood cancer patients and survivors, who experience treatment-related pain and distress.

Methods

10 youth (ages 9-17, 5 females) completed a fMRI task that involved viewing distress-inducing videoclips (e.g., a child fighting with a parent or receiving an injection). Prior to each clip, participants received MAB meditative instructions (e.g., “focus on your breath”), non-meditative instructions (“count backwards from ten”) or were instructed to passively view the clip. Participants rated their distress after each clip using a Likert scale, and distress and cortico-limbic activity were compared between instructions.

Results

There were no significant differences in distress ratings between instructions (p’s>0.05). There were significant effects on cortico-limbic activity such that MAB meditative relative to non-meditative instructions were associated with lower activity in regions associated with pain and emotional arousal (i.e., insula, anterior cingulate, $pFWE$<0.05).

Conclusions

While non-meditative techniques (e.g., distraction) are commonly used in pediatric patients to alleviate pain and distress, our results suggest MAB meditative techniques may have additional benefit for modulating neural mechanisms underlying pain and distress (e.g., cortico-limbic regions).

Evaluation of a martial-arts meditative intervention for high-risk schoolchildren: use of an objective marker of anxiety risk

Aneesh Hehr[1], Amanpreet Bhogal[1], Sean Minton[2], Charis Wiltshire[2], Sterling Winters[2], Cassandra Wanna[2], Anais Stenson[2], Tanja Jovanovic[2], Christine Rabinak[1], Allesandra Iadipaolo[1], Natalie Hardy[1], Shelley Paulisin[1], Autumm Heeter[1], Cindy Cohen[3], Jamila Carrington Smith[3], Marc Cohen[3], Peter Davenport[3], Michael Hunt[3], Richard Plowden[3], Elimelech Goldberg[3], Naami Kosofsky[4], **Hilary A. Marusak[1]**

[1]Department of Pharmacy, [2]Department of Psychiatry and Behavioral Neurosciences, [3]Kids Kicking Cancer, [4]Wurzweiler School of Social Work

Background:
Relative to their more affluent counterparts, lower income children are at higher risk of anxiety and associated negative outcomes (e.g., educational underperformance, social isolation). We are conducting a study to test whether a martial-arts based meditative intervention can reduce stress and anxiety in a predominantly low-income sample of schoolchildren. Here, we aim to establish an objective marker of anxiety that can be used to evaluate the effectiveness of this intervention. In particular, we will examine fear-potentiated startle (FPS), a widely used physiological marker of risk for anxiety, at a baseline (pre-intervention) visit.

Methods:
N=21 3rd and 4th grade students (8-10 years, 9 female) completed a differential fear conditioning paradigm, wherein one conditioned stimulus (CS+) was paired with an aversive airblast to the larynx. A second stimulus (CS-) was never paired with the aversive outcome. FPS was measured using the eye-blink component of the acoustic startle response. In particular, we recorded electromyogram activity of the right *orbicularis oculi* muscle in response to a startle probe (106 dB white noise burst).

Results:
We expect no difference in baseline FPS in children receiving the martial-arts intervention vs. a control intervention. Following the intervention, we expect that children receiving the martial-arts (but not the control) intervention will show a decrease in FPS. Exploratory analyses will examine effects of age, sex, and baseline anxiety on FPS.

Conclusion:
Determining the effects of a martial arts-based intervention on objective markers of anxiety may provide new insights into approaches for reducing anxiety and stress in high-risk children.

Funding Source: Kids Kicking Cancer, D. Dan and Betty Kahn Foundation

Title: NEUROIMMUNE ACTIVATION HAS SEX-SPECIFIC EFFECTS ON LEARNING AND MEMORY CONSOLIDATION PROCESSES IN MICE

Author List: Posillico, C. K., M.S., Garcia-Hernandez, R.E., B.S., Tronson, N. C., Ph.D.

Institution: Department of Psychology, University of Michigan, Ann Arbor, MI, 48109

Abstract: Activation of the neuroimmune system has been implicated in disorders of memory such as post-traumatic stress disorder and Alzheimer's disease. Neuroimmune signaling may also contribute to sex- and gender-differences in the prevalence of these disorders, in which women have more than 2-fold higher prevalence than men. Our lab has previously shown sex differences in both the pattern and time course of central cytokine induction following acute peripheral immune stimulation as well as sex differences in memory deficits long after a subchronic immune challenge. To determine the impact of acute immune activation state on hippocampal-dependent memory tasks in both male and female mice we used intracerebroventricular (ICV) administration of a viral mimic, polyinosinic:polycytidylic acid (poly I:C) and (A) identified the neuroimmune response in both sexes, and (B) determined the impact of neuroimmune activation on learning and memory in both sexes. Both males and females showed a significant immune response to poly I:C (20ug) compared to saline-treated controls. Poly I:C treatment significantly increased levels of hippocampal cytokines, including IL-1β, IL-6, CXCL10, and CCL2, between 30 minutes and 24 hours in both sexes, albeit with sex differences in the precise patterns and timing of activation. To determine the effects of central administration of poly I:C treatment on learning and on memory consolidation, mice were treated with poly I:C either 4 hours prior to contextual fear conditioning or immediately after training. Poly I:C before training caused learning deficits in female, but not male mice. In contrast, males but not females showed specific impairments of consolidation. These differences in the learning and memory processes affected may be due to sex differences in patterns or time course of neuroimmune activation, or to differences in interactions with memory mechanisms. To determine how neuroimmune activation changes the cellular and circuit level activation induced by context fear conditioning, we measured cFos activation in the hippocampus following poly I:C administration and context fear conditioning. As expected, training on context fear conditioning increased cFos+ cells. Surprisingly, poly I:C administration enhanced this effect, and females exposed to both context fear conditioning and poly I:C showed significantly higher levels of cFos within the hippocampus, despite decreased fear conditioning. Collectively, these findings demonstrate that males and females are both vulnerable to disruption of memory by poly I:C, although these effects may be via different mechanisms in males and females.

THE RELATIONSHIP BETWEEN EMPATHY, OPTIMISM, AND STRESS AFTER COMPETITION, Pruitt, R., Robinson, T., Howard, E., Everett, D., Durrett, K., Jositas, L., Cole, D., Kaganac, H., Matchynski-Franks, J.J., Rochester University, Rochester Hills, MI

Abstract:

The present study provides new insight into understanding the effects of optimism on reactions to winning or losing a competition. Optimistic explanatory style (OptES) is defined by Seligman as explaining good events as permanent, pervasive and personal, while explaining bad events as temporary, specific, and external. OptES has been associated with higher academic and athletic success, and reduced risk of stress and illness. Empathy has been related to general optimism, but not specifically to OptES. We hypothesized that more OptES individuals would be less affected by losing a game, and predict higher than actual performance in a game. We also hypothesized they would have higher empathy, health, and academic and athletic achievement-perception, and lower reported stress. Student athletes and non-athletes competed in a boat-making competition for a $20.00 prize. After completing the boat game, they were asked to predict how well they did compared to others. Based on their performance, they were divided into groups and told they were the current winner, in the bottom 20%, or had the worst score. Physiological stress measures were given before the competition, after the competition, and after they were told their ranking. Participants also were given Toronto empathy scale, Seligman's optimism scale, stress and health questionnaires and a demographic form including questions on academic and sport-related achievements. OptES did not predict game perception, life-stress, empathy, health, academic or sport-related achievement, as hypothesized. However, OptES for good events did predict lower blood pressure after the game partially supporting the hypothesis. Additional unexpected findings suggest empathy is predictive of increased life-stress and illness. Overall these findings suggest that more research is needed to understand if OptES is as predictive of psychological health, and academic and athletic success as previously reported.

Effects of a class IIa histone deacetylase inhibitor on locomotor activity and anxiety-like behavior in male and female rats

Nareen Sadik, Lauren E. Chaby PhD, Nicole Burson, Arman Harutyunyan, Ashley Wheeler, Pearl James, Shane A. Perrine PhD

Department of Psychiatry and Behavioral Neuroscience, Wayne State University School of Medicine

Histone deacetylases (HDACs) are a group of enzymes that remove acetyl groups from histones to condense DNA and regulate gene expression, thereby shaping behavior and cognition. A subclass of HDACs, class IIa, mediate fear behavior and lasting cognitive change. Here, we tested the role of class IIa HDACs in locomotion and anxiety like-behavior, using a pharmacological class IIa HDAC inhibitor, MC-1568, in adult male and female Sprague-Dawley rats. Rats were injected with a single intraperitoneal injection of MC-1568 at different doses, 0.5 or 5 mg/kg or the drug vehicle solution as a control. After a delay to allow for changes in class IIa HDAC levels, behaviors were assessed with open field and novel object tests. We found that females had greater locomotor behavior than males, but class IIa HDAC inhibition did not affect locomotor activity in either sex in the open field assay. However, class IIa HDAC inhibition increased the amount of time spent in the center of the open field arena, suggesting it reduced anxiety-like behavior. Class IIa HDAC inhibition also reduced the latency to approach an object in the novel object test in both males and females. These results suggest that reduced class IIa HDAC activity decreases anxiety-like behavior independent of sex. Overall, our results support the role of class IIa HDACs as key epigenetic regulators of behavior, and emphasize the importance of future exploration of class IIa HDACs as regulators of anxiety.

The Impact of Aging and Executive Control on the Ability to Make Corrective Actions for Obstacle Collision Avoidance

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[1]Department of Kinesiology & Integrative Physiology; [2]Department of Cognitive & Learning Sciences, Michigan Technological University

The objective of this research is to identify the neurocognitive mechanisms that contribute to our ability to make rapid evasive actions in response to sensory feedback during an ongoing movement, and to establish how these mechanisms are impacted by aging. Rapid motor corrections allow us to make evasive actions to avoid knocking over objects in a cluttered workspace, and navigate around other people in a crowded room. In the current study, we used a robotic device (Kinarm, BKin Technologies) to apply unpredictable visual "cursor shifts" while participants reached for visual targets and tried to avoid visible haptic obstacles. The obstacles were positioned to the right and left of a straight hand path from the start position to the target, and upon contact with the participant's cursor a repulsive force was applied by the robot to simulate a collision with a real obstacle. On each trial the cursor briefly disappeared behind a rectangular occluder positioned in front of the start position, and emerged either unperturbed, or shifted by a small, medium, or large distance to the left or right of a straight line to the target. The medium-shift trials placed the cursor in a collision course with one of the obstacles and required a rapid movement correction to avoid the obstacles. For the no-shift and small-shift trials, and the large-shift trials a movement correction was not necessary to guide the cursor between or around the outside of the obstacles, respectively. We recruited 18 younger adults (M = 20.4, SD = 1.5 years old) and 18 older adults (M = 72.4, SD = 5.5 years old) to test the prediction that older adults would perform less optimal corrections than younger adults. Overall, older adults collided with the obstacles more frequently than younger adults, especially in the large jump condition. Additionally, when successfully navigating around or between the obstacles, movement time was generally slower in the older compared to younger adults, especially in the large jump condition. We also administered a battery of perceptuomotor, processing speed, and executive control tasks to identify the factors that best predict impairments in performance on the obstacle avoidance task in later adulthood. We focused our multiple regression analyses on the behavior during the large jump condition, which is unique in requiring participants to switch hand paths around the obstacles to be most efficient. The results revealed that for older adults processing speed and executive control were significant predictors of the frequency of obstacle collisions, and when successfully navigating around the obstacles, movement time was predicted by executive functioning in the older group. The results of this study add to a growing literature examining the impact of aging, and age-related cognitive decline, on adaptive motor behavior. The data also contribute to our understanding of the nature of cognitive contributions to rapid feedback control.

Development

Authors: Katelyn Babcock, Dominique Gutierrez, Jesse Flath, and Shasta Sabo

Effects of ASD-Associated Mutation in Dendritic Spine Development

Autism Spectrum Disorders (ASD) affect millions of individuals and are characterized by social deficits, repetitive movements, and restricted interests. Several *de novo* mutations have been identified through whole exome sequencing and are implicated as causative for those individuals. One such mutation is a single nucleotide substitution (IVS9-2A>G, CCD8662.1) at the canonical 3' splice site of exon 10 within the GRIN2B gene. We predict that this GRIN2B mutation produces an early stop codon, and the resulting GluN2B protein becomes truncated. Our previous research has indicated that the truncated mutant protein is translated and can co-assemble with wildtype GluN1 and GluN2B subunits. *In vitro* studies from our lab have shown abnormal dendrite development and extreme morphological changes due to the ASD-associated mutation. The focus of this project is to understand whether this change in dendrite length is accompanied by a concomitant change in dendritic spines. Cortical neurons from Sprague-Dawley rats were cultured (P0-1) and transfected (DIV2) with plasmids encoding GFP-tagged wild-type or mutant GluN2B and a tdTomato fill. Immunocytochemistry was performed to visualize dendritic spines, and neurons were then imaged using confocal microscopy. We find that dendritic spine density is unchanged in the mutant when compared to wild-type neurons. Consequently, the number of potential synapses should be reduced in mutant neurons when compared to wildtype neurons since mutant dendritic arbors have reduced overall dendrite length and complexity. Understanding the dysfunction behind *de novo* mutations associated with ASD is vital to understanding the many ways ASD can manifest itself and for discovering common underlying mechanisms of ASD symptoms.

Title: Determining the Function of *Gata3* in Differentiating Hair Cells

Authors: Paige Blinkiewicz and Dr. Jeremy Duncan

Mammalian HDR syndrome is characterized by hyperparathyroidism, deafness, and renal disease and is caused by *Gata3* haploinsufficiency. We have previously shown that early deletion of *Gata3* in the inner ear of mice results in abnormal development of inner ear neurosensory epithelia. However, *Gata3* expression begins in the otocyst and spans from early embryogenesis through adulthood. To better understand the function of *Gata3* at later postnatal time points we conditionally knocked out *Gata3* when hair cells are just starting to differentiate utilizing the *Atoh1-cre* mouse line, and shortly after differentiation of the inner hair cells utilizing the *Fgf8-cre* mouse line. Absence of *Gata3* at the time of hair cell differentiation results in a disorganized arrangement of hair cells, in the middle and apical regions while the base appears normal. While the hair cells appear disorganized, the stereocilia arrangement, organization of supporting cells, and organization of neurons appear normal. Knocking out *Gata3* later in hair cell differentiation results in a similar, but less severe pattern of hair cell disorganization.

Title: Uncovering the Temporal Functions of Histone Demethylase KDM5C in Neurodevelopment and Behavior

Authors: Bonefas, Katherine; Iwase, Shigeki

How does one's environment during development shape behavior in adult life? This is in part accomplished by chromatin modifiers, such as KDM5C, which can alter the structure of chromatin and influence gene expression. KDM5C is a lysine demethylase that removes di- or tri-methylation on Histone 3 Lysine 4. Humans with mutations in KDM5C present with heightened aggression, ASD, and intellectual disability. These phenotypes are recapitulated in *Kdm5c*-KO mice, however mice that lose KDM5C in the adult forebrain do not display these behaviors. This suggests loss of KDM5C during neurodevelopment is sufficient to produce long-lasting neurological consequences. To better understand the mechanism by which KDM5C impacts neurodevelopment, I will transiently alter KDM5C expression through the novel Shield1 system. When fused to a DD domain, KDM5C will be degraded unless in the presence of the small molecule Shield1. Contrastingly, KDM5C fused to a LID domain is stable and destabilized when bound to Shield1. By transiently applying Shield1 during early development in *Kdm5c*-DD or -LID mice, I will ultimately shed light upon the unique temporal relationship between chromatin modifiers and neuronal function.

INVESTIGATING THE ROLES OF ERBB4 DIRECT NUCLEAR SIGNALING IN BRAIN DEVELOPMENT USING NOVEL MOUSE MODELS

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ErbB4 is a receptor tyrosine kinase (RTK) that plays important roles in nervous system development and function. All RTKs signal through canonical phosphorylation-mediated downstream signaling cascades. However, alternative splicing generates an ErbB4 isoform that includes a cleavage site for tumor necrosis factor-α-converting enzyme (TACE). This isoform, called ErbB4-JMa, undergoes ligand-induced sequential cleavage by TACE and the presenilin/γ-secretase complex, releasing a soluble ErbB4 intracellular domain (E4ICD) that can translocate to the nucleus to regulate transcription directly. Although there is evidence that E4ICD direct nuclear signaling is important in several aspects of development and brain function, this has not been formally tested in vivo. To address this gap in knowledge, I have used CRISPR/Cas9 gene editing to create two novel mutant mouse lines in which E4ICD signaling is abolished. In one mouse line a point mutation in the TACE cleavage site renders ErbB4-JMa TACE-uncleavable (named ErbB4-TUC), and in the other a frame shift deletion in leads to nonsense-mediated degradation of ErbB4-JMa isoform specifically (named ErbB4-JMa$^{-/-}$). The novel mutant mice have been shown to wean viable offspring with normal size litters and have undetectable levels of E4ICD formation, indicating that ErbB4 cleavage has been abolished. Using these mice, I am investigating the specific roles of direct nuclear signaling by E4ICD in the intact organism. These studies will provide insights into the importance of direct nuclear signaling by ErbB4 in brain development and organogenesis.

Selective Elimination of *Neurod1* from Spiral Ganglion Neurons Reveals Importance in Axon Development and Projection

Hearing loss is the most common sensory disorder in society, yet current treatments are reliant on the preservation of the afferent neurons that project from the cochlea to the hindbrain. There is much to discover regarding development and projection of the afferent spiral ganglion neurons (SGNs) from the organ of Corti to the cochlear nucleus in the hindbrain. SGNs rely on the expression of different basic helix-loop-helix (bHLH) transcription factors for proper development, like *Neuronal differentiation 1* (*Neurod1*). In previous studies, *Neurod1* has been knocked out of the entire sensory portion of the inner ear, rather our study that selectively eliminates *Neurod1* from the SGNs. To assess whether the role of *Neurod1* in spiral ganglion formation is cell intrinsic or cell extrinsic, we have generated a unique self-terminating Neurod1-cre mouse line (*Neurod1-cre: Neurod1 f/+*) to selectively eliminate *Neurod1* from SGNs. Both peripheral and central projections of the neurons are currently being examined using immunohistochemistry and lipophilic dye tracing techniques. Our preliminary findings have shown a peripheral phenotype and aberrant central projections in mutants to suggest that *Neurod1* works in a cell-intrinsic manner to promote SGN development and projection to the cochlear nucleus.

Title: Cortical Hyperexcitation During Early Development Results in Autism Behavioral Phenotypes

Authors: Medendorp, W. E., Pal, A., Waddell, M.L., & Hochgeschwender, U.

Early development is marked by spontaneous neuronal activity that occurs without the input of sensory experience. This spontaneous activity has been demonstrated in select pathways to refine foundational neural circuits before sensory input. By manipulating this activity in genetically-targeted pyramidal neurons within the cortex, we can experimentally test its role in formation of specific neural circuits. Many psychiatric disorders are thought to be neurodevelopmental, stemming from malformation of neural circuits in early development. Autism disorders in particular have been associated with increased cortical excitation leading to a cortical imbalance of excitation to inhibition. Early disruptions to cortical activity may underlie the later manifestation of E/I imbalance resulting in behavioral changes typical of disorders such as autism. We report here on the first unbiased testing of the role of developmental over-excitation in altering adult behavior and circuit dynamics.

We took advantage of Bioluminescent Optogenetics (BL-OG), where light stimulation of an optogenetic element is achieved either through bioluminescence emitted from a tethered luciferase upon application of a chemical substrate or through application of physical light via fiber optics, for chemogenetic activation during development and for optogenetic interrogation in adult animals. Mice conditionally expressing LMO3, a fusion of sbGLuc and VChR1, were crossed with Emx1-Cre transgenic mice, thus limiting expression of LMO3 to cortical pyramidal neurons. By delivering CTZ intraperitoneally during post-natal days 4-14, over-excitation was induced in the cortical pyramidal neurons of developing mouse pups. During adulthood, mice were tested behaviorally, and assessed for electrophysiological changes in circuit dynamics. Behavioral phenotypes are consistent with autism spectrum disorders, including social deficits and repetitive behaviors. Optogenetic circuit interrogation demonstrates disrupted cortico-striatal circuitry, as well as altered E/I balance in the cortex. The results of this research directly implicate cortical over-excitation during early postnatal development to behavioral phenotypes characteristic of autism disorders.

PACAP Receptor Polymorphism Effects on Functional Organization of the Limbic System in Youth

Authors: Stephanie E. Morgan, Hilary A. Marusak, Farrah Elrahal, Craig Peters, Allesandra S. Iadipaolo, Kyle J. Burghardt, **Christine A. Rabinak**

Affiliations: Department of Pharmacy Practice, Wayne State University

Pituitary adenylate cyclase-activating polypeptide (PACAP) is a protein involved in the hypothalamic-pituitary-adrenal (HPA) axis. Previous studies in adults link genetic variation in the PACAP receptor, PAC1, to risk of stress-related disorders. The *PAC1* CC genotype is associated with reduced functional connectivity (FC) between limbic brain areas associated with HPA axis regulation (e.g., hippocampus, amygdala). Although stress-related disorders frequently begin in youth, no pediatric studies have tested for potential links between the *PAC1* polymorphism and variation in limbic-based functional organization.

Forty-five youth, ages 6-17 (26 females) participated in this functional magnetic resonance imaging study. DNA was extracted from saliva, and Taqman Genotyping was performed for rs2267735. Resting-state FC within and between amygdala and hippocampus were calculated and compared between gene groups (CC vs. G-alleles). Graph theory measures were calculated to compare functional segregation and integration of limbic-based connections, between groups.

No group differences in FC were observed. However, there were group differences in graph measures of limbic-based functional integration such that youth with the CC genotype demonstrated lower efficiency of the right hippocampus, compared to G-alleles. Additionally, there was a sex-by-genotype interaction such that females with the CC genotype showed lower efficiency of the right amygdala compared to CC males. Lower efficiency of the hippocampus may reflect poorer control over the HPA axis, and underlie risk for stress-related pathology. Sex-by-genotype effects may indicate an interaction between PACAP and sex hormones. Our results suggest that the *PAC1* polymorphism influences limbic-based brain organization earlier in life than previously demonstrated.

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Exploring the role of PARP1 in transcriptional regulation of neurodevelopment

Megan Nelson, Pablo Sardi, Gabriel Corfas

Cortical development is a highly ordered and temporally controlled process, and its disruption can result in psychiatric disorders, cognitive dysfunction, and neurodegenerative diseases. The precise timing of cortical development is regulated in part through the similarly precise timely transcription of key genes. Several transcriptional control mechanisms involve a protein called Poly (ADP-ribose) Polymerase 1 (PARP1). This enzyme uses NAD as a substrate to add ADP-ribose polymers (PAR) to itself, other proteins, and chromatin in a process known as PARylation. Interestingly, mutations in genes that effect PARylation are associated with cognitive dysfunction and neurodegeneration. Previous unpublished experiments from our lab also indicate that PARP1 has a role in regulation of astrogenesis during cortical development via ErbB4 nuclear signaling. Taken together, these studies suggest that PARP1 has a role in proper development and function of the brain, but the effects of PARP1 loss on neurodevelopment *in vivo* remain understudied.

Using a constitutive PARP1 knockout mouse model, I seek to understand the impact of PARP1 loss on gene expression, cell fate, proliferation, and patterning of the cerebral cortex. To define the impact of PARP1 in gene expression in the embryonic cortex, we performed RNAseq of E15.5 PARP1 knockout cortex. We found upregulation in several genes involved in neuronal migration (n=4 for wildtypes and KOs). Subsequent experiments verified changes in gene expression with RT-qPCR, including the Cajal-Retzius cell marker *Reln.* Mechanistically, we found that the enzymatic activity of PARP1 is crucial for repression of *Reln* expression *in vitro*. Surprisingly, we were unable to detect large differences in brain architecture in PARP1 knockout mice, including cell proliferation, neuronal migration, or cortical layering. Given our lab's data surrounding PARP1 interaction with the tyrosine kinase receptor ErbB4, which is known to repress astrogenesis through its ligand NRG1, we are investigating the role of PARP1 in repression of astrogenesis during cortical development. Preliminary experiments show that PARP1 knockdown impairs NRG1/ErbB4 mediated repression of *GFAP in vitro* and PARP1 knockout P0 cortex (n=11) overexpresses *GFAP* relative to wild-type controls (n=10). Together, these findings indicate PARP1 regulates transcription in the embryonic brain and the loss of its activity results in alterations in cell fate.

Failure to Extinguish Fear in Trauma-Exposed Children with a Common Variant in the Cannabinoid Receptor 1 Gene

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Background: Deficits in fear extinction are implicated in the pathophysiology of anxiety disorders such as posttraumatic stress disorder. Recent studies suggest that signaling via the cannabinoid receptor 1 (CB1R) is essential for fear extinction. A common variant in the gene encoding the CB1R (*CNR1*) has been linked to poor extinction learning in adults. However, anxiety disorders typically begin in childhood. The present study examines the effect of *CNR1* on fear extinction learning and its later recall in children.
Methods: 37 children (6-11 years) underwent a novel two-day fear extinction learning and memory recall experiment in virtual reality. Skin conductance responses (SCRs) were collected and genotyping was performed for *CNR1* (rs2180619). Of note, a subset of children reported previous exposure to trauma (e.g., violence, medical).
Results: Overall, there were no group differences in extinction learning or subsequent memory recall (p's > 0.05). However, within the trauma-exposed group, children with the AA genotype showed poorer extinction learning and extinction recall compared to C allele carriers, evidenced by higher SCRs to an extinguished cue ($p = 0.05$).
Conclusion: Genetic differences in the endocannabinoid system may contribute to a reduced ability of to extinguish learned fear among trauma-exposed children. Failure to extinguish may increase risk of anxiety.

Keywords (3): Fear conditioning, endocannabinoid, anxiety

Neural Correlates of Inhibitory Control in Adolescents with Symptoms of Food Addiction

Camille Phaneuf, Laura Cope, Ashley Gearhardt, Mary Heitzeg, Jillian Hardee

Introduction

Neural correlates of food addiction in adults have been found in studies using the Yale Food Addiction Scale (YFAS) in conjunction with fMRI, and inhibitory control has been used as a behavioral proxy in the addiction literature. However, research combining these methods to consider adolescent food addiction has yet to be conducted. This project aims to investigate the relationship between inhibitory control, addictive-like eating, and brain regions implicated in executive functioning in an adolescent population. It is predicted that adolescents demonstrating weaker inhibitory control will endorse food addiction symptomatology, as opposed to adolescents demonstrating stronger inhibitory control.

Methods

Seventy-six right-handed participants, aged 8.2 to 17.8 years, were recruited from the Michigan Longitudinal Study (MLS). Participants performed a go/no-go task during fMRI and completed the YFAS for Children (YFAS-C), after which they were categorized into two groups according to their YFAS-C scores. Individual analysis was completed using a general linear model; the main contrast of interest was correct no-go versus correct go trials, calculated for second-level group analysis.

Results

A two-sample *t*-test revealed significant group differences for CRvsGO ($p < 0.001$; uncorrected with a cluster-wise threshold of $p < 0.05$ FWE) in three primary clusters when comparing the Control and YFAS-C groups, all exclusively in the left hemisphere: the middle temporal gyrus/occipital gyrus, the precuneus/calcarine sulcus, and the inferior frontal gyrus. Specially, the YFAS-C group showed deactivation in these clusters.

Discussion

Differences in inhibitory control are apparent in food addicted adolescents, as determined by the YFAS-C and as visualized in the middle temporal gyrus, posterior cingulate, and inferior frontal gyrus. While these differences are perhaps due to task demands, developmental changes in inhibitory control or sustained attention circuitry have more explanatory power.

Keywords: Response inhibition; food addiction; development; adolescence; left middle temporal gyrus; left middle occipital gyrus; left precuneus; left calcarine sulcus; left posterior cingulate, left inferior frontal gyrus

Title: Characterizing the Over-expression of Gata3 in Differentiating Hair Cells
Authors: Sydney Sheltz-Kempf, Jeremy Duncan

Neurosensory hearing loss is one of the most common sensory disorders and is characterized by flat epithelia resulting from sensory cell death within the Organ of Corti. It has been previously shown that *Atoh1* is necessary for the differentiation and survival of hair cells, but its function is dependent on prior and continued expression of transcription factors such as *Eya1/Six1, Sox2* and *Gata3*. Of these genes mentioned, the function of *Gata3* is not well-understood due to its widespread expression in the otic placode that continues throughout the prosensory epithelia. Interestingly, *Gata3* expression is even located in differentiating hair cells. In order to further elucidate the role of *Gata3* in inner ear sensory development, we are overexpressing *Gata3* in differentiating hair cells using a murine Atoh1-cre line, and in inner hair cells only using a murine Fgf8-cre line. By comparing the cellular morphology and hair cell organization within the Organ of Corti between the controls with normal endogenous expression of *Gata3* to the mutants with an additional allele of *Gata3* expression at postnatal time points P0 and P7, we analyze the specific level of *Gata3* required for proper sensory development. While there does not appear to be a significant difference between controls and mutants, a previous study suggests that two alleles of *Gata3* need to be overexpressed in order to analyze a measurable phenotype.

SPIRAL GANGLION NEURONS RELY ON FZD3 FOR AXONAL PATHFINDING, Stoner, Z; Elliot, K; Ketchum, E; Duncan, J. Western Michigan University

Auditory perception is the result of complex and precise neuronal circuitry that allows us to perceive, process, and respond to external sound stimuli. Spiral ganglion neurons receive auditory information from tonotopically organized cochlear hair cells and transmit this information to precise locations within the cochlear nuclei of the hindbrain. The projection of sensory afferents to their peripheral (hair cell) and central (cochlear nuclei) targets is a highly orchestrated process that results in the generation of a tonotopic sensory map. Peripheral guidance mechanisms have been well researched; however, we have relatively little information on the molecular cues associated with central guidance. Previous work has indicated that diffusible factors likely play an important role in central pathfinding. *Frizzled3* (*Fzd3)* which is part of the Wnt/PCP signaling pathway is expressed in the organ of Corti, spiral ganglion neurons, and cochlear nuclei. Wnt ligands which are released from the hindbrain may be binding to *Fzd3* present on afferent neurons and acting as a molecular guidance cue. We demonstrate that there are disorganized central projections in *Frizzled3* (*Fzd3*) null mutants indicating that Wnt/PCP signaling is required for spiral ganglion neurons to make proper wiring decisions in the cochlear nuclei. Further we show that this central phenotype is due to *Fzd3* expression in a specific cell type.

EFFECTS OF NEONATAL PROCEDURAL PAIN AND MATERNAL ISOLATION ON CELL PROLIFERATION IN POSTNATAL RAT BRAINS, Timmerman, B, Mooney-Leber, S, Rana, F, Sustaita, E, Brummelte, S. Wayne State University Department of Psychology

Preterm birth accounted for almost 10% of all U.S. live births in 2016. Children born preterm often display impaired cognitive, behavioral, motor, and brain development compared to full-term peers. Prior studies have found a relationship between these alterations and the number of neonatal stressors that preterm infants experience. Utilizing a rodent model, our study investigated how stressors that preterm infants commonly experience in the Neonatal Intensive Care Unit (NICU), particularly procedural pain and reduced maternal care, may alter brain development.

Male and female rat pups were exposed to stressors in 4 sessions per day on postnatal days 1 through 4 (PD 1 – 4). Stress exposure was done via repeated needle pricks, maternal isolation, repeated needle pricks followed by maternal isolation, or neither (touch control group). Animals were sacrificed at PD8 and brain slices were processed via immunohistochemistry for Ki67, a marker of cell proliferation.

Preliminary results indicate that pups who received procedural pain and isolation had significantly lower cell proliferation in the dentate gyrus compared to pups who received just isolation. No significant differences between groups were found in the frontal cortex or medial prefrontal cortex, and no sex differences were observed. Our results indicate that neonatal pain and isolation may have a synergistic effect on cell proliferation in the dentate gyrus of 8-day-old rat pups. Future research will investigate additional markers of brain maturation as well as explore the consequences of these early life stressors for adult animals.

Integrative Physiology and Behavior; Motivation and Emotion

Role of Melanin-Concentrating Hormone Neurons in the Integration of Sleep and Reproductive Physiology. Beekly, B.; Vanini, G.; Elias, C. University of Michigan Department of Molecular and Integrative Physiology.

Episodic release of gonadotropin releasing hormone (GnRH) from the hypothalamus and consequent pulsatile release of luteinizing hormone (LH) from the pituitary are necessary for reproductive development and function. Data from human children shows that an increase in LH release during non-rapid eye movement (NREM) sleep precipitates puberty. Interestingly, in sexually mature men and women, LH release is *inhibited* by sleep. Despite the clear importance of the relationship between sleep and the HPG axis, the neuronal circuits linking sleep and gonadotropin secretion are unknown. Neuropeptidergic control of arousal is complex, but there is a well-established role for melanin-concentrating hormone (MCH) in inducing sleep, especially REM sleep. Our lab and others show that MCH neurons also project to areas implicated in reproductive control such as the medial preoptic nucleus and the median eminence, where GnRH neuron cell bodies and terminals, respectively, are located. However, the role of these connections is unclear. There is evidence to support that MCH is able to both stimulate and inhibit LH. These discrepant data could be explained by the existence of distinct groups of MCH neurons with different roles.

In rats, two populations of MCH neurons have been characterized: a medial population expressing cocaine- and amphetamine-related transcript (CART) with projections to cortex, hypothalamic nuclei, and brainstem nuclei associated with REM sleep; and a lateral, CART-negative group that projects more caudally in the brainstem. **We hypothesize that CART-positive MCH neurons, hodologically poised to dually regulate both sleep and the reproductive axis, are orchestrating the tight temporal relationship between these two systems.** Functional characterization of these populations will be more efficiently accomplished with the power of mouse genetics. Thus, we have begun by verifying whether divisions of MCH neurons are similar in mice and rats. We use a combination of immunohistochemistry and viral tracing methods to show that a medial population of MCH neurons in mice projects to the medial and ventrolateral preoptic areas. These structures contain GnRH neuronal cell bodies and GABAergic suppressors of the arousal system, respectively. We also demonstrate that these neurons are codistrubuted with CART neurons and likely express CART, as data from rats would predict. Interestingly, our initial studies suggest that the MCH/CART system is sexually dimorphic, which has not previously been documented. Therefore, moving forward, we will also immunohistochemically analyze the expression of sex steroid receptors in CART+/MCH+ neurons. Ultimately, we aim to develop a mouse model to target CART+/MCH+ neurons and probe their ability to modulate both sleep and reproduction with *in vivo* EEG recordings and analysis of LH release using direct and indirect measures such as estrus cyclicity, pubertal timing, and serum LH concentration in pulse bleeds. This research will help define the genetic and functional heterogeneity of MCH neurons and contribute to a working model of how sleep and the HPG axis interact to promote normal pubertal development and reproductive capacity.

Opioid Exposure following Traumatic Brain Injury Exacerbates Behavioral and Neuroinflammatory Outcomes

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Traumatic brain injury (TBI) is linked with higher-risk opioid use, which may result from post-TBI opioid treatment. Both TBI and the opioid, morphine, induce reactive oxygen species (ROS) and glial-mediated inflammation. Considering this, we hypothesized that post-TBI morphine exposure increases opioid responding after injury by synergistically enhancing ROS and glial activation. C57Bl/6 mice (8-10 weeks old) received a closed-skull impact or sham surgery. Thirty days later, morphine reward responding was assessed using conditioned place preference (CPP) and two-bottle choice drinking. Compared to sham, TBI mice displayed a significant 1.5-fold higher preference for the morphine-paired environment in CPP. Regarding morphine consumption, both sham and TBI mice displayed a marked preference (~80%) for morphine intake when paired against a quinine tastant control. However, only TBI mice continued to show a morphine preference (~70%) when measured against its vehicle (0.2% sucrose solution, no quinine), a response that was significantly greater than that of sham controls (~30% preference) under these conditions. To assess early changes in oxidative generation, tissue ROS was measured from TBI and sham mice treated with morphine or saline for 7 days post-injury using a fluorometric assay. While TBI-saline mice exhibited increased ROS levels compared to respective sham controls, ROS accumulation was significantly greater in the TBI-morphine cohort relative to all other groups. Our data also support the hypothesized exaggerated glial recruitment in TBI-morphine mice, with reward-related cortical areas showing a markedly enhanced Iba-1 expression (microglial marker) in tissue from TBI-morphine mice undergoing CPP compared to all other injury/treatment groups. Together, our data model heightened preference to morphine post-TBI and suggest alterations in oxidative and inflammatory mediators may underlie this vulnerability. This work was supported by the resources and facilities of the John D. Dingell VAMC.

Age as a Determinant in MDPV and Methamphetamine-Induced Locomotor Sensitization

Burroughs, Rachel L. and Baker, Lisa E.

Recreational use and abuse of illicit synthetic cathinones ("bath salts"), pose a serious public health risk. A substantial body of preclinical research has characterized the locomotor stimulant, discriminative, and reinforcing effects of synthetic cathinones, with a particular emphasis on 3,4-methylenedioxypyrovalerone (MDPV). Collectively, previous findings have documented that the psychopharmacology of MDPV is similar to that of other commonly abused psychostimulants, such as cocaine and methamphetamine (METH). Despite the popularity of concomitant use of MDPV with other stimulants, few preclinical studies have assessed the behavioral effects of these drug mixtures. Moreover, no published studies have assessed age effects on MDPV's locomotor stimulant effects. The present study characterized the locomotor stimulant effects of repeated daily treatment with MDPV alone or concomitantly with methamphetamine to determine if age influenced the development or expression of locomotor sensitization. Male Sprague-Dawley rats aged two months (N=36), seven months (N=32), or 12 months (N=36) were randomly assigned to one of four treatment groups: 1.0 mg/kg METH, 1.0 mg/kg MDPV, 1.0 mg/kg + 1.0 mg/kg MDPV, or saline. Treatments were administered for seven consecutive days and locomotor activity was monitored for 60 min pre-injection and 60 min post injection. Following a 10 day drug washout period, rats in each treatment group were re-assessed for the expression of sensitization to a challenge dose of 1.0 mg/kg MDPV. In 2 month old rats, MDPV and MDPV+METH produced drug-induced increases in activity that were significantly augmented on day 7 compared to day 1. In contrast, drug-induced activity was lower on day 7 compared to day 1 in older animals treated with MDPV or MDPV +METH, although METH-induced locomotor sensitization in 12 month rats. None of the treatment groups in any age group exhibited a statistically significant augmented response to MDPV administered after the 10 day washout. A two-way ANOVA (age, treatment) on activity following the post-washout MDPV challenge indicated statistically significant effects of age and treatment, but multiple comparisons revealed statistical significance only between each group compared to the sal-sal (i.e., drug naïve) treatment group.

3,4-Methylenedioxypyrovalerone (MDPV) Discrimination in Adult Female Sprague-Dawley Rats

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3,4-Methylenedioxypyrovalerone (MDPV), one of several synthetic cathinones, is a popular constituent of illicit psychoactive “bath salts.” In preclinical studies utilizing drug discrimination methods with male rodents, MDPV has been characterized as similar to both cocaine and MDMA, although some discrepancies have been noted in study outcomes, perhaps due to methodological differences. The aim of the current study was to evaluate the discriminative stimulus effects of MDPV in female rats. Twelve adult female Sprague-Dawley rats were trained to discriminate 0.5 mg/kg MDPV from saline using a resetting fixed ratio 20 schedule of food reinforcement. Stimulus substitution was assessed with MDPV (0.05-0.5 mg/kg), cocaine (2.5-10 mg/kg), MDMA (0.75-3 mg/kg), and methamphetamine (0.1-1 mg/kg). Sch 23390, a D1 dopamine receptor antagonist and haloperiodol, a D2 DA antagonist were assessed for stimulus antagonism. Cocaine, MDMA, and methamphetamine all produced dose-dependent increases in MDPV-lever responses with full substitution at the highest dose of each substance. MDPV discrimination was attenuated by Sch 23390 and this effect was surmountable by increasing the MDPV dose. Preliminary results indicate haloperidol attenuates MDPV discrimination, though these experiments are still in progress. The current findings are generally consistent with previous published studies with male rats trained to discriminate MDPV.

Sex-Specific Effects of Stress: ΔFosB and its Role in the Development of Depression

Sadhana Chinnusamy

Women represent the majority of patients affected by depression, but preclinical studies, such as rodent stress models, have historically used male animals and ignored sex differences in depression-like behaviors (Iñiquez et al, 2017). A newer method, the sub-chronic variable stress (SCVS) paradigm, induces depressive-like behaviors specifically in female mice (Brancato et al, 2017), allowing us to test the molecular mechanisms driving sex differences in mood disorders. In the brain, the ventral hippocampus (vHPC), a region involved in learning and memory, sends neuronal projections to the nucleus accumbens (NAc), a major component of the reward pathway, and the basolateral amygdala (BLA), which regulates fear and anxiety behaviors. In the NAc, expression of the transcription factor ΔFosB, which accumulates after chronic stimulation, is necessary for resilience to stress-induced depression-like behavior (Vialou et al, 2010). We know that heightened activity of vHPC neurons directly projecting to NAc drives stress-induced behaviors in males (Bagot et al, 2015), and we thus hypothesized that ΔFosB in vHPC-NAc projecting neurons promotes resilience to stress. Therefore, we used the SCVS model to induce stress in both male and female mice, and as expected, only female mice developed anhedonia, as measured reduced preference for sucrose solution over water. We then used immunohistochemistry and Western Blotting to measure ΔFosB induction in vHPC-NAc and vHPC-BLA neurons. We predict differences in ΔFosB induction in these pathways between stressed and non-stressed animals and between sexes. This study will provide insight into the molecular etiology of depression, as well as the potential for uncovering sex-specific treatments for mood disorders in the future.

Title: Differences in locomotor activation during exposure to binge-like toluene exposure during the periadolescence period in swiss-webster mice.

Authors: Cameron J. Davidson, Michael Naddaf, & Scott E. Bowen

Affiliations: Department of Psychology, Wayne State University

Abstract: Inhalants, such as toluene, are one of the most commonly abused compounds among early adolescents. Inhalant abuse during adolescence has long lasting neurobiological and neurobehavioral consequences such as impaired decision-making, increased risk taking, and drug use. While preclinical models have shown behavioral effects for abused inhalants like toluene, there is a lack of systematic evaluation of toluene exposure during adolescence. In our translational model of inhalant abuse, male adolescent Swiss-Webster mice (N=364) were exposed to toluene vapor in concentrations of 0, 2000, or 4000 parts per million (ppm) for 30 min/day (postnatal days 28-32). Toluene exposures occurred within a static-exposure chamber fitted with infrared locomotor sensors which noninvasively recorded locomotor activity during exposure. Our results demonstrated that acute toluene exposure resulted in dose-dependent increases in locomotor behavior (4000 ppm > 2000 ppm > 0 ppm (Control)). Repeated exposures to 4000 ppm toluene resulted in further increases in locomotor behavior across days of exposure. This outcome was not observed with repeated exposures to 2000 ppm, which showed decreased locomotor behavior. Overall the results observed show that repeated exposure to “abuse-like” toluene concentrations produce a differential pattern of locomotor behavior including both desensitization (2000 ppm) and sensitization (4000 ppm). These biphasic, motor increasing and decreasing effects of toluene (as a function of exposure concentration), may reflect the CNS-depressant drug-like effects of abused solvents, which may impact the effects of other drugs of abuse later in life.

DETERMINING THE EFFECT OF CELL TYPE-SPECIFIC VTA SGK1 MANIPULATION ON DRUG REWARD

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Drugs of abuse are known to regulate activity of the mesolimbic dopamine (DA) system. Specifically, drug-induced changes in ventral tegmental area (VTA) cellular activity and gene regulation contribute to behavioral outputs associated with addiction. Our previous work has determined that serum- and glucocorticoid-inducible kinase 1 (SGK1) catalytic activity and phosphorylation at Ser78 are increased by chronic, but not acute, administration of cocaine and morphine. Furthermore, I have shown that viral overexpression of SGK1 mutants in the VTA of adult mice produce behaviorally relevant effects on drug reward, assessed by cocaine conditioned place preference (CPP) and voluntary morphine intake using a two-bottle choice task. Specifically, intra-VTA infusion of a catalytically inactive SGK1 mutant (K127Q) significantly decreases cocaine CPP and morphine intake, suggesting that decreasing VTA SGK1 activity is sufficient to decrease drug reward. Intra-VTA infusion of SGK1 S78A, a mutant that prevents phosphorylation at Ser78, significantly decreases cocaine CPP and morphine intake, suggesting that VTA SGK1 pSer78 is necessary for drug reward and intake. To more fully understand the role of VTA SGK1 in behaviors relevant to addiction, I am now manipulating SGK1 expression in a cell type-specific manner to determine whether SGK1 activity and phosphorylation in DA or GABA neurons drives the observed behavioral effects. Utilizing novel Cre-dependent viral constructs, I am currently assessing the impact of altered SGK1 activity in VTA DA neurons on drug reward. In parallel, using a floxed-SGK1 mouse line crossed with a DAT-Cre driver line, I am determining the impact of DA SGK1 knockout on reward behavior. These studies will allow for identification of the specific cells and circuits that are critical for SGK1-mediated effects on drug reward and intake. This work will increase our understanding of the role of VTA SGK1 activity in drug-related behaviors, a necessary step in assessing the feasibility of SGK1 inhibition as a novel therapeutic avenue for addiction.

Sex-specific effects of early life stress on hippocampal neurogenesis in the pig

Durga K, Duque-Wilckens N, Moeser A, Robison AJ

Depression is a leading cause of disability worldwide, yet available treatments are ineffective for nearly half of treated patients. Depression patients often display reduced hippocampal volume, and many animal models of depression display a reduction in neurogenesis that is reversed by chronic exposure to antidepressants like fluoxetine. Using a pig model, we assessed whether exposure to early life adversity, a major risk factor for the development of depression later in life, affects neurogenesis. Early weaned (15 days post partum) or late weaned (28 days post partum) female, castrated male, and intact male pigs were euthanized at 20 weeks of age and brain tissue was immediately harvested. We performed immunohistochemistry in dentate gyrus to detect the protein doublecortin, a marker of differentiating neurons. We found a significant reduction in doublecortin labeled cells in early weaned females compared to the late weaned females in the dentate gyrus. We also used western blot to detect expression of the *FosB* gene, which is necessary for neurogenesis in mice. We found that early weaned females had an increased level of hippocampal FosB and ΔFosB, while these *FosB* gene products were reduced in the early weaned castrated males. Together, these data suggest that early weaning has sex specific effects on hippocampal neurogenesis later in life, and that this effect may be mediated by gonadal hormones. Ongoing studies include assessing FosB and ΔFosB in the prefrontal cortex and the nucleus accumbens, as well as examination of neuroinflammation throughout the brain.

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ACADEMIC STRESS PREDICTS ABNORMAL EEG LATERALIZATION

This study sought to examine the psychological and physiological implications of the presentation of positive and negative images during both high and low academic stress conditions. Further, this study aimed to expand upon past research that has demonstrated an increase in right frontal alpha activity during periods of elevated academic stress. We hypothesized that those with elevated academic stress would have increased right frontal activity and negative (unpleasant) images would trigger an increase in alpha activity as shown on electroencephalogram (EEG). Participants were divided into groups based on self-reported academic stress conditions; low stress conditions being times without exams, high stress during a week where participants had three or more exams. Following a baseline EEG reading, the participants were presented with selected images from the International Affective Picture Rating Scale (IAPS) containing pleasant, unpleasant, and neutral photo stimuli. Participants were asked to rate both their happiness and discomfort levels when viewing each image on a Self-Assessment Manikin Scale (SAMS). Total, alpha, and beta activity were analyzed in the left and right hemispheres of the brain during baseline EEG measures and presentation of the SAMS rating scale. Statistical analyses revealed that right frontal activity was elevated in those with higher academic stress as hypothesized. However, we did not observe increased reactions to negative images. Results may be useful for identifying groups most affected by academic pressures and contribute to the development of stress prevention techniques.

Examining processing of absent gustatory stimuli in Disrupted-in-schiphrenia-1 mice

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Department of Psychology[1], Neuroscience Program[2], Michigan State University

Schizophrenia is a chronically debilitating disease and while animal models have been somewhat successful in mirroring certain endophenotypes associated with cognitive and negative symptoms, positive symptoms have received significantly less attention. This is partly due to a lack of appropriate animal behavioral models to examine psychosis in animals. Using mice in which dominant-negative Disrupted-in-Schizophrenia-1 is expressed throughout central nervous system circuitry (DN-DISC1-PrP), the capacity for an auditory conditioned stimulus (CS) to evoke processing of an absent sucrose solution was examined. At test, during CS presentations, DN-DISC1-PrP mice consumed more water and displayed a licking profile that is more typically revealed while ingesting a sweet-tasting solution. DN-DISC1-PrP mice also displayed greater c-fos expression in the insular (gustatory) cortex when consuming water in the presence of the CS. This capacity for the CS to more readily substitute for the taste features of the absent sucrose solution in DN-DISC1-PrP mice was attenuated following systemic treatment with the antipsychotic haloperidol. Conversely, social isolation during adolescence promoted the manifestation of these effects. Results suggest that it is possible to examine reality testing in animal models through the use of associative learning procedures; furthermore, these effects are dopamine-mediated, and appear dependent on strong activation of the insular cortex in our transgenic mouse model of neuropsychiatric illness.

Cellular compartment analysis of temporal activity by fluorescent in situ hybridization (catFISH) in the rat transcardially perfused brain

Ali Gheidi, Vivek Kumar, Christopher J Fitzpatrick, Rachel L Atkinson

Background: Cellular compartment analysis of temporal activity by fluorescent in *situ* hybridization catFISH allows high spatiotemporal resolution mapping of immediate early genes in the brain in response to internal/external stimuli. One caveat of this technique and indeed other methods of *in situ* hybridization is the necessity of flash-freezing the brain prior to staining. Often however, the mammalian brain is transcardially perfused to use the brain tissue for immunohistochemistry, the most widely-used technique to study gene expression. *New Method:* We have developed a technique, modified from that of Guzowsky and Worley, 2001, that allows the catFISH method to be used in adult rats that have been transcardially perfused with 4% paraformaldehyde. *Results* c-Fos activity induced by either an auditory tone or status epilepticus was visualized using the catFISH procedure. We see clear distinction of the compartmental distribution of c-Fos mRNA in the nuclei and cytoplasmic regions of the rat prefrontal cortex, hippocampus and amygdala. Furthermore, the qualitative proportion of c-Fos compartmentalization is similar to previous reports of c-Fos expression pattern in rodents navigating novel environments. *Conclusion:* c-Fos catFISH on perfused rodent brains is an attractive addition to the traditional histological methods using fluorescently labeled riboprobes, and opens several avenues for future investigations.

From Exposure to Healing: Tracking Mental Health Trajectory in Syrian Refugees and Impact of Body-Based Treatments

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Background: Exposure to civilian war trauma, life in camps, and stress of migration has negatively impacted mental health of refugees. Within one month of resettlement, our team identified 32.2% of adults who screened positive for posttraumatic stress disorder, 40.3% screened positive for anxiety, and 47.7% screened positive for depression. As these conditions are not static and symptoms severity changes over time, we asked how mental health of Syrian refugees changes following a year of resettlement in the US. Knowing this high prevalence of mental health concerns, we also structured a body-based intervention program to overcome barriers to traditional treatments in the Syrian refugee community, which include accessibility, language, and cultural stigma.

Methods: 69 adults (35M; 34F) between the ages of 18 and 65 (mean age 34) responded to self-report questionnaire data assessing trauma exposure, posttraumatic stress, anxiety, depression, and somatic symptoms. 16 children (8M, 8F; mean age 10) participated in a dance/movement therapy program and responded to self-report questionnaire data assessing posttraumatic stress and anxiety symptoms. A small case sample (n=5) of adults also participated in body-based treatments.

Results: Most common trauma exposures included fire or explosion and combat or exposure to warzone. There was no significant change in posttraumatic stress, anxiety, or depressive symptoms from arrival to year 1 for adults. In adults, severity of mental health symptoms predicts somatic symptoms—the most commonly reported being back pain. For adults who participated in mindful yoga (women) or exercise (men), all moved from suprathreshold to subthreshold on the HSCL-25 measure of anxiety and depression after 6 weeks of active intervention. For children who participated in dance/movement therapy, a significant decrease in posttraumatic stress symptoms ($t(15)=3.238$, $p=.006$; $d=.8095642$) and anxiety symptoms ($t(15)=3.628$, $p=.002$; $d=.9070915$) was observed. Additionally, over the course of intervention, a decrease in a pro-inflammatory cytokine IL-18 was observed ($t(11)=2.409$, $p=.035$).

Conclusions: The data from a sample of Syrian refugee adults who have resettled in Southeast Michigan demonstrate high prevalence of anxiety and depression, with minimal improvements one year following resettlement. Ongoing data collection will assess changes in symptoms for those non-high-stress adults and will also integrate biological and environmental data. The results thus far indicate a need for greater mental health resources for refugees in order to achieve better psychosocial outcomes, and the pilot intervention data show promise for use of dance/movement therapy, mindful yoga, and other exercise-based treatments in this population.

Cocaine differentially affects locomotor behavior in male and female rats, and in females estrous cycle mediates the behavioral response.

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1: Wayne State University, School of Medicine, Department of Psychiatry and Behavioral Neurosciences
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Background: Chronic exposure to cocaine induces unique gene transcription and neuroplastic changes in synaptic proteins, but the effect on epigenetic regulators such as histone deacetylase (HDAC) proteins remains to be understood. Locomotor sensitization to psychostimulants is a hallmark of drug-induced neuroplasticity and results in increased locomotor behavior over repeated exposure. Furthermore, these neuroadaptations have been shown to be sex-dependent but information on underlying mechanisms for this sex difference is lacking.

Methods: Male and female Sprague Dawley rats were given three daily injections of 15 mg/kg of cocaine or saline for 14 days. Locomotor activity was recorded on days 0, 1 and 14 and the percent sensitization from day 1 to 14 was calculated. Females estrous cycle on day 14 was determined by vaginal lavage. All rats were euthanized immediately after their last behavioral session and brains were frozen to allow for HDAC protein measurements in future experiments.

Results: Females displayed significantly greater locomotor activity in response to cocaine on day 1 compared to males; however, males had significantly greater activity on day 14 than day 1. Females did not show significant sensitization in ambulatory behavior ($F(1,35) = 5.83$, $p = .021$ gender x day interaction). Females in proestrus and estrus cycles had significantly greater activity than those in diestrus and metestrus during the first 10 minutes following the first cocaine administration on day 14 ($F(3,13) = 6.75$, $p = .041$).

Conclusion: This experiment demonstrates sex-dependent differences in neuroplastic adaptations to chronic binge-pattern cocaine as males behaviorally sensitized to repeated days of cocaine injections while females showed no evidence of behavioral (locomotor) sensitization. Additionally, females responded differently to cocaine depending on their estrous cycle during the last day of repeated cocaine. These differences may be explained by the interaction of cycling hormones and HDAC proteins, as it has been shown estrogen influences the transcription of HDAC4. Ongoing molecular studies are measuring HDAC4 levels to explore the mechanisms that underlie the behavioral effects of cocaine.

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d-Amphetamine Effects on Impulsive Choice in a Rodent Model of Delay Discounting

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Impulsive behavior is a common symptom underlying several psychological disorders including addiction and attention deficit hyperactivity disorder. Delay discounting is an experimental procedure used to measure impulsive behavior across several species. For cases related to attention deficit hyperactivity disorder, individuals are often prescribed stimulant medications to attenuate symptoms related to inattention, hyperactivity, and impulsivity. However, preclinical investigations have reported mixed results related to the effects of psychostimulants on impulsive behavior. The present study utilized a delay discounting procedure to determine the effects of d-amphetamine on choice behavior in rats. Eight adult male Sprague-Dawley rats were trained to respond on each of two levers under a fixed ratio 1 schedule of food reinforcement and subsequently assigned a delayed reinforcement lever based on response bias during initial training. Delay discounting sessions consisted of five blocks of eight trials. The first two trials of each block were denoted as forced-choice trials and the last six were free-choice trials. During each trial, rats were given 30 seconds to respond, with a 30 second inter-trial interval. A series of four incrementing baseline delays were used, culminating in a terminal delay series of 0, 10, 20,40, and 60 seconds in the subsequent drug exposure phase. Rats were injected with d-amphetamine (1 or 2 mg/kg, i.p., four days with each dose) 5 min. before each test session during an eight day drug exposure phase. Subsequently, a four day post-drug phase was implemented. A two-way repeated measures mixed-model of the terminal baseline data did not reveal any significant effect between drug groups prior to beginning the first drug phase. A significant between group effect of delay at 0, 10, and 20 seconds was observed during the first drug phase (2 mg/kg). Between group comparisons during the second drug phase (1 mg/kg) revealed a significant difference only at the 40 second delay among the two treatment groups. Four days of d-amphetamine (2 mg/kg) produced significant augmentations in responding for the immediate reinforcement when compared to saline treatment. These data suggest d-amphetamine at a dose higher than those typically assessed in delay discounting procedures produce robust augmentations in impulsive choice at specific delays. Several other between group or condition comparisons approached statistical significance, but were subjected to high variability due to the small sample size and limited degrees of freedom. These limitations may be overcome with an increased sample size and procedures that account for drug-induced response disruption during experimental sessions. Nevertheless, the present study adds to existing literature demonstrating a potentiation of impulsive behavior following amphetamine administration that may be counterintuitive to its prescribed use.

TITLE: Circuit-specific Hippocampus ΔFosB Expression Mediates Resilience in Chronic Social Defeat Stress

AUTHORS: Manning, C.E., Eagle, A.E., Williams, E.S., Wirtz, A.J., Carabello-Perez, D.T., Chinnusamy, S., Kurdziel, P.A., Neve, R.L, Mazei-Robison, M.S., Robison, A.J.

ABSTRACT: Stress contributes to mood disorders in some individuals while others are resilient. As hippocampus (HPC), and especially its projections to the nucleus accumbens (NAc), plays a crucial role in stress responses, we investigated whether HPC expression of the transcription factor ΔFosB drives resilience to stress. Using the chronic social defeat stress (CSDS) model of depression, we demonstrate that general inhibition of ventral HPC ΔFosB promotes a depression-like phenotype. Using a novel dual-virus CRISPR system, we show that silencing the *FosB* gene specifically in HPC neurons projecting to NAc prevents resilience to CSDS in male mice. Critically, deletion of the *FosB* gene in hippocampal neurons projecting to amygdala did not affect resilience, and the effects in the HPC-NAc circuit could be prevented by overexpressing ΔFosB into the same neurons in which *FosB* was deleted. These data are some of the first to demonstrate the circuit-specific role of a gene in a model of neuropsychiatric disease, and we are now extending this work to study both males and females in an additional stress/depression models, including subchronic variable stress.

Andrew McLocklin, Ben Fry, Alex Johnson

Abstract: Effects of Dopamine D2 Receptor Antagonism on Effortful Decision Making

Dopamine and its effects are a hot topic in the psychological and neuroscience fields. Dopamine has been shown to influence learning and decision-making. We sought to determine how dopamine receptor antagonism affects decision-making when presented with a choice between a low value reward, and a high value reward that gradually requires more effort to obtain. Manipulations were done through intraperitoneal injections of haloperidol, a dopamine D2 receptor antagonist. Mice received injections of a low (0.1 mg/kg) as well as a high (0.25 mg/kg) dose of haloperidol, and performance was compared to that observed under saline control injection conditions. Mice received two tests under each of the drug conditions and the order in which they were tested was fully counterbalanced. After each injection, mice were run through an effortful decision-making task, which consisted of two levers being presented; one associated with a high value (20% sucrose solution) reward, and a second with a low value reward (5% sucrose solution). The amount of effort required to obtain the high reward increased from 1 lever response at the start of the test, to 40 responses by the end. We found that haloperidol impaired effortful discounting as well as increased omissions in a dose-dependent manner. These results confirm that dopamine plays a robust role in the maintenance of effort and decision making.

EFFECT OF SUCROSE VS. HIGH FRUCTOSE CORN SYRUP DURING DIFFERENT PATTERNS OF ACCESS ON CONSUMPTION, RESPONDING FOR FOOD, AND SENSORY SPECIFIC SATIETY

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Sweet solutions can stimulate overconsumption, alter subsequent behaviors, and contribute to obesity. Specific types of sweet solutions such sucrose and high fructose corn syrup (HFCS) may differentially influence consumption. The pattern of solution access may also affect fluid consumption. The purpose of this study is to determine if type of solution (sucrose vs. HFCS) and pattern of access induce binge-like consumption. Additionally, we look at whether the above variables interact to induce sensory specific satiety (SSS). Female Sprague-Dawley rats were divided into 4 groups. Group one received intermittent access to 3.20% sucrose (Intermittent Sucrose). Group two received intermittent access to 4.26% HFCS (Intermittent HFCS). Group three received daily 24hr access to HFCS (Daily HFCS). Group four received only water (Control). The sweet fluids were equicaloric and all groups were given water and chow ad libitum. After 12 weeks of home-cage drinking, rats lever-pressed for chocolate pellets on an FR1 schedule followed by an increasing response requirement (FR1 for first 10 reinforcers, FR3 for next 10 reinforcers, and FR5 for all subsequent reinforcers- FR135 schedule). For SSS testing, rats had 15 min access to food pellets (chow or chocolate) followed by 20 min progressive ratio operant sessions with delivery of chocolate pellets as reinforcers. Results showed that Intermittent Sucrose rats consumed more solution than Intermittent HFCS rats which consumed more solution than the remaining groups after initial 30 min access and longer 24 hr access period. Although binge-like consumption emerged, the effect failed to generalize to sweet chocolate pellets in an operant paradigm; responding for chocolate pellets did not differ across groups during the FR1 or the FR135 schedule. During SSS testing, Daily HFCS and Control rats demonstrated SSS via more responding when chow pellet access preceded responding for chocolate pellets vs. chocolate pellet access preceded responding for chocolate pellets. However, the Intermittent Sucrose and Intermittent HFCS failed to demonstrate SSS. In conclusion, the type of solution and pattern of access play a role in the overconsumption of the sweet solutions and can induce binge-like consumption as indicated by 30 min consumption levels. Although the sucrose and HFCS solutions contained an equal number of calories, the sucrose solution may have been more palatable and cause greater consumption. Pattern of sweet solution/food access or consumption may contribute more to obesity as suggested by our finding that rats with a history of intermittent solution access failed to express SSS. Future studies should investigate measures for a daily sucrose group to further look at the effects of pattern of access on SSS.

Aberrant dopamine projections to mesostriatal brain areas associated in Disrupted-in-schizophrenia-1 mice.

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Altered function of the brain's dopaminergic pathways, which traffic the neurotransmitter dopamine (DA), is tied to several psychiatric disorders; most notably, schizophrenia. Here, we used a transgenic mouse model for neuropsychiatric illness known as Disrupted-In-Schizophrenia 1 (DISC-1). The DISC-1 mutation was initially identified as a genetic risk factor for neuropsychiatric illness in humans. In the current study, we made use of a transgenic mouse model in which dominant negative expression of DISC-1 is driven by the prion protein promoter (DN-DISC1-PrP). These transgenic mice were crossed with mice that selectively express Cre-recombinase within tyrosine-hydroxylase (TH; the rate-limiting enzyme for DA synthesis) cells, resulting in DN-DISC1-PrP X TH-Cre mice. We wanted to examine changes in dopaminergic circuitry as a result of the DISC-1 mutation. Thus, mice received unilateral injections of a Cre-mediated anterograde tract tracer, Ad-syn-mCherry into the ventral tegmental area (VTA). Accordingly, only cells that express TH go through Cre-mediated recombination and thus selective expression of the tracer is observed in infected dopamine cells and terminals. This expression pattern was compared to Ad-syn-mCherry-treated TH-Cre mice that did not express the DISC-1 mutation. We noted increased density of fibers in striatal and pallidal circuitry in DN-DISC1-PrP X TH-Cre mice, which may be consistent with overactive dopaminergic signaling noted with schizophrenia. Overall, this viral-mediated transgenic labeling technique may be useful for elucidating genetic alterations in circuits that are known to be affected in neuropsychiatric illness.

Effect of Eccentric Exercise on Motor Learning and Emotional Intelligence Abilities in Older Adults.

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Previous research has shown that as little as 12 weeks of exercise can lead to improvements in both physical fitness and cognitive function in older adults. It is unclear whether those improvements translate to improvements in other domains that rely on cognitive function such as motor skill learning and emotional processing. Additionally, the factors that might underlie individual differences in the extent to which older adults improve cognitive function and physical fitness are not clear. For our study, we recruited 11 healthy older adults (65-85 years old) to perform 12-weeks of eccentric leg exercise (Eccentron). Our aim was to assess whether this kind of low to moderate intensity of exercise would lead to improvements in cognitive function, emotional intelligence abilities and motor learning (reduced susceptibility to proactive interference). In the motor learning (visuomotor rotation) task, we found the control group experienced more interference compared to the exercise group ($F(59, 590)=1.453$, $p=0.019$, $\eta p2 = 0.127$). In addition to displaying relatively superior adaptation to the interference, the exercise group also displayed a higher level of emotional intelligence abilities. They had a higher STEM-F score (emotion management score for fear) in the post-test ($M= 5.35$, $SD=1.02$), as compared to the pre-test ($M= 4.66$, $SD=1.06$); $t(10)= -2.560$, $p = 0.028$, while there was no such improvement displayed by the control group. The exercise group also displayed a higher level of accuracy in the Emotion Perception task in the post test ($M= -1.36$, $SD=3.13$) compared to the control group ($M=1.36$, $SD=2.83$); $t(20)=-2.137$, $p = 0.045$. A few interesting correlations were also observed: the proactive interference effet was moderately correlated to reaction time for emotion identification in the Emotion perception task. $r = -0.468$, $p = 0.028$ and positively correlated with the emotion management score of sadness for the control group $r = 0.66$, $p = 0.027$. The findings highlight the effectiveness of this type of exercise for improving motor learning and emotional intelligence skills. Potential future areas of study could include other age groups, combination with motivational techniques and looking at other mediating variables like personality and cardiovascular fitness levels.

Influence of estrus cycle and interactions with Melanin Concentrating Hormone on interval timing in rats

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Feeding behaviors have traditionally been studied within a context of circadian timing; however, interval timing (i.e., timing in the milliseconds to minutes range) may play a central role, as this distinct form of timing is critical for learning and decision-making. In this manner, interval timing may inform decisions to engage in food-related behaviors, such as food-seeking or consumption. Notably, no studies have examined signals controlling appetite regulation on interval timing. In this study, we examined the role of the lateral hypothalamic (LH) feeding peptide Melanin concentrating hormone (MCH) in an interval timing task. Male and female Sprague Dawley rats received targeted LH injections of a Designer Receptor Exclusively Activated by Designer Drugs (DREADD) packaged within an adeno-associated virus driven by the MCH promoter, pMCH. Placement of an intracerebroventricular cannula enabled central delivery of the synthetic ligand, clozapine-N-oxide (CNO), and activation of LH MCH cells during the interval timing task. In the peak interval task, rats first learned to respond on a lever for sucrose reward following the passage of a 20 s target criterion. Next, intermixed probe trials were introduced: in these trials, the lever extended but no sucrose was provided, regardless of lever responding. Probe trials allowed measurement of maximum lever responding across a trial, and enabled assessment of the subject's interval timing function. Under control conditions, a normal distribution of lever responding that peaked near the 20 s criterion duration indicated that all rats showed intact temporal performance. Interestingly, stimulation of LH MCH neurons selectively modified the right-hand side of the timing function in females, whereas it had no effect on the timing function in males. In females, this effect of prolonged peak rate responding was specific to estrus cycle stage. Moreover, CNO activation of MCH neurons restored responding in diestrus females to the behavioral phenotype observed both during proestrus/estrus and to that observed in males. Finally, analysis of estrus cycle stage and task performance under vehicle conditions revealed that diestrus females show a leftward shift in the timing function. Collectively, these findings suggest that MCH may affect performance in interval timing tasks in a sex-specific manner—in females, this depends on estrus cycle stage. Furthermore, estrogen itself may be an important modulator of interval timing.

ACTIVATION OF THE VENTRAL TEGMENTAL AREA SUPPORTS THE EXPRESSION OF SOCIAL PLAY BEHAVIOR IN JUVENILE RATS

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The ventral tegmental area (VTA) is an essential component of the mesocorticolimbic dopamine reward system and an important node of the Social Decision-Making Network (O'Connell & Hofmann, 2011). As such, the VTA is interconnected with brain regions implicated in the expression of social play, a highly rewarding behavior predominately displayed by juveniles and expressed by nearly all mammalian species. Using juvenile male and female rats, we investigated the recruitment of the VTA following social play exposure (Experiment 1), how temporary inactivation of the VTA affected the expression of social play (Experiment 2), and how extracellular levels of neurotransmitters in the VTA change during social play exposure (Experiment 3). In Experiment 1, single-housed juveniles were exposed, in their home cage, to an age- and sex-matched unfamiliar juvenile for 10 min ("Play" condition) or received similar handling but no partner ("No Play" condition). Fos and tyrosine hydroxylase (TH) immunohistochemistry was used to determine activation of the VTA and its dopaminergic neurons in response to social play. Subjects in the play condition had greater Fos induction in the rostral and mid VTA than subjects in the no play condition; there was no effect of play exposure on Fos induction in the caudal VTA. Likewise, subjects in the play condition had greater Fos induction within TH-positive VTA neurons than subjects in the no play condition, although the occurrence of double-labeled neurons was very low. In Experiment 2, subjects received, in counterbalanced order, bilateral infusions (0.3 uL/side) of vehicle (aCSF) or the GABA-A receptor agonist muscimol (10 ng/side) into the VTA 20 min prior to exposure to the 10 min social play test (as described for the "Play" condition above). Temporary inactivation of the VTA with muscimol selectively decreased the expression of social play behavior while leaving social investigation intact. In Experiment 3, intra-VTA in vivo microdialysis was used to measure extracellular levels of glutamate, GABA, and dopamine in response to social play exposure (aCSF perfused at 3 uL/min; 10-min dialysate samples). Social play was associated with dynamic changes in the extracellular levels of all three neurotransmitters. Together, these data suggest that activation of the VTA supports the expression of social play behavior in juvenile male and female rats. Research supported by NIMH R01MH102456 to AHV.

Conditioned place preference with low dose mixtures of 3,4-methylenedioxypyrovalerone (MDPV) and 3,4-methylenedioxymethamphetamine (MDMA) in male and female Sprague-Dawley rats.

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3,4-Methylenedioxypyrovalerone (MDPV) is a novel synthetic cathinone reported to have a high abuse potential and to produce adverse medical consequences when used recreationally. Preclinical research indicates the psychopharmacology of MDPV is comparable to both cocaine and 3,4-methylenedioxymethamphetamine (MDMA). MDPV is commonly used as a substitute or in combination with other psychostimulants, which may be a contributing factor to MDPV-related toxicity. Despite the prevalence of concomitant use of synthetic cathinones and other psychostimulants, few studies have investigated the combined behavioral effects of these substances. The current study evaluated the combined effects of MDPV and MDMA in a rodent model of conditioned place preference (CPP). Adult male (n=72) and female (n=60) Sprague-Dawley rats underwent an eight-day biased CPP procedure. Treatment groups consisted of saline, MDPV (1 or 3.2 mg/kg), MDMA (3 mg/kg), 1 mg/kg MDPV + 3 mg/kg MDMA, or 3.2 mg/kg MDPV + 3 mg/kg MDMA. Activity was monitored during all conditioning trials. To assess evidence for CPP, difference scores were calculated by subtracting time spent in the drug-paired chamber pre-conditioning from time spent in the same chamber post-conditioning. Activity levels during drug conditioning trials were highest among the 3.2 mg/kg MDPV-treated animals. A two-way ANOVA on the difference scores indicated a statistically significant treatment effect. Sex and the treatment by sex interaction were not statistically significant. Although difference scores were higher in all MDPV and MDPV+MDMA treatment groups compared to the saline control groups, only the females treated with 3.2 mg/kg MDPV + 3 mg/kg MDMA exhibited statistically significant evidence for CPP. Interestingly, 3 mg/kg MDMA appeared to attenuate the locomotor stimulant effects of 3.2 mg/kg MDPV but increase its effects on conditioned reward. These findings indicate females are more sensitive to the rewarding effects of MDPV and these effects may be enhanced by co-administration of MDPV and MDMA. Moreover, concurrent use of MDPV and MDMA may pose an enhanced risk for abuse, particularly in females.

THE ROLE OF DIMINISHED MOTIVATION IN EXTINGUISHING FEAR RESPONSES TO ENVIRONMENTAL STIMULI

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Organisms integrate environmental stimuli with internal states (e.g. motivation, pleasure, fear) to modulate decisions for approach versus defensive behavior. Stressors can affect this decision-making process by decreasing reward-seeking behavior, which can heighten an organism's chance for survival in certain contexts. Deficits in modulating reward-seeking behavior, such as an inability to diminish a fearful response when stimuli are no longer predictive of a previously aversive outcome (fear extinction, FE), can be maladaptive and are core characteristics of many anxiety disorders. Previous studies have demonstrated significant variations in the ability for FE learning between *wild-type* mouse strains (Camp et al., 2009). In particular, the 129S1-inbred mouse strain (129S1) exhibits maladaptive persistent fear (measured as freezing) to a stimulus (a conditioned tone) that no longer predicts an aversive outcome (a footshock). However, 129S1 who have never received a footshock paired to a stimulus also show increased freezing (Cazares et al., 2019). As a result, our hypothesis is that FE deficits in the 129S1 are partially driven by lowered tendencies to perform motivated behavior, which consequently biases their behavior towards defensive responses (vs. approach). To begin to test this hypothesis, we characterized tendencies of 129S1 (relative to C57BL/6, which do not exhibit FE deficits) by monitoring their exploration in home-cage like environments and novel environments, as well as exploration of novel objects. We find that 129S1 exhibit significantly less exploration in both home cage and novel environments. Additionally, when presented with two novel objects, 47% of tested 129S1 spend all of their exploration time on only one of the two objects. In contrast, all of the C57BL/6 explore both of the novel objects. To determine whether the hypo-motivated phenotype in the 129S1 is modulated by the HPA axis response, we are currently testing whether basal and mildly-stressed corticosterone levels differ between strains. Furthermore, we are also testing whether treatment with the selective serotonin reuptake inhibitor, fluoxetine, modulates exploration levels. Our data demonstrating both heightened defensive behavior and diminished innate motivation in the 129S1 mice serve as a basis for establishing this strain as a model of anhedonia.

Name: Cole Showers (Michigan State University)
Title: Determination of effective *in vivo* delivery of SGK1 inhibitor in the VTA to study its effect on SGK1 catalytic activity and phosphorylation in drug-related behaviors

Drug addiction is a psychiatric condition with increasing prevalence in the United States in recent years. Despite being a common disorder, a large amount remains unknown about the neuroadaptations that occur in drug addiction. Previously, our lab has identified a novel protein, serum- and glucocorticoid-regulated kinase 1 (SGK1), that is upregulated in the ventral tegmental area (VTA) upon chronic drug administration. More specifically, we have found that SGK1 phosphorylation and catalytic activity is increased under chronic drug administration. The ventral tegmental area (VTA) is a brain region that plays a key role in drug reward behavior. Therefore, the aim of my project is to characterize if *in vivo* administration of an SGK1 inhibitor can decrease SGK1 catalytic activity and SGK1 S78 phosphorylation. We used a binge cocaine paradigm in mice, followed by direct infusion of the SGK1 inhibitor, GSK 650394, into the VTA via stereotaxic surgery. VTA was then microdissected and processed by western blotting technique to analyze changes in SGK1 S78 phosphorylation and SGK1 catalytic activity. We found no significant difference in catalytic activity levels between the GSK and control treated mice. We hypothesize that this is due to GSK having difficulty getting inside the neuronal cell, preventing GSK from inhibiting SGK1 catalytic activity. In future experiments, strategies to expand transmembrane delivery of GSK will be investigated. One potential strategy includes the use of cyclodextrin, a macrocyclic molecule made of glucose that is often utilized to increase drug delivery.

Validation of VTA SGK1 knockout mice for use in morphine neuroadaptation studies

Ali Stark, Marie Doyle, Michelle Mazei-Robison

According to the National Institute on Drug Abuse, over 40,000 people per year die of an opioid overdose. Opiate abuse in part results from neuroadaptations in the ventral tegmental area (VTA), and our lab previously demonstrated that chronic morphine exposure increases catalytic activity of the protein serum- and glucocorticoid-regulated kinase 1 (SGK1). However, as the VTA is composed of ~60% dopaminergic (DA) neurons and ~35% GABAergic neurons, the neuronal cell type of interest remains unknown. To answer this question, we are using novel mouse models with cell type-specific manipulations of SGK1. To determine the effect of SGK1 gene knockout in DA neurons, I am isolating VTA RNA from mice with a transgenic knockout of SGK1 from all DA neurons (FlxSGK1xDAT-Cre). Following qPCR to analyze SGK1 mRNA levels, we predict reduced expression in DA KO mice than in controls. To create a VTA- and cell type-specific manipulation of SGK1, I will validate a Cre-dependent virus which overexpresses a catalytically inactive version of SGK1 (AAV-DIO-SGK1-K127Q). This virus will be injected into the VTA of mice which express Cre recombinase in either DA (DAT-Cre) or GABA neurons (VGAT-Cre). I will validate viral expression using immunohistochemistry in VGAT-Cre mice and function using Western Blotting for SGK1 catalytic activity in both VGAT-Cre and DAT-Cre mice. We predict that K127Q overexpression mice will show a decrease in SGK1 catalytic activity compared to GFP controls. After validation of these models, mice will undergo the morphine two-bottle choice test to assess VTA SGK1 manipulation on drug reward behavior.

AN EVALUATION OF SUB-CHRONIC KETAMINE EXPOSURE ON RODENT MEMORY AS ASSESSED BY THE ODOR SPAN TASK

This study sought to evaluate the impact of sub-chronic ketamine treatment on performance in the odor span task (OST), believed to be a behavioral assessment of working memory in rodents. The OST uses odor stimuli, which are more salient to rats than visual or auditory stimuli. Reinforcement is attained by the subject when a response to a novel scent stimulus is emitted; responses to previously presented scents are not reinforced. The subject must be able to recall the scents already contacted to successfully select the novel scent stimulus. A new scent is added with each correct trial, so as the session progresses, the memory load increases. In two separate experiments, 10 male and six female Sprague-Dawley rats aged at least 90 days were trained on the OST. Subjects advanced to the Drug Phase after a total of 25 training sessions if they achieved either a) at least three spans of five or greater in the last ten training sessions, - or - b) four longest runs of five or greater in the last ten training sessions. All subjects met one or both of these criteria. Training was suspended and the NMDA receptor antagonist ketamine was administered according to a ten-day sub-chronic dosing regimen at 30 mg/kg daily. OST performance was re-assessed after a two-day drug washout period. Females acquired the task in fewer trials compared to males. However, in contrast to previous findings that ketamine disrupts working memory (Rushforth, Steckler, & Shoaib, 2011), it failed to do so in this study. Ketamine-treated males (n = 5) did not show any deficits in performance compared to the saline-treated males (n = 5). Preliminary results indicate there was slightly more disruption in post-injection performance for both the ketamine (n = 3) and the saline-treated (n = 3) females. Insofar as the OST is a model of working memory, these preliminary findings indicate ketamine's effects on working memory are tenuous.

Characterizing reproductive function in POMC-deficient mice

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The pro-opiomelanocortin (*Pomc*) gene encodes POMC, which is differentially processed to produce adrenocorticotrophin, beta-endorphin, and three melanocyte-stimulating hormones, among other peptides. POMC neurons are principally located in the arcuate nucleus (Arc) of the hypothalamus, where they are essential in the control of food intake, energy expenditure and body weight. Several different homozygous null mutations in the *POMC* gene have been shown to cause early-onset obesity and adrenal cortical insufficiency in a small number of humans.

Pomc expression in Arc neurons is regulated by two distal enhancers. Mutations in these enhancers selectively reduce the amount of *Pomc* mRNA and POMC peptides in Arc neurons, but not pituitary cells. Furthermore, estrogen receptor alpha can bind to one of these enhancers *in vitro*, and about 25% of Arc POMC neurons express this receptor. Mice with combined deletions of both enhancers (FNΔ1Δ2) have less than one percent of Arc *Pomc* mRNA compared to wildtype mice. Like other mouse models of obesity, FNΔ1Δ2 mice are infertile, but it is unclear whether their reproductive disruption is due primarily to POMC-deficiency in the brain or is secondary to obesity.

We are comparing aspects of reproductive function in wildtype and FNΔ1Δ2 female mice, including day of vaginal opening, day of first estrus, estrus cyclicity and fertility. In addition, we are using a related, conditional mutant mouse model (FNΔ2) in which *Pomc* gene expression can be restored by the action of a tamoxifen-inducible Cre-ERT2 transgene after the mice have developed obesity. Because humans with mutations in the *POMC* gene also experience disruptions in timing of puberty, or a cessation of pubertal development, understanding more about how hypothalamic POMC-deficiency impacts reproduction in mice may help to develop therapies for humans impacted by similar mutations.

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SLEEP AND MIDBRAIN DOPAMINERGIC NEURONS ARE MODULATED BY THE PRESENCE OF A NEST

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Prior to sleep, animals perform various sleep-preparatory behaviors, yet little is known about their contribution to sleep physiology. Due to the high prevalence of sleep disorders and drawbacks of available pharmacological interventions, there is a strong need for a better understanding of the ecological and evolutionary contexts of sleep. Nest building is a sleep-preparatory behavior performed by many species, including all great apes and mice. In this study, we aimed to determine whether the presence of a nest modulates sleep. Specifically, we investigated the effects of the presence/absence of a nest on sleep/wake architecture and activity in wake-promoting midbrain dopaminergic (VTA-DA) neurons in mice. First, we recorded EEG/EMG activity over 24 hrs (n=14, 7 males and 7 females) in the presence/absence of a nest. We found that, in the presence of a nest, mice exhibited a decreased latency to both NREM and REM sleep and spent significantly more time asleep during the inactive/light phase. Moreover, NREM and REM episodes were significantly longer in the presence of a nest, and mice exhibited fewer transitions between arousal states. Together, our findings support the hypothesis that the presence of a nest facilitates and consolidates sleep. Next, we aimed to elucidate the neuronal circuit elements underlying sleep dysregulation in the absence of a nest. VTA-DA neurons are implicated in the regulation of motivational aspects of arousal, and their activation can potently promote wakefulness and suppress sleep and sleep-related behaviors. To determine whether the lack of a nest hyperactivates VTA-DA neurons, we simultaneously recorded EEG/EMG data and neuronal activity (using calcium-dependent fiber-photometry recordings) from VTA-DA neurons while manipulating the nest (n=8 Th-Flpo mice, 4 males and 4 females). We found that nest removal significantly increased population activity in VTA-DA neurons. These findings suggest that the lack of a nest suppresses and fragments sleep through hyperactivation of midbrain dopaminergic neurons, and that reduced activity in these neurons is a prerequisite for sleep. Taken together, our findings provide the first evidence for a role of sleep-preparatory behaviors in the facilitation and consolidation of sleep and could shape the development of novel treatments for sleep disorders.

MALES AND FEMALES DIFFER IN THEIR SENSITIVITY TO MATERNAL BUFFERING OF FEAR

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Background: Beginning in infancy, social cues play a crucial role in the regulation of emotional state and behavior. In addition to providing their child with nutrition, warmth, and safety, mothers can suppress amygdala activity, cortisol release, emotional distress, and fear learning in their child, an effect termed "maternal buffering." When infant rats undergo fear conditioning in the presence of a calm mother, they do not acquire the association between the neutral cue and mild foot shock. There is an emerging literature on sex differences in expression of fear and in the social regulation of fear in adults; however, sex differences in maternal buffering of fear have not received much attention. Here, we examined whether maternal presence differentially modulates fear in female and male rat pups.

Methods: All experiments were conducted using Sprague-Dawley rats. Rat mothers were anesthetized with 50 mg/kg ketamine and 5 mg/kg xylazine. Once dams were fully anesthetized, they were placed in a clean cage lined with an absorbent blue pad. Postnatal day 13 (P13) pups were then placed in the cage with the mother and given a 10-min adaptation period. Following the adaptation period, pups received 11 presentations of a 30-s peppermint odor (CS) and a 1-s, 0.5-mA tail shock unconditioned stimulus (US) (Lafayette scrambled shock generator), with an intertrial interval (ITI) of 4 min. Peppermint odor was delivered by a flow dilution olfactometer (2 L/min flow rate) at concentration of 1:10 peppermint vapor. Paired odor–shock pups received a shock overlapping with the last second of the 30-s odor presentation. On P18, pups were placed in clear plastic boxes and given a 3-min adaptation period. A 30-second CS odor was presented to the pups 3 times with an ITI of 2 minutes. Pup behavior was recorded via video camera. Neuronal activation and endocrine response will be examined in future experiments.

Results: In females, we found a significant main effect of maternal presence ($p = 0.008$); females that were conditioned in the presence of an anesthetized mother ($n = 6$) froze significantly less at test than females that were conditioned in the absence of an anesthetized mother ($n = 7$). However, we did not observe significant differences between males that were conditioned in the presence of an anesthetized mother ($n = 7$) and males that were conditioned in the absence of an anesthetized mother ($n = 5$) ($p = 0.75$).

Conclusions: Our data demonstrate that female pups are more susceptible to maternal buffering of fear, and suggest that sex differences in social regulation of emotion emerge very early in life. Caregivers buffer their child's emotional response to stressful events and protect their child's developing brain from the deleterious effects of stress. A better understanding of the mechanisms underlying maternal buffering of fear may help clinicians improve treatment outcomes for children that have experienced trauma.

Neural Excitability, Synapses, and Glia

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The Effects of Aging and Exercise on Glial Cell Line-Derived Neurotrophic Factor Content in Rat Hearts

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Heart function is regulated by the sensory nervous system and the two branches of the autonomic nervous system, the sympathetic and parasympathetic nervous systems. Neural development, plasticity, and survival of these branches of the nervous system are controlled by neurotrophic factors such as the glial cell line-derived neurotrophic factor (GDNF). Studies in our laboratory have shown that aging can shift the innervation pattern in mesenteric vessels from a balanced sensory/sympathetic pattern to a predominately sympathetic pattern and this is associated with an increase in blood pressure. However, the same studies suggest that exercise is associated with an increase in GDNF protein content in mesenteric vessels, a restored balance of innervation, and a lowering of blood pressure. Little is known about how aging and exercise impacts neurotrophic factor expression in cardiac muscle. Therefore, the purpose of this study was to measure GDNF protein content in the heart of rats at different ages and to investigate the effects of exercise on GDNF protein levels. Control groups consisted of 4-week-old, 14-week-old, 6-month-old, 12-month-old, and 18-month-old sedentary rats (5 or 6 animals per group). In the exercise study, 4-week-old, 6-month-old, and 12-month-old rats were placed in cages with access to voluntary running wheels and were allowed to exercise for 10 weeks (4-week-old), or 6 months (6- and 12-month-old). Sedentary and exercised animals were euthanized and cardiac tissues were removed. Each heart was divided into three parts: right atria (RA), left atria (LA), and ventricles (VT). Tissues were processed and neurotrophic factor protein content was measured using enzyme-linked immunosorbent assay. GDNF protein content in right atria (0.95 ± 0.14pg of GDNF/mg of tissue), left atria (0.91 ± 0.29pg of GDNF/mg of tissue), and ventricles (0.54 ± 0.35pg of GDNF/mg of tissue) of 4-week-old animals was significantly higher than that in heart chambers of older animals (6-month-old sedentary RA: 0.17 ± 0.02pg, LA: 0.14 ± 0.07pg, VT: 0.03 ± 0.02pg of GDNF/mg of tissue; 12-month-old sedentary RA: 0.02 ± 0.01pg, LA: 0.018 ± 0.012pg of GDNF/mg of tissue, VT: Non-detectable; 18-month-old sedentary RA: 0.000172 ± 0.000170pg, LA: 0.00128 ± 0.00236pg of GDNF/mg of tissue, VT: Non-detectable). The results show that exercise significantly increased GDNF protein content in all heart chambers, in all age-matched groups (14-week-old sedentary: RA: 1.20 ± 0.17pg , LA: 1.30 ± 0.14pg, VT: 1.27 ± 0.10pg of GDNF/mg of tissue and 14-week-old exercised: RA: 2.97 ± 0.27pg , LA: 2.06 ± 0.23pg, VT: 2.57 ± 0.32pg of GDNF/mg of tissue; 12-month-old exercised RA: 0.25 ± 0.07pg, LA: 0.11 ± 0.03pg, VT: 0.02 ± 0.01pg of GDNF/mg of tissue; 18-month-old exercised RA: 0.18 ± 0.21pg, LA: 0.04 ± 0.03pg, VT: 0.006 ± 0.006pg of GDNF/mg of tissue). These results suggest that GDNF protein content decreases with aging in all heart chambers and that exercise increases GDNF protein content in all heart chambers. The observed lowering of GDNF content with age may contribute to altered neural function observed in aged individuals, while the increased GDNF expression observed following exercise could help explain beneficial effects of exercise on cardiac innervation.

René N. Caballero-Floran, Andrew D. Nelson, Paul M. Jenkins

Title: Lithium partially restores presynaptic GABAergic signaling deficits in the Ank3 W1989R mouse model

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Abstract:

Multiple genome-wide association studies (GWAS) have shown that the *ANK3* gene is one of the most significant risk loci for bipolar disorder (BD). The *ANK3*gene encodes ankyrin-G, an adaptor protein that is involved in the formation of the axon initial segment (AIS), nodes of Ranvier, and GABAergic synapses. Recently, we have generated a mouse model with a W1989R mutation in *Ank3*, which abolishes the interaction between ankyrin-G and GABARAP necessary for ankyrin-G-¬dependent stabilization of postsynaptic $GABA_A$ receptors. We have shown that the *Ank3* W1989R mice have striking reductions in inhibitory currents in cortex and hippocampus compared to control mice resulting in increases in the intrinsic excitability of pyramidal neurons. Importantly alterations in inhibitory signaling have also been seen in BD patients. Consistent with this idea, we recently identified a BD family carrying the *ANK3* W1989R variant in our patient cohort in the Heinz C. Prechter Bipolar Research Program at the University of Michigan. The proband is a Caucasian male with type I BD characterized by recurrent mania and depression with a successful treatment with lithium. In these studies, we have treated *Ank3* W1989R mice for 21 days with chow containing lithium carbonate until serum levels reach the therapeutic range and used voltage clamp and current clamp whole cell electrophysiology recordings to measure inhibitory postsynaptic currents in cortical and hippocampal pyramidal neurons. Our results showed a 21 day lithium treatment partially reverses the defect in spontaneous inhibitory post-synaptic current (sIPSC) frequency, while not significantly affecting sIPSC amplitude. Since sIPSC frequency is a measure of presynaptic GABA release probability, we hypothesize that lithium is increasing activity of parvalbumin-positive GABAergic interneurons. In summary, these results suggest that the *ANK3* has an important role in the control of cortical and hippocampal neuronal excitability and dysfunction of this pathway may contribute to the imbalance of circuits seen in BD patients. In addition, our work suggests that lithium may act to increase the presynaptic GABA release in our model, perhaps resulting from increased excitability of parvalbumin-positive interneurons.

USE OF SITE-DIRECTED MUTAGENESIS TO PROBE THE SUBSTRATE BINDING SITE WITHIN SYSTEM x_c^-

Kevin Catalfano and Leah Chase

System x_c^- is a heterodimeric transporter that functions as a Na^+-independent antiporter exchanging extracellular cystine for intracellular glutamate. The transporter functions as an obligate heterodimer and is comprised of two proteins, xCT, which functions specifically in amino acid exchange, and 4F2HC, which appears to play a role in transporter stability. System x_c^- belongs to the SLC7 family of transporters, which is further subdivided into the cationic amino acid transporters (CAT) and the L-type amino acid transporters (LAT), the latter of which is the subfamily which includes System x_c^-. In recent years, there have been advances in our understanding of the structure of the SLC7 transporters as crystal structures of bacterial homologs of CAT and LAT transporters have been published. As a result, we can now begin to ask important questions about the mechanism by which System x_c^- exchanges cystine and glutamate across the membrane. Previous studies in the Chase lab have demonstrated that System x_c^- is a Cl^--dependent. Specifically, we have shown that transport of one molecule of cystine requires more than one Cl^- ion, and that Cl^- must bind to the transporter prior to cystine. This substrate specificity is novel among the SLC7 family of transporters, therefore, the goal of this study is to identify the amino acids within xCT that are important in Cl^- binding and substrate binding and exchange. Previous studies have suggested that Cys 327 is important for transport activity and this residue is also conserved in the bacterial homolog. We have also shown that chloride dependence of cystine transport is pH dependent. The $K_{0.5}$ of chloride increases as the pH increases, suggesting that the Cl^- binding affinity decreases at higher pH. Using this collective information, we have begun to examine the structure of xCT to identify the most likely binding site for cystine and glutamate. Thus far, we have used Chimera 1.13.1 and Pyrx to dock cystine into its most energetically favorable position on xCT, and examined the binding site for residues with pH-dependent side groups. We have identified His 373, Tyr 244, Arg 135 and Cys 327 (previously determined to be important in cystine binding using site-directed mutagenesis) in the binding pocket. We are currently identifying putative Cl^- binding sites within xCT in the presence and absence of docked cystine. Once our modeling studies are complete, we intend to use site-directed mutagenesis to test the hypotheses that these residues are important in cystine and/or chloride binding and transport. *This research is supported by Schaap Endowed Funds for Undergraduate Research.*

Repeated variable stress exposure in mid-adolescence attenuates behavioral and epigenetic effects of trauma-like stress in early adulthood

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Stressful experiences have diffuse effects on the adolescent transition into adulthood, and can regulate vulnerability to trauma and psychopathology in adulthood through lasting epigenetic modifications including histone deacetylase expression. Feedforward effects of adolescent stress can impair health, academic success, and social function, yet conflicting literature describes a capacity for adolescent stress to promote either risk or resilience to adverse effects of subsequent stress exposure on cognition. Here, we used a rodent model to determine how repeated variable stress exposure in mid-adolescence affects a cognitive deficit of trauma-like stress (single prolonged stress) in early adulthood. Additionally, we measured brain region specific levels of histone deacetylase (HDAC) 4 and 5, which control gene transcription and have been linked to long-term memory and vulnerability to posttraumatic stress disorder. We found that exposure to trauma-like stress in adulthood induced an extinction-retention deficit, characteristic of posttraumatic stress disorder, however this cognitive deficit was eliminated by prior stress exposure in mid-adolescence. Changes in histone deacetylase levels in several brain regions mediating fear learning were consistent with this pattern; only animals exposed to adult trauma-like stress in the absence of adolescent stress showed reduced HDAC4 and HDAC5 levels in the hippocampus, infralimbic cortex, and prelimbic cortex. Adolescent and adult stress did not have detectable effects on HDAC4 or HDAC5 in the amygdala, suggesting that adolescent stress history regulates the epigenetic effects of subsequent stress with regional-specificity. Overall, our results demonstrate that adolescent stress can program cognitive and epigenetic responsivity to adult trauma, and support the need for more comprehensive understanding of the lasting effects of adolescent experiences on HDAC expression and their role in regulating vulnerability to psychopathology throughout the lifespan.

Homeostatic Metaplasticity in Hippocampal Networks

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Synaptic scaling is essential for buffering prolonged destabilizing levels of activity in neural networks, but what factors determine how this form of plasticity can be repeatedly engaged are unknown. Here, we show in rat hippocampal neurons that a prior history of synaptic scaling, suppresses future scaling adaptations, revealing an important constraint on a network's ability to repeatedly compensate for activity shifts. On a mechanistic level, preliminary results suggest that prior scaling influences future scaling upstream from AMPAR accumulation at synapses but downstream from activity-dependent signaling, suggesting that altered gene transcription may underlie suppression. Consistent with this idea, profiling transcriptional dynamics with Bru-seq reveals a subset of genes that are differentially regulated by scaling history. Together, these data reveal a novel homeostatic metaplasticity mechanism whereby a history of synaptic scaling modifies the ability of network to implement future homeostatic adaptations.

Novel interaction between ankyrin-B and NaV1.2 is disrupted by autism spectrum disorder *de novo* mutations in *SCN2A*

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Department of Pharmacology

Ankyrins are a family of scaffolding proteins that organize ion channels, transporters, and cell adhesion molecules to the plasma membrane necessary for neuron excitability and function. One family member, ankyrin-G (*ANK3*), clusters the ion channel NaV1.2 (*SCN2A*) to the axon initial segment (AIS) early in development, which is the site of action potential generation. Late in development, NaV1.2 is replaced with NaV1.6 at the AIS and NaV1.2 localizes to the dendrites and modulates excitability; however, the mechanisms that underlie NaV1.2 dendritic localization are unknown. Another ankyrin family member, ankyrin-B (*ANK2)*, is expressed in the soma, dendrites, and distal axon. I hypothesize that ankyrin-B directly interacts with NaV1.2 in the dendrites of mature neurons to maintain proper neuronal excitability. To test this hypothesis, I overexpressed the NaV1.2 II-III loop, the known ankyrin binding region, in HEK293T cells. Using immunoprecipitation, I showed that ankyrin-B is capable of binding to the NaV1.2 II-III loop *in vitro* and mutation of the binding region abolished this interaction. To determine whether ankyrin-B and NaV1.2 interact *in vivo*, I immunoprecipitated Nav1.2 with ankyrin-B from P60 mouse brain. Lastly, I tested whether various *SCN2A de novo* human variants within the NaV1.2 II-III loop affected binding between ankyrin-B and NaV1.2 and found E1115K prevented their interaction. Future experiments will be conducted to evaluate neuron excitability in ankyrin-B heterozygous mice. *ANK2* and *SCN2A* are both strongly associated with ASD, thus this work may provide insight to common mechanisms that contribute to ASD.

Neural activity-dependent transcription start sites
Garay PM, Funk OH, Kwan KY, Iwase S

Transcription of a gene begins at the transcription start site (TSS). Over half the genes in the mammalian genome possess more than one TSS, raising the question of their purpose. The selection of TSS can impact cellular function in multiple ways, including changes in the translational efficiency of the RNA and the encoded N-terminal protein sequence. It has been shown that different TSS of a given gene are used distinctly across cell types and across development, but whether neurons leverage the utility of multiple TSS to undergo the process of synaptic plasticity remains obscure.

Using novel experimental and analytical toolsets, we discovered 40 genes in which a change in neural activity switched the relative usage of the gene's TSS. This pattern of expression rarely has been described, one prominent exception being *Bdnf*. The discovered genes encode chromatin regulators, transcription factors, and synaptic proteins critical for synaptic plasticity. The use of these TSS is greatly conserved in human neurons, and 14/40 genes have been associated with autism or neurodevelopmental disorders. Unlike *Bdnf*, whose alternative TSS yield transcripts encoding identical proteins, in 38/40 genes of interest, the alternative TSS encodes a distinct protein N-terminus, suggesting TSS-switching may impact protein function. In 8 genes, the alternative protein N-terminus contains an altered signal peptide, implying that alternative TSS may function to redirect their protein product to distinct cellular organelles. Current work has validated the use of alternative TSS *in vivo*. Our discovery and characterization of these TSS inform our hypothesis that activity-dependent TSS are critical for synaptic plasticity. Future aims include determining the use of these TSS in single neuronal subtypes, testing the impact of TSS on protein subcellular localization, and testing the relevance of TSS to synaptic plasticity.

STRUCTURAL AND FUNCTIONAL CHANGES OF PYRAMIDAL NEURONS IN PRIMARY MOTOR CORTEX AT THE SITE OF AN IMPLANTED MICROELECTRODE ARRAY

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Multielectrode arrays (MEAs) are implemented as chronic brain implants that allow neuron activity to be recorded for novel research and diagnostic purposes. The implementation of MEAs is restricted by variability in signal strength and quality thought to be the result of interactions with surrounding brain tissue, where gliosis and neurodegeneration are known to occur following MEA insertion. However, the link between the tissue response and neuron-specific changes in excitability are unknown. Our studies explore potential mechanisms of signal loss by characterizing dendritic morphology and intrinsic excitability of pyramidal neurons closely associated with an MEA. We employed fluorescence microscopy and patch-clamp electrophysiology in brain slice preparations containing a polyimide-based MEA device, harvested 1 week or 6 weeks after insertion into the primary motor cortex (M1) of Sprague-Dawley rats. Pyramidal neurons with somas <100 μm (Near-Device) and >500 μm (Distant-Device) from the device surface, sham-implant and non-implanted controls were investigated. Results show that Near-Device neurons have decreased spine density and limited dendritic arborization compared with controls. Whole-cell intracellular recordings showed two observations in near-device neurons: (1) neurons typically displayed a depolarized resting potential, lower input resistance, and lack of action potentials in response to current injection, or (2) neurons had a hyperpolarized resting potential, but varied in input resistance and spike firing properties. These data suggest that MEA insertion does impact dendritic architecture, while also altering the excitability of near-device cells. The results propose a novel role of local structural and functional plasticity surrounding MEAs related to signal loss and instability.

The Acute Effects of Melatonin on Striatal Dopamine Release: Progressive Electrochemical Analysis in an *Ex Vivo* Mouse Model Utilizing Fast Scan Cyclic Voltammetry

Kevin Hughes

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Abstract

The caudate putamen is a sub region of the basal ganglia, containing neural tracts important for cognition, reward learning, and voluntary motor function. Dopamine (DA) signaling received from the dopaminergic neurons of the substantia nigra pars compacta mediate locomotion, degradation of which is the characteristic neuropathology for Parkinson's disease (PD). PD is an initially neurovegetative motor disorder but can progress to include cognitive impairments as well. Sundowner's syndrome (SS) has been observed in patient populations with neurodegenerative diseases, characterized by the decline of cognition into evening hours. Due to the circadian influence which the hormone melatonin has on the sleep-wake cycle, attention has been drawn to its relationship with SS. While melatonin has been observed to decrease DA release, the real-time measurement of acute melatonin exposure on DA release within the caudate has yet to be studied. Utilizing various techniques of fast scan cyclic voltammetry (FSCV) in an *ex vivo* mouse model, we observed a decrease in DA release upon exposure to supraphysiological concentrations of melatonin. Results from this experiment support previous literature suggesting that activation of presynaptically expressed melatonin receptor 1 (MT1) plays an important physiological role in downregulating DA release. Additionally, results suggest that 1-hour of MT1 activation is a sufficient time-frame for significant downregulation of DA availability. This research seeks to deepen understanding of the complex roles melatonin has on neurotransmission.

Andrew D. Nelson, René N. Caballero-Florán, Jean Carlos Rodríguez Díaz, Melvin G. McInnis, Lori L. Isom, Chao Wang, Kevin S. Jones, Paul M. Jenkins

Title: A Loss-of-Function Variant in *ANK3* from a Family with Bipolar Disorder Causes Altered Forebrain Circuitry

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Abstract:

Bipolar disorder is a highly prevalent brain disease that affects approximately 1-2% of the general population worldwide. *ANK3*, which encodes the ankyrin-G protein, is one of the most significant genes linked to bipolar disorder through genome-wide association studies (GWAS); however, the functional effects of bipolar disorder-associated *ANK3* variants on brain circuitry are not known. GABAergic circuits are critical for the synchronization and higher order function of brain networks. Defects in this circuitry are linked to neuropsychiatric diseases, including bipolar disorder, schizophrenia, and autism. Previous work in cultured neurons has shown that ankyrin-G plays a key role in the regulation of GABAergic synapses on the axon initial segment and somatodendritic domain of pyramidal neurons where it interacts directly with the $GABA_A$ receptor associated protein (GABARAP) to stabilize $GABA_A$ receptors. Here, we generated a knockin mouse model expressing a mutation that abolishes the ankyrin-G/GABARAP interaction (*Ank3* W1989R) to understand how ankyrin-G and GABARAP regulate GABAergic circuitry *in vivo.* Coronal brain sections from homozygous *Ank3* W1989R mice showed a striking reduction in forebrain GABAergic synapses. In addition, whole-cell patch clamp recordings of miniature inhibitory postsynaptic current (mIPSCs) revealed a decrease in both the frequency and amplitude of GABA-mediated currents. *Ank3* W1989R mice also displayed smaller kainate-induced gamma oscillations, suggesting disruptions in network synchronization. Moreover, *Ank3* W1989R pyramidal neurons demonstrated reduced dendritic spine density and shorter axon initial segments likely as compensatory mechanisms to attempt to maintain homeostasis of neuronal excitability. Finally, we identified this variant, *ANK3* W1989R, in a family with bipolar disorder, suggesting a potential role of this variant in disease. Our results highlight the importance of ankyrin-G in regulating forebrain circuitry and provide novel insights into how *ANK3* loss-of-function variants may contribute to bipolar disorder in human patients.

Adult Zebrafish Astroglial Response to Olfactory Organ Damage in the Olfactory Bulb

Jackson Scheib and Christine Byrd-Jacobs, Ph.D.

The brain requires a degree of neuroplasticity to rewire and repair damaged neurons after injury. Humans have evolved to have a limited degree of neuroplasticity, which inhibits the ability to recover from brain trauma. Zebrafish, however, are renowned for their neuroplasticity, and their olfactory system is an excellent model for this. Glia are major mediations of neuroplasticity. Astrocytes, a type of glia, maintain homeostasis within the brain and react during brain trauma in a process termed astrogliosis which is characterized by an increase of cell branching, hypertrophy, and proliferation. Astrogliosis, in mammals, can be beneficial or neurotoxic especially if the trauma is repetitive. Since zebrafish are highly neuroplastic by nature, it is unlikely that astrogliosis will be neurotoxic. It is unknown if damage to the periphery will cause downstream astrogliosis, and if this is repetitive, if astrocytes will retain their astrogliosis morphology.

To induce neuronal trauma to zebrafish, we damaged the peripheral olfactory organ repeatedly for seven days and labeled astroglia in the olfactory bulb using antibodies against glial fibrillary acidic protein. Our hypothesis is that mechanical damage to the olfactory organ will cause astrogliosis in the olfactory bulb. When we measured the amount of astroglial staining in the olfactory bulb and a consistently found glomeruli throughout the timepoints, we found a significant increase of staining in the affected bulb when compared to the internal control, which is indicative of an increase of astrocyte branches, size, and/ or number. Interestingly, this effect was only seen at earlier time points. In addition, astroglial proliferation was observed after 24 hours after the first insult to the olfactory organ. These data lead us to believe that astrogliosis does occur in the presence of peripheral damage, but this process attenuates by later time points. Further exploration of the mechanism of zebrafish astrogliosis may lead to novel medical treatments.

GENE EXPRESSION CHANGES IN MULLER GLIA AFTER STIMULATION OF RETINAL PIGMENT EPITHELIUM WITH AN ALPHA7 NICOTINIC ACETYLCHOLINE RECEPTOR AGONIST

Authors
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Abstract

The adult mammalian retina does not typically regenerate. As a result, there is no cure for neurodegenerative diseases, such as glaucoma. We have found dedifferentiation of Müller glia (MG) occurs in the adult mammalian retina after application of an α7 nicotinic acetylcholine receptor (nAChR) agonist, PNU-282987 (PNU), to retinal pigment epithelial (RPE-J) cells in adult rodents (Webster, et al., 2019). RNAseq was performed in a MG cell line to determine changes in gene expression profiles following contact with RPE-J cells treated with PNU. RPE cultures were treated for 24 hours with 100 nM PNU and thoroughly washed to remove any residual PNU. MG were then exposed to treated RPE cells using a transwell system for 8, 12, 24 and 48 hours. A separate RPE culture was treated with DMSO for 48 hours as a vehicle control. Total RNA extracted from MG was sent to GeneWiz for Next Generation Sequencing (RIN>7) and basic bioinformatics was performed to determine significant changes in expression following transwell culture with treated RPE-J cells compared to control conditions. Up or down-regulated genes were compared with published literature of MG dedifferentiation that occurs in lower vertebrate regeneration or during early mammalian development. Between 8-12 hours, upregulation was observed in heparin-binding epidermal-like growth factor (HB-EGF) (log_2 fold change (FC) increase of 1.92). HB-EGF is rapidly induced in MG residing at an injury site in the neuronal retina of zebrafish and has been found to regulate the expression of Ascl1 (Goldman, et. al., 2012). After 48 hours, significant upregulation was found in genes Ascl1 (log_2 FC increase of 3.99) and Lin28a (log_2 FC increase of 4.77). Both Ascl1 and Lin28a are rapidly induced in dedifferentiating MG following injury in zebrafish. Ascl1 is a transcription factor essential for retinal regeneration in zebrafish (Goldman, et. al., 2008). Lin28a is an RNA binding protein whose expression has been found to share characteristics with embryonic stem cells (Nimmo & Slack, 2009). Importantly, downregulation was observed in BMP4 (log_2 FC decrease of -1.43) after 8 hours, which is a glial differentiation signal found to be necessary to fully repress glial gene expression for neurogenesis in early development in mice (Ueki, et al., 2015). These results suggest that MG are dedifferentiating in response to PNU-treated RPE-J cells. Analyzing these changes in gene expression could lead us to further our understanding of the barriers associated with adult mammalian neurogenesis and provide potential strategies to treat neurodegenerative diseases.

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Histone H3 Lysine 4 Methyltranferases and Synaptic Scaling

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Activity-dependent changes in gene expression play a critical role in enduring forms of synaptic plasticity and memory, but the molecular mechanisms that link neural activity with transcriptional control remain poorly understood. Methylation of histone 3 at lysine 4 (H3K4me) is a chromatin modification that marks transcriptionally active areas of the genome, and is placed by the KMT2 family of histone methyltransferases. Interestingly, single mutations in five out of the six KMT2s are associated with neurodevelopmental diseases in humans, implying non-redundant roles for these enzymes in neurons and brain development. However, the roles of these enzymes in post-mitotic neurons remain largely unknown. To ask how these H3K4me enzymes function in enduring forms of synaptic plasticity, we conducted a systematic analysis of KMT2 family members in synaptic scaling, a long-lasting homeostatic form of plasticity critically dependent on gene transcription. Whole-cell voltage-clamp recordings in rat primary hippocampal neurons following RNAi-mediated knockdown of each KMT2 family member surprisingly revealed unique roles for all six enzymes in synaptic scaling. Moreover, our results identify a clear division of labor among H3K4me enzymes in different facets of scaling, with KMT-2A and -2B (Trx in fly) required for downscaling, KMT-2C and -2D (Trr in fly) required for upscaling, and KMT-2F and -2G (dSet in fly) necessary for both up- and down-scaling. Hence, while 6 distinct methyltransferases all mediate a single chromatin mark (H3K4Me), our results reveal non-redundant roles for these enzymes in activity-dependent transcription required for enduring forms of synaptic plasticity. These findings thus provide a novel framework to understand how intricate patterns of chromatin regulation link neural activity with downstream control of gene expression.

MICROGLIAL PROLIFERATION PATTERNS FOLLOWING DAMAGE TO THE OLFACTORY BULB IN ADULT ZEBRAFISH

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The plasticity of the zebrafish olfactory system is a useful model for examining immune cell response after injury. Microglia are the resident immune cells that respond to damage in the CNS. We previously demonstrated the time course of the microglial response to olfactory bulb (OB) injury in adult zebrafish; however, it is unclear whether the response is from proliferating resident microglia or peripheral migration. We hypothesize that after damage, there will be an increase in resident microglia, followed by the influx of peripheral macrophages, rather than localized cellular proliferation.

A direct lesion to the OB in the whole fish was compared to a direct lesion to the isolated brain in culture removed of all afferent input. 4C4 antibody was used to label microglia, and PCNA antibody was used to label proliferating cells. There were some proliferating microglia in both OBs at most time points after injury, with a notable increase at 12h and 24h after injury in the whole fish. In the isolated brain, there were few to no proliferating microglial profiles in either OBs. Significant increases in activated microglial profiles following 1, 4, and 12h after injury to the isolated brain suggest that microglia can respond to signals without afferent input or peripheral influence, up to a certain time after injury. Our findings suggest that local proliferation may not be a major contributor to the microglial response to OB injury. Further work is required to explore microglial proliferation patterns and their potential role in recovery and regeneration after injury.

Neurodegenerative Disorders and Injury

The ability of lower vertebrates to regenerate entire organs is an intriguing phenomenon that has various beneficial implications for improving human health. Zebrafish have shared and conserved features with mammals, making them an ideal model to study regeneration. Intranasal irrigation with Triton X-100 produces severe degeneration of the olfactory epithelium, followed by rapid regeneration. We hypothesize that following chemical lesioning of the olfactory epithelium there will be changes in proliferation patterns that lead to recovery of the epithelium. We determined the amount of newly born cells and neurons in adult zebrafish by intraperitoneally injections of 50 μL/g body weight of BrdU, and the right naris was irrigated with TX-100. Anti-BrdU was used to label newly born cells and anti-HuC/D to label immature and mature neurons. We assessed recovery after 1, 3, 5 and 7 days following damage. In control fish, scattered BrdU+ profiles were present deep in the epithelium of the trough region, with few profiles in the side regions of the olfactory organ. Colocalization of BrdU and HuC/D was present in control fish. One day after TX-100 treatment, there was obvious thinning of the epithelium; many BrdU+ profiles were observed in the trough region, with very few in the side region and there was a decrease in Hu C/D staining. By three days, Hu C/D staining increases and the epithelial thickness appeared to return to control level; BrdU+ profiles were observed at various levels of the olfactory epithelium in both the trough and side regions. This appears to be evidence of differentiation and migration of olfactory sensory neurons. At five and seven days, BrdU profile numbers were substantially diminished, even below controls levels, and Hu C/D labeling was similar to control levels. Further investigation will focus on understanding the mechanisms involved in regenerating neurons after damage, with an overall goal of facilitating recovery from neurodegenerative diseases and traumatic brain injuries.

Abstract

LASTING BENEFITS OF STIMULATING TRANSPLANTED STEM CELLS IN UNILATERAL 6-OHDA LESIONED RATS

by Kevin Anderson

Of the treatments for Parkinson's disease (PD), a neurodegenerative disorder resulting from the degradation of dopaminergic (DA) neurons in the substantia nigra, stem cell transplantation demonstrates high potential. Preclinical studies demonstrate that intrastriatal transplantation of DA cells derived from stem cells attenuate PD symptoms; however, clinical data indicates that recurrence of symptoms may begin after 10-15 years. Curiously, several postmortem analyses suggest that the grafted cells were still releasing DA and that pathology spread was minimal. We theorized that a gradual adaptive response is occurring due to imprecise regulation of grafted cells. With lack of contextually relevant afferent stimuli, the phasic activity of these cells may become lost to the noise of imprecise DA control. We also theorized that by driving the cells optogenetically *in vivo*, we could potentially promote cellular integration and derive more behavior support than transplantation alone. With a previous study, we serendipitously discovered that when one stimulates the cells early on—approximately 5 days post-transplant—it led to reduction of PD symptoms up to 2 days later even without any further stimulation. We thus decided to conduct a follow-up study to see if we could replicate these results as well as determine if these benefits required behavioral context to occur. To start, all rats were injected unilaterally with nigrostriatal 6-OHDA, tested for limb bias pre-transplant, and grafted with light-sensitive DA neuron-like cells derived from mesenchymal stem cells. After 5 days, the animals according to group were either stimulated while swimming (*Stim-Swim*), not stimulated while swimming (*NoStim-Swim*), or were stimulated while sitting at rest (*Stim-NoSwim*). Swimming-related limb bias was then assessed 2 days and 7 days later, without stimulation. We thus explored whether stimulation alone, behavioral context, or both factors were important in mediating the extended reduction of motor deficits. Preliminary data suggests that early stimulation reduces deficits for up to 7 days. This effect is nearly absent in the NoStim-Swim group, suggesting that laser stimulation is responsible for this reduction. Curiously, the Stim-NoSwim group is showing reduced limb bias similar to the Stim-Swim groups, suggesting that in our paradigm, behavioral context has minimum impact compared to stimulation. Further data collection will determine how reliable this effect is, but current indications imply stimulating tranplanted cells early might promote long-term integration. This strategy may thus extend the longevity of stem cell transplants in human PD patients.

RECOVERY AND MORPHOLOGICAL REMODELING OF THE ZEBRAFISH OLFACTORY BULB FOLLOWING A FOCAL EXCITOTOXIC LESION

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Brain lesions are the leading cause of long-term disability worldwide. Patients suffering from these lesions frequently present incomplete recovery, since adult mammalian brains have a limited repair capacity following damage. On the other hand, zebrafish (Danio rerio) has an extraordinary regenerative potential since it effectively repairs brain lesions throughout the lifespan. The olfactory bulb is a highly plastic brain region that swiftly adapts in response to odors and to damage to the peripheral olfactory organs; however, the recovery and morphological remodeling of the zebrafish olfactory bulb following direct injury has not been studied. In this work we establish a new paradigm of focal excitotoxic lesion in the zebrafish olfactory bulb and investigate the recovery and remodeling of the lesioned olfactory bulb in time. We used adult zebrafish of both sexes and produced a focal excitotoxic lesion in the right olfactory bulb by injecting 1 µl of 15mM quinolinic acid (QA). We performed histological, immunohistochemical, stereological and morphological analyses of olfactory bulb sections following 1, 7, 15 and 21 days post lesion (dpl) in order to study damage, repair and morphological remodeling parameters following lesion. Although the exact location of the lesion varied, all lesioned bulbs exhibit a consisting wound of similar volume. Our results show that a QA focal excitotoxic lesion greatly damages the olfactory bulb and reduces its volume. This lesion causes apoptotsis, neuron loss, and a disruption of bulbar olfactory sensory axons. By 21 dpl, we observed significant repair, morphological remodeling and bulbar volume recovery, as well as cell number and axonal morphology reestablishment. To our knowledge, these results are the first to report a time-course characterization of the recovery of the lesioned olfactory bulb in zebrafish.

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Inherited tauopathy mutation alters the interaction between tau and protein phosphatase 1

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The tau protein is implicated in a variety of neurodegenerative disorders characterized by progressive degeneration of axons and neurons closely associated with pathologically-modified forms of the protein. The majority of the cases are sporadic but an important subset is caused by mutations, like P301L, within the tau gene itself. Due to the highly toxic nature of these mutations they are often used to model tau dysfunction but the mechanisms of toxicity remain poorly understood. By identifying the effect of mutant tau we hope to better understand toxic mechanisms engaged by pathological tau in all tauopathies. Several lines of evidence link tau toxicity to disruptions in axonal transport that may induce degeneration of axons, a common and early hallmark of disease. Previously, we showed that conformation changes in pathological tau activate a protein phosphatase 1 (PP1) and GSK3β signaling cascade that inhibits axonal transport in the squid axoplasm but exactly how tau engages this pathway remained unknown. We hypothesized that FTDP-17 mutations enhanced the tau-PP1 interaction and activation of PP1. To study this, we used WT full-length tau and a P301L mutant version of the protein to determine the nature of tau's interactions with the PP1γ isoform. We used pulldown assays and bioluminescence resonance energy transfer (BRET) assays to find that tau directly interacts with PP1 while the P301L mutation significantly enhanced the interaction. We also used phosphatase activity assays to find that tau increases PP1 phosphatase activity but no significant differences exist among WT and P301L tau. Expression of P301L, but not WT, tau altered axonal transport in primary rat hippocampal neurons. This effect was rescued through shRNA-mediated knockdown of PPγ. These results support a direct interaction between tau and PP1 that can enhance phosphatase activity and cause PP1γ-dependent disruptions to axonal transport. This suggests that P301L tau may induce neuronal toxicity through an increased functional protein-protein interaction with PP1γ.

Biochemical Characterization of Human Tau Expressed in a *Drosophila* Model of Tauopathy

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Program Theme C: Neurodegenerative Disorders and Injury

Alzheimer's disease and other closely related neurodegenerative disorders known as tauopathies are characterized by the accumulation of abnormally phosphorylated and aggregated forms of the microtubule-associated protein tau. Current research suggests that although neurofibrillary tau tangles themselves may not be toxic to neurons, smaller, intermediate tau filaments, including oligomers and/or multimers, may be toxic. The properties of tau oligomers and multimers are not well understood. Currently, there are established methods to isolate and identify tau oligomers in human and mouse models using sarkosyl extraction of insoluble tau from cultured neurons, followed by protein resolution using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). These methods, however, have not been established for use in *Drosophila melanogaster*. The aim of this project is to adapt the established protocols for sarkosyl extraction and SDS-PAGE used for human and mouse models for use with human tau expressed in *Drosophila*. Preliminary results indicate that our extraction and separation techniques are effective in isolating soluble and insoluble tau from flies. Comparing the ratios of soluble and insoluble tau from flies expressing wild-type and mutant forms of human tau will allow us to establish a relationship between the biochemical characteristics of tau and observed *in vivo* toxicity. Overall, this project establishes a protocol to analyze tau oligomers in *Drosophila* using sarkosyl extraction and SDS-PAGE.

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Revealing Functional Brain Activity Following Excitotoxic Injury to Retinal Ganglion Cells in a Diurnal Rodent Model

Nolan Krause, Ciara Brennan, John Dyke, Garrett Fogo, & Andrew J. Gall

Abstract: Intrinsically photosensitive retinal ganglion cells (ipRGCs) transmit light signals to the brain and contribute to non-image forming vision, such as synchronizing circadian rhythms to the light-dark cycle. Our lab recently showed that ipRGCs are resistant to excitotoxic damage and remain functional following N-methyl-D-aspartic acid (NMDA) administration to the retina, in a diurnal rodent, the Nile grass rat (Arvicanthis niloticus). Importantly, whereas non-image forming vision remained functional due to the survivability of ipRGCs, image-forming vision was significantly impaired due to damage to traditional retinal ganglion cells (RGCs). Specifically, RGC damage led to behavioral deficits in the Morris Water Maze, a test that requires rodents to use visual cues in order to find a hidden platform. We hypothesized that brain areas that are critical for image-forming vision in NMDA-treated grass rats would have significantly less neuronal activity than controls. To test this hypothesis, we used cFos, a marker for neuronal cell activation, to visualize neuronal activity in the brains of NMDA-treated grass rats and controls. We predicted that the primary visual cortex (V1), a brain region that is involved in image-forming vision, would exhibit significantly less cFos in NMDA-treated grass rats vs. controls. In contrast, we predicted that the suprachiasmatic nucleus (SCN) and intergeniculate leaflet (IGL), brain areas that are involved in non-image forming vision, would exhibit no difference in the amount of cFos in NMDA-treated grass rats vs. controls. Staining of brain tissue using cFos is complete, and we are currently mounting brain tissue on slides. We will image the brain areas and count the number of cells expressing cFos in V1, SCN, and IGL. Altogether, the present study aims to reveal the functionality of retinorecipient brain regions that are involved in visual functions following excitotoxic injury to RGCs in a diurnal rodent model, the Nile grass rat.

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Effects of low-dose developmental dieldrin exposure on neuroinflammation and α-synuclein aggregates in the mouse nigrostriatal pathway

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Abstract

Human and animal studies have shown that exposure to the organochlorine pesticide dieldrin is associated with increased risk of Parkinson's disease. Although previous work demonstrated that developmental dieldrin exposure increases neuronal susceptibility to MPTP toxicity in male C57BL/6 mice, the mechanisms driving this increased susceptibility are not well characterized. Male mice developmentally exposed to dieldrin display enhanced MPTP toxicity compared to mice treated with MPTP alone, showing greater induction of glial fibrillary acidic protein (GFAP) and α-synuclein (α-syn) expression. This suggests that dieldrin-induced changes in neuroinflammation and α-syn may underlie increases in neuronal susceptibility. Here, we tested the hypothesis that low-dose developmental dieldrin exposure induces changes in neuroinflammatory markers and α-syn, thereby increasing vulnerability of the nigrostriatal pathway to dopaminergic toxicity. Starting at 8 weeks old, female C57BL/6 mice were exposed to 0.3 mg/kg dieldrin by feeding every 3 days, continuing throughout mating, gestation and lactation. At 12 weeks of age, male and female pups from independent litters were sacrificed, and striatum and substantia nigra were dissected. To determine whether sex-specific changes in neuroinflammation or α-synuclein underlie male-specific enhanced vulnerability, both sexes were included in analyses. We assessed markers of neuroinflammation via targeted expression assays to test if developmental exposure to dieldrin led to induction of neuroinflammatory pathways in the striatum and substantia nigra. In addition, we analyzed α-syn aggregation by western blot in non-denaturing and non-reducing conditions to test whether exposure leads to changes in α-syn species. We identified changes in both systems, demonstrating that developmental dieldrin exposure produces "sub-toxic" changes in these pathways that produce a phenotype of increased vulnerability. In a parallel study, we identified sex-specific DNA methylation changes in genes related to the development and maintenance of the nigrostriatal pathway. Taken together, these data suggest that developmental dieldrin exposure leads to persistent changes in phenotype that may contribute to the development of Parkinson's disease.

Decision tree analysis identifies changing patterns, potential drug interactions, and biomarkers associated with opioid deaths

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Opioid abuse is now the primary cause of accidental deaths in the US. The cyclical nature of illicit drug use is well established, with a current resurgence of heroin abuse and fentanyl's emergence as a major cause of drug-related deaths. The present study examined forensic toxicological data from the Wayne County Medical Examiner's Office from 2012-2017 to better understand abuse trends and to explore potential lethality of specific drug-drug interactions. Our analyses revealed clear changes in opioid abuse and significant variations in trends of abused drugs over a six year period, including the growing relevance of fentanyl and its analogs as highly significant causes of lethality starting in 2014. The number of fentanyl-related cases surpassed those with heroin in 2017, making it more predictive of death due to drugs than previously reported. Only a small number of cases involved fentanyl alone, suggesting that it is more commonly used as an adulterant of other drugs of abuse, which may account for higher rates of co-abuse. We also observed that the presence of specific fentanyl analogs fluctuates over time. We used Chi-square Automatic Interaction Detector (CHAID) decision-tree analysis to investigate the relationship between the presence in blood of any particular drug and the determination of a drug or drugs as a cause of death. This analysis identified blood biomarkers of specific drug or drug combinations and suggested potentially deadly interaction between methadone and the commonly used anti-depressant citalopram. Decision-tree analyses also showed the presence of fentanyl plus the benzodiazepine midazolam was diagnostic for in-hospital deaths following serious medical illness and interventions that included these drugs. The data presented resulted from a systematic collection and unbiased analyses of forensic toxicological results. They highlight the continued abuse of prescription opioids, growing abuse of heroin and the danger of fentanyl and its analogs. Our local trends largely mirror those of the most recent findings from the CDC on fentanyl abuse, as well as other trends of co-abuse reported around the USA for cocaine, benzodiazepines and other drugs of abuse. These data highlight the power of decision tree analyses not only in determination of cause of death, but also as a key surveillance tool to identify previously unknown drug interactions and inform drug abuse treatment and public health policies for combating the opioid crisis.

NON-INVASIVE OPTOGENETIC STIMULATION IN A RAT MODEL OF SPINAL CORD INJURY

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Spinal Cord Injury (SCI) is an injury that can result in paralysis below the site of injury. Out of all of the common methods used in medicine, most of the spinal cord injury treatment procedures have negative trade-offs that could permanently damage neurons. We are going to couple a non-invasive method to target neurons after injury along with swim therapy. In the case of this study we will be able to target interneurons of the spinal cord by combing an adeno associated virus (AAV) serotype 2/9 with a synapsin promoter to restrict gene expression and subsequent stimulation to interneurons of the spinal cord. The AAV also expresses a light-emitting luciferase fused to a channelrhodopsin, which is light sensitive. Neural cells will be stimulated by the addition of Coelenterazine (CTZ), which is the luciferase substrate. CTZ injections will ultimately lead to activation of synaptic networks of neurons that express the excitatory luminopsins. The Human Synapsin Promoter (hSYN1) can drive expression of the luminopsin, LMO3, which is stimulated by CTZ in adult rats. This will result in functional connectivity across the site of injury, aiding in the recovery of locomotion below the site of injury. After the interneural spinal cord stimulation, rigorous swim testing and therapy will be conducted in order to analyze the effects of the swim testing. Constant gait analyses will be done in order to evaluate the progression of the gait of the rats after injury and throughout the treatment.

Low-dose Developmental Dieldrin Exposure Alters DNA Methylation at Genes Related to Parkinson's Disease in Mouse Midbrain

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Abstract

Human and animal studies have shown that exposure to the organochlorine pesticide dieldrin is associated with increased risk of Parkinson's disease (PD). Despite previous work showing a link between developmental dieldrin exposure and increased neuronal susceptibility to MPTP toxicity in male C57BL/6 mice, the mechanism mediating this effect has not been identified. Here, we tested the hypothesis that developmental exposure to low-dose dieldrin increases neuronal susceptibility via genome-wide changes in DNA methylation. Starting at 8 weeks of age, female C57BL/6 mice were exposed to 0.3 mg/kg dieldrin by feeding every 3 days for 30 days prior to mating; maternal dieldrin exposure then continued for the duration of gestation and lactation. At 12 weeks of age, pups were sacrificed and midbrains were dissected. Offspring DNA was then isolated and dieldrin-related changes in DNA methylation were assessed via reduced representation bisulfite sequencing (RRBS). We identified a number of significant differentially methylated CpGs (DMCs) and differentially methylated regions (DMRs) by developmental dieldrin exposure (FDR<0.05). Furthermore, stratification by sex showed that dieldrin exposure had distinct effects on the male and female epigenome. In the male mice, we found dieldrin-related differential methylation within the gene body of the imprinted *Grb10* locus. *Grb10* encodes an adaptor protein that interacts with Grb10-interacting GYF Protein 2 (GIGYF2), a protein encoded in the PARK11 locus that may play a role in PD-related neurodegeneration. Meanwhile, in the female mice, gene ontology pathway analysis showed an enrichment for differential methylation at genes related to central nervous system development. These genes clustered together in an interaction network, and one of the identified loci – *Nr4a2* – encodes a transcription factor that has previously been implicated in PD pathogenesis and development of the monoaminergic phenotype. In targeted RNA-sequencing analyses, a protein-coding *Nr4a2* transcript showed marginally significant differential expression by dieldrin exposure in female mice (p=0.06). Combined, these data suggest that developmental dieldrin exposure can establish a poised epigenetic state at genes related to PD, and that sex-specific epigenetic changes at these loci may contribute to the development of neurodegenerative disease.

COMPARISON BETWEEN BOVINE AND OVINE SOURCES OF GANGLIOSIDE GM1 AS A POTENTIAL TREATMENT OF HUNTINGTON'S DISEASE IN YAC128 HD MICE MODEL

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Huntington's disease (HD) is a neurodegenerative genetic disorder caused by mutations in the HTT gene containing an abnormally long polyglutamine (CAG) stretch. This leads to excessive production and accumulation of mutant huntingtin protein (mHtt) in the brain. The excessive mHtt protein accumulation causes progressive motor, psychiatric, and cognitive decline and eventually leads to neuronal death. Given that only palliative treatments are available and no effective treatment has been approved, researchers are looking at a variety of potential therapeutics for HD, including the use of GM1 ganglioside. GM1 ganglioside is a sialic acid-containing glycosphingolipid that is found abundantly in the outer leaflet of the neuronal membrane in the brain. GM1 gangliosides play a vital role in cell signaling, calcium homeostasis and cell-cell interactions in the brain. In HD patients, GM1 ganglioside levels are decreased, which contribute to HD symptomology and disease progression. Previous studies found that treatment with GM1 ganglioside obtained from bovine sources improves motor dysfunction in HD rodent models. However, GM1 gangliosides that are obtained from ovine sources are more economical than bovine sources and the goal of our experiment were to test whether the ovine source can produce similar effects in improving motor and cognitive dysfunctions in HD. In our current study, we used intraventricular osmotic pumps to deliver GM1 gangliosides that are obtained from ovine and bovine sources for six weeks in eight to nine months YAC128 mouse model. We used rotarod-, open field-, catwalk-, Barnes maze-, novel-object-recognition- and elevated-plus- maze- tasks to measure non-motor parameters. Our premiliminary results indicate that both bovine and ovine sources of GM1 tended to reduce behavioral deficits in YAC128 mice on the rotarod-, open fields-, Barnes maze-, but not on novel-object-recognition-, catwalk-, and elevated-plus-maze- tasks.

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Title: *In vivo* redox regulation of ΔFosB's structure-function relationship in Alzheimer's

Authors: H. Lynch, C. McCornack, V. Bali, G. Rudenko, E.J. Nestler, A.J. Robison

Abstract:
Brain function is regulated by a variety of factors, and this regulation occurs partly at the level of gene expression within neurons. These regulating factors can alter the reduction/oxidation balance within neurons (redox state); an effect associated with various neuropathologies, including Alzheimer's Disease (AD), but the mechanisms by which redox state controls gene expression in neurons is unknown. Many neurons critical for such activities as memory, mood, and motivated behaviors orchestrate expression of select critical genes through transcription factor ΔFosB, a stable splice variant of the *FosB* gene. We show that the redox-dependence of the structure-function relationship of fos-family proteins found *in vitro* is also conserved in ΔFosB *in vivo*; a characteristic that is preserved across the brain. Under non-reducing (oxidizing), fully denatured conditions, immunoprecipitation followed by Western blot reveals a shift in the molecular weight of ΔFosB from 37kDa to ~75kDa and ~150 kDa; potentially representing the binding of ΔFosB to other proteins through disulfide bridge formation between cysteine residues that has been demonstrated *in* vitro. We demonstrate that JunD and Smad3 are examples of potential binding partners. In contrast, under reducing conditions, ΔFosB remains at 37kDa, indicative of no covalent complex formation. Taken together, these data suggest that ΔFosB complex formation in the brain is directly regulated by redox state through disulfide bonds. Better understanding how the structure-function relationship of ΔFosB is regulated by redox state may ultimately allow us to use ΔFosB as a therapeutic target for diseases associated with an altered redox state, like AD.

Title: Effects of Age and Exercise on Density of Sympathetic Innervation in Rat Vasculature
Authors: Kori L. Mecklenburg, Gabriel Alves, Alberto F. Cintrón-Colón, John M. Spitsbergen

Hypertension is a condition that affects nearly 75 million people in the United States. To better understand this mostly idiopathic condition, the role of neurotrophic factors on arterial innervation and consequent blood pressure changes must be understood. Nerve growth factor (NGF) and glial cell-line derived neurotrophic factor (GDNF) have been shown to support development and maintenance of the sympathetic nervous system. The aim of this study was to reveal how density of sympathetic innervation changes in mesenteric arteries over time and with six months of voluntary exercise. Additionally, this study aimed to reveal the localization of GDNF and NGF alongside these changes in innervation pattern. To accomplish this, density of sympathetic innervation was measured in male Sprague Dawley rats. Densities were measured in a group of four-week-old sedentary rats, an exercised and sedentary group of one-year-old rats, and an exercised and sedentary group of 18-month-old rats. The density of sympathetic innervation was significantly less ($p < 0.05$) in the sedentary 18-month-old group (2.74 ± 0.319 grid crossings per $900\mu m^2$) than in the four-week-old group (3.735 ± 0.276 grid crossings per $900\mu m^2$). There was no difference in density of innervation between the four-week-old group and the 18-month-old exercised group suggesting that exercise blocked the decrease in sympathetic innervation. GDNF and NGF do not appear to be localized within sympathetic fibers. NGF and GDNF appear to be localized around adipocytes but more research must be conducted to confirm this result and its implications. Blood pressure increased throughout age and was significantly lower in age-matched exercised rats. Results suggest that exercise may promote a youthful pattern of sympathetic innervation. More studies must be done to investigate how these changes affect blood pressure. In the future, studies will contain female rats and explore how changes in sensory innervation pattern of arteries occur alongside changes in sympathetic innervation pattern. Understanding the role of neurotrophic factors in arterial control is crucial for understanding mechanisms behind conditions like hypertension.

Title: STN DBS REDUCES LEWY BODY-LIKE ALPHA-SYNUCLEIN INCLUSION FORMATION TRIGGERED BY INTRASTRIATAL FIBRIL INJECTION

Kathryn M Miller, Christopher J Kemp, Joseph R Patterson, Anna C Stoll, Kathy Steece-Collier, Kelvin C Luk, Michael R Kubic and Caryl E Sortwell

Objectives:
Whether deep brain stimulation (DBS) of the subthalamic nucleus (STN) is disease-modifying for Parkinson's disease (PD) remains unclear. A hallmark of PD pathology is the progressive accumulation of alpha-synuclein (alpha-syn) inclusions (Lewy bodies). To date, only a single report has examined whether Lewy body pathology is impacted in PD subjected by DBS (PMID: 27911008). While no effect was observed, this study was limited by the 14-year disease duration prior to DBS, long after the establishment of Lewy body pathology. No preclinical study has examined whether STN DBS can prevent the formation of alpha-syn aggregates. The distinct alpha-syn aggregation phase in the alpha-syn PFF model can be leveraged to examine the impact of STN DBS on accumulation of Lewy body-like pathology. Adult male rats received intrastriatal injections of alpha-syn preformed fibrils (PFFs) and were implanted with electrodes in the STN during the same surgical session. Three days later, animals were randomly assigned to receive either stimulation or no stimulation for a period of 30 days. Stereological assessment of nigral neurons possessing phosphorylated alpha-syn (pSyn), truncated phosphorylated alpha-syn (pSyn*), and fibrillar alpha-syn (F2) inclusions served as the primary outcome measures. STN DBS in rats reduced the formation of alpha-syn aggregates by ≈30% ($p \leq 0.022$). Quantification of tyrosine hydroxylase immunoreactive (THir) nigral neurons and examination of BDNF-trkB signaling (ELISA) also was conducted and showed no stimulation-related differences. Collectively, our preliminary findings suggest that STN DBS may be disease-modifying by decreasing alpha-syn pathology.

The Use of Curcumin Encapsulated Dendrimer as an Anti-Inflammatory Agent for Glioblastoma Therapy in C57BL/6J Mice.

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Abstract:

Glioblastoma (GB) is an aggressive form of brain tumor. Currently there are no viable treatment options outside of radiation therapy and chemotherapy for this disease. Less than 30% of patients with GB live longer than two years post-diagnosis. GB is known to cause significant inflammation leading to increase in tumor size and metastasis. Therefore, reducing inflammation in GB could be a potential therapy for the cancer. To achieve this we are utilizing dendrimer nanoparticles (D) with curcumin (a known phytochemical having anti-inflammatory property) encapsulated within the dendrimers. Dendrimer nanoparticles are multi-branched, star-shaped macromolecules with nanometer-scale dimensions. Dendrimers can be defined by three components. The first being a central core, the second being an interior dendritic structure (the branches), and finally an exterior surface with functional surface groups. The varied combination of these components yields dendrimers of different shapes and sizes with shielded interior cores that are ideal candidates for applications in both biological and materials sciences. What makes dendrimers potentially very useful for the treatment of many diseases is that fact that the shielded interior cores can carry cargo within them. For this study, we used Cystamine core (S=S) dendrimer, which has the ability to split into dendrons (halves) in the presence of glutathione, which is present at high concentrations in the GB cells. This will enable the curcumin cargo to be delivered within the GB cells thereby rendering their anti-inflammatory properties. In addition to Curcumin, when the dendrimer splits, it is converted into its reduced form and forms thiol groups (-SH), having the anti-inflammatory effect. Our *in vitro* study showed that curcumin causes GB cell death while specifically sparing other types of cell such as stem cells and neurons at certain concentrations. In addition, we have showed that Curcumin as well as the cystamine dendrimers show anti-inflammatory effects in cells. Following this, our *in vivo* study involved use of mice having tumor induced by Gl261 cells. Following treatment with dendrimers and dendrimer encapsulated curcumin showed (1) a decrease in astrocytes within the brain suggesting a decrease in inflammation around the tumor cite; (2) a decrease in the amount of activated microglia within the brain, also suggesting a decrease in inflammation; (3); and a significant increase in lifespan of mice with GB. Overall our study shows that the use of curcumin encapsulated dendrimer nanoparticles is a viable treatment for GB due to the anti-inflammatory effect and longer lifespan that has been observed.

DUAL PROTECTIVE ACTION OF αA-CRYSTALLIN ON RETINAL GLIAL AND NEURONAL CELLS DURING METABOLIC STRESS

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Purpose: Diabetes initiates retinal neurodegeneration through a multitude of mechanisms implicating neuronal and non-neuronal cells. We recently demonstrated that the molecular chaperone αA-crystallin is a potent neuroprotector for retinal neurons, but the mechanism by which αA-crystallins exerts retinal neuroprotection in early diabetes is still unclear. The present study was carried out to assess the interaction between αA-crystallins and other known neuroprotective mechanisms during metabolic stress.

Method: Differentiated R28 cells, a model of retinal neurons, as well as rMC-1, a Müller glial cell line, or primary Müller glial cells from WT and Ko-αA-crystallin mice were transfected with plasmids encoding either wild-type (WT), phosphomimetic (148D), or non phosphorylatable mutants (148A) of αA-crystallin on the 148 residue, as well as constitutively active (CA) or dominant negative (DN) forms of Akt. The cells were then either exposed to serum starvation (SS), high glucose (25mM) or high glucose with TNF-alpha (100ng/ml) (HG+T) in order to assess the effect of αA-crystallin and Akt on cell survival (cell viability assays) as well as induction of inflammation (inflammatory markers expression by qPCR).

Results: Elevated levels of inflammatory markers in SS group were diminished in *αA-WT* (IL-1β- 67%; MCP-1-26%; IL-6-12%) and further in *αA-148D* (IL-1β- 70%; MCP-1-30%; IL-6-15%) as compared to EV. The increase of IL-1β, MCP-1 & IL-6 were blunted in HG+T by *αA-WT* (IL-1β-17%; MCP-1-180%; IL-6-36%) and to a greater extent by *αA-148D (* IL-1β-21%; MCP-1-202%; IL-6-45%) when compared to EV. The overexpression of CA-Akt in combination with αA-WT or αA-148D mutant, or by itself significantly decreased the SS-induced (*αA-WT -49%; αA-148D-62%; CA-Akt alone-40%)* and HG+T-induced (*αA-WT -61%; αA-148D-66%; CA-Akt alone-88%)* cell death *compared to the EV.* Of note, DN-Akt co-transfection with αA-crystallin plasmids did not show any reduction in cell death in the SS or HG+T group as compared to EV. Similarly, overexpression of αA-148A alone or in combination with Akt did not have any protective effect in either of the metabolic stress.

Conclusion: The data strongly suggest that αA-crystallin plays a dual neuroprotective role in the retina under metabolic stress, via reduction of the inflammatory response of Müller glial cells and interaction with the pro-survival Akt pathway in neurons.

INDUCED REGENERATION USING AN ALPHA7 NICOTINIC ACETYLCHOLINE RECEPTOR AGONIST IN A GENETIC MOUSE GLAUCOMA MODEL

Authors

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Abstract

Glaucoma is a degenerative retinal disease characterized by loss of vision due to progressive loss of retinal ganglion cells (RGCs). Previous studies in this lab have shown that the application of a specific α7 nicotinic acetylcholine receptor agonist, PNU-282987 (PNU), to the murine eye induces neurogenesis of numerous retinal cell types, including retinal ganglion cells (Webster et al., 2017). The aim of this study is to characterize the short-term and long-term effects of PNU in a glaucoma model. To address this issue, the effects of PNU were analyzed in a DBA/2J mouse model that auto-induces a glaucoma-like condition in adulthood. These mice manifest an elevated intraocular pressure (IOP) starting at 6 months, followed by loss of ganglion cells. We hypothesized that PNU would act to regenerate new retinal neurons in a genetic mouse model of glaucoma. PNU (1mM) and BrdU (1 mg/mL) were applied as eye drops for 2 weeks to male and female DBA/2J mice at various ages (3, 6, and 10 months) to determine the regenerative effects that occur at each of these time points. After two weeks of treatment, the retinas from both the control animals and from the treatment animals were removed, fixed, processed using IHC procedures, and RGC regeneration was assessed and compared. Antibodies against BrdU were used to identify new cells. Retinal ganglion cells were identified using antibodies against Thy-1.2. Cell counts were obtained using confocal images of flat mounted retinas. Four images were taken of the retinal ganglion cell layer of each retina, each image taken from a different quadrant of the retina and RGC counts were averaged. A one-way ANOVA was used to compare the number of cells between the different time points. IOP measurements were obtained under control and treated conditions. IOP measurements in untreated animals significantly changed from an average of 10.79 to an average of 19.79 mmHg ($p \leq 0.01$; n=8). Results showed a significant loss of retinal ganglion cells between the 3-month-old and the 10-month-old control mice ($p \leq 0.01$; n=5). The average number of RGCs dropped from 310($\pm$21) in 3 month DBA/2J animals to 221($\pm$12) in 10 month DBA/2J animals, a loss of about 30%. Similarly, the 10-month-old DBA mice had about 25% fewer cells than non-DBA control mice at the same age ($p \leq 0.01$; n=5). The addition of PNU caused about a 15% increase in the number of retinal ganglion cells in all time points compared to the untreated DBA/2J retinas ($p \leq 0.01$, n=4). These were verified to be new cells using BrdU labeling. PNU treatment had no effect on IOP measurements. These results suggest the future clinical potential of PNU-282987 in the treatment of degenerative retinal diseases.

Functional characterization of recombinant αA–crystallins as potential therapeutics for neurodegeneration–associated cell death in diabetic retinopathy (DR).

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Diabetic retinopathy (DR) is the primary ocular complication associated with diabetes and the primary cause of vision loss in the working-age population worldwide. Despite recent progress especially in regards to the success of anti-VEGF molecules for the treatment of advanced stages of DR, there remains a dramatic need for therapeutic treatment options for the early neurodegenerative conditions. While the retinal neurodegeneration associated with the early stages of DR is now well recognized, no therapeutic strategies are available. α–Crystallins (αA and αB) are well-studied small heat shock proteins that function as molecular chaperones, preventing non-specific protein aggregation and cell death in response to a multitude of stress conditions. Studies from our lab validate the neuroprotective potential of exogenous αA–crystallin, as conditioned media from αA–crystallin overexpressing glial cells prevented stress induced apoptosis of neuronal cells in culture. Additionally, our work with human donor retinal samples has revealed a progressive loss in αA–crystallin chaperone function in DR through alteration of its phosphorylation on T148. Preliminary studies involving *in vitro* chaperone assays on αA–crystallin phosphomimetic, αA–T148D, have revealed its enhanced chaperone activity. Furthermore, supplementation of recombinant wild type αA–crystallin reduced metabolic stress induced apoptosis in differentiated retinal neural-like cells. Our current study demonstrates that the anti-apoptotic effect of the αA–T148D is associated with a significantly enhanced chaperone activity. Overall, the study strongly supports the use of αA–crystallins as proteins of therapeutic interest for the treatment of retinal degeneration associated with early stage DR.

Analyzing Toxic Tau Degradation and Clearance *in a Drosophila* Model of Tauopathy

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Program Theme C: Neurodegenerative Disorders and Injury

Alzheimer's disease (AD) is a neurodegenerative disorder associated with two pathological hallmarks: β-amyloid plaques and neurofibrillary tangles (NFTs). Studies have shown that the microtubule-associated protein tau becomes toxic in AD, producing a soluble, highly phosphorylated, aggregated form of tau. Our research suggests clearance of toxic tau species are important to preventing cell death. There are two normal cellular pathways used by cells to recycle and/or degrade proteins: the ubiquitin-proteasomal system (clearance of healthy proteins with short half-lives) and the autophagic-lysosomal system (clearance of large cell components with long half-lives). Full length, non-toxic tau (~65 kD) is thought to be cleared via ubiquitin-proteasomal degradation. Toxic-tau aggregates are thought to be cleared via the autophagic-lysosomal pathway. We have optimized a method for culturing tau-expressing neurons, along with staining and immunofluorescence for confocal microscopy analysis. Previous culturing conditions had numerous fungal infections, produced clumped cells lacking neuronal processes (axons and dendrites), and had non-specific staining. We optimized a protocol reducing infections, distributing cells across the coverslip, increasing cellular processes, and staining specifically for cellular structures and tau, leading to reliable confocal analysis. The next steps of this project will analyze the pathway for clearance of toxic tau species in living *Drosophila* neurons by taking a pharmacological approach, using drugs to inhibit or promote the autophagic pathway. Discovering the underlying mechanism of tau clearance in AD will unveil a fundamental system in diseased neurons and bring researchers closer to enhancing health, lengthening lifespan, and reducing illness.

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PHOSPHOMIMETIC AND NON-PHOSPHORYLATABLE MUTANTS OF α-CRYSTALLIN EXHIBIT DIFFERENCES IN SOLUBILITY, Sluzala, ZB & Fort PE, University of Michigan Neuroscience Program

α-Crystallins are molecular chaperones expressed in the lens and retina. Our lab has demonstrated a neuroprotective role of one of the members of this family, αA-crystallin, in the retina. We have shown that one mechanism by which αA-crystallin protects cells is by interacting with the pro-apoptotic protein Bax and sequestering it in the cytoplasm. In mouse models of diabetes, however, the neuroprotective ability of αA-crystallin is progressively impaired. We have previously shown that the solubility of αA-crystallin is substantially decreased in both mouse models of diabetes and human samples from donors with diabetic retinopathy. Additionally, while overall αA-crystallin protein content increased, we recently showed that its phosphorylation on T148 is dramatically decreased in both the retina from rodent models and diabetic human donors. This leads us to hypothesize that phosphorylation of T148 controls, at least in part, the chaperone and neuroprotective functions of αA-crystallin. This hypothesis is supported by our recent data that R28 retinal neurons overexpressing either the phosphomimetic (T148D) or wild-type (WT), but not the non-phosphorylatable (T148A) αA-crystallin were strongly protected from various cellular stresses. In order to further characterize the mechanism by which pT148 controls the neuroprotective function of αA-crystallin in differentiated R28 retinal neurons, we investigated differences in solubility following transfection with the WT, T148A, or T148D. To determine how metabolic stress may affect the solubility of any of the constructs, subsets of cells from each construct were serum-starved for 4 and 24 hours. Levels of soluble and insoluble αA-crystallin were determined by immunoblot analysis. Immunoblot results showed a higher level of insoluble αA-crystallin in T148A cells, and an equal, if not lower level of insoluble αA-crystallin in the T148D cells. Collectively, these results further substantiate the hypothesis that phosphorylation of T148 affects the solubility of αA-crystallin, and further implicate pT148 in the regulation of αA-crystallin's protective role.

Microglial depletion to attenuate synucleinopathy-triggered neuroinflammation

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Parkinson's disease (PD) is a neurodegenerative disorder that is characterized by the presence of proteinaceous alpha-synuclein (α-syn) inclusions (Lewy bodies), the progressive loss of the nigrostriatal dopamine (DA) neurons and markers of neuroinflammation. These pathological features can be recapitulated *in vivo* using the alpha-synuclein preformed fibril (PFF) model of synucleinopathy. Our lab has revealed that the peak of α-syn inclusion formation and neuroinflammation in the substantia nigra pars compacta (SNpc) is two months post PFF injection (PMID 29843738), long before neurodegeneration is evident. These results suggest a role for neuroinflammation in the progression of PD and that activated microglia could represent a potential target for novel therapeutics. The goal of this project is to determine whether depletion of endogenous microglia can impact the process of α-syn aggregation and/or microglial activation. Pexidartinib (PLX3397), a colony stimulating factor-1 receptor (CSF1R) inhibitor, has been shown to decrease microglia levels (PMID 24742461). The CSF1R is known to be integral to the activation, survival, and proliferation of microglia. Male Fischer 344 rats were injected intrastriatally with either alpha-synuclein PFFs or vehicle. Rats were fed chow containing Pexidartinib (600ppm) or control chow for a period of 2 months. Preliminary data demonstrates a 42% depletion of ionized calcium-binding adapter molecule 1 immunoreactive (iba-1-ir) microglia within the substantia nigra of PLX3397 treated animals as compared to controls. A depletion of greater magnitude was observed when rats were fed PLX3397 as well as the CYP3A4 inhibitor Ritonavir. Ritonovir co-administration also resulted in increased PLX3397 levels in plasma, CSF and brain. Post mortem tissue outcome measures will include immunohistochemistry combined with stereological assessment to determine number of iba-1-ir and major histocompatibility complex II immunoreactive (MHC-IIir) microglia, α-syn inclusion-containing neurons in the SNpc and status of the nigrostriatal system. These studies will determine whether PLX3397-mediated microglial depletion alters the accumulation of α-syn inclusions and/or the inclusion-triggered neuroinflammation. Future long-term studies will examine the impact of PLX3397-mediated microglial depletion on nigrostriatal degeneration induced by synucleinopathy. Positive results would support a strategy in which Pexidartinib, currently being assessed in multiple Phase II-III clinical trials, could be repurposed to attenuate the neuroinflammation and progression of nigrostriatal degeneration in PD.

THE ROLE OF NEUROINFLAMMATION ON THE CLEARANCE OF MACROMOLECULAR SOLUTES IN THE RAT BRAIN

Suresh, S, Larson, J, Jenrow,K, Central Michigan University

Previous reports suggest that neuro-inflammation increases steadily in the aging brain and is associated with an age-related decrease in the clearance of macromolecular waste from the brain parenchyma. Impaired waste clearance has also been reported in a variety of neurodegenerative disease and traumatic brain injury models, where chronic neuroinflammation is a common underlying pathology. Here we investigate whether chronic neuroinflammation in the absence of other brain pathologies is sufficient to impair waste clearance from the rat brain, and whether this promotes the accumulation of endogenously-derived macromolecular waste and/or cognitive impairment over time. Using a rat model of chronic neuroinflammation induced by lipopolysaccharide (LPS), the clearance kinetics of two fluorescently-tagged dextran tracers from the brain parenchyma, and ultimately into the blood serum, were assayed 8-weeks post-induction. Cognitive function was assayed between 30 and 36-weeks post induction, using the open field, novel object recognition, and contextual fear conditioning assays. The expression and distribution of aquaporin-4 (AQP4) and amyloid β (Aβ) proteins were subsequently assayed within selected brain regions. Relative to controls, chronic neuroinflammation significantly impaired the clearance kinetics of both dextran tracers such that their concentrations were significantly increased in the brain parenchyma and significantly decreased in blood serum. Behaviour analysis revealed significantly enhanced fear memory and a trend toward context generalization in the LPS group, consistent with a heightened state of anxiety. Chronic neuroinflammation was associated with a loss of aquaporin channel polarization on astrocytic end-feet processes within the prefrontal cortex and hippocampus, and significantly elevated Aβ in the hippocampus, where punctate Aβ deposits were evident within perivascular space. Our results suggest that chronic neuroinflammation is capable of compromising waste clearance from the brain and promotes the pathological accumulation of macromolecular waste products and impaired cognitive function.

ATRAZINE CAUSES OLFACTORY SENSORY NEURON APOPTOSIS IN CRAYFISH (*ORCONECTES VIRILIS*) FOLLOWING ENVIRONMENTALLY RELEVANT EXPOSURES,

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The herbicide atrazine is heavily applied in agricultural areas in the Midwestern United States and can run-off and seep into surrounding aquatic habitats where concentrations can reach over 300 ppb. Previously, our lab has shown that 96-hour exposures to 80 ppb atrazine cause lasting deficiencies in the chemoreception of food and mate odors. Due to the fact that atrazine impairs chemosensory responses, the goal of this study was to determine the effect of atrazine on olfactory sensory neurons located in the lateral antennules of crayfish. In this experiment, we treated crayfish with atrazine for 10 days with ecologically relevant concentrations of 0, 10, 40, 80, 100 and 300 ppb. Following treatments, the proximal portion of the lateral antennules were cryosectioned. We used a TdT mediated dUTP nick-end labelling (TUNEL) to determine if any cells had DNA damage and were thus undergoing apoptosis. We have found that as atrazine concentrations increase, the number of TUNEL-positive cells in the lateral antennules increases. Overall, our data suggests that atrazine exposure causes degeneration of olfactory sensory neuron clusters and apoptosis, leading to impairments in chemosensory abilities.

THE ALPHA7 NICOTINIC ACETYLCHOLINE RECEPTOR AGONIST, PNU-282987, ACTIVATES RETINAL PIGMENT EPITHELIUM CELLS TO INDUCE NEUROGENESIS AND REGENERATION IN ADULT MAMMALS

Authors

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Abstract

Neurodegenerative diseases of the eye cause blindness and are incurable. Application of PNU-282987 (PNU), an α7 nicotinic acetylcholine receptor agonist (nAChR), causes neurogenesis of the retina in the adult mammal (Webster et. al. 2017). These new neurons arise from the Müller glia (MG) which mirrors regeneration in other vertebrates. However, the MG lacks α7 nAChRs. Our objective was to better understand where PNU acts in the mammalian eye to initiate neurogenesis and determine if PNU causes regeneration of new retinal ganglion cells (RGCs) in a glaucoma model. Our hypothesis was that PNU activates retinal pigment epithelium (RPE). To test this, RPE-J cell lines were treated with PNU for 24 hours before extensive washing. After 72hr in culture, the supernatant was collected and injected into both male and female adult mouse eyes. BrdU (1mg/mL) eyedrops were given for 7 – 14 days. IHC demonstrated incorporation of BrdU in all three nuclear layers after supernatant injection. In the INL, treated-RPE supernatant induced 50% (±10) BrdU-positive cells. To validate that PNU works through the α7 nAChRs in the RPE-J cell line, cells were pre-treated with an α7 antagonist, MLA, before PNU treatment. MLA decreased BrdU-positive cells to less than 5% (±1) which represents a significant reduction from those treated with PNU-modified supernatant (*n=6, p≤0.01*). To analyze potential genes involved in this process, RNAseq was performed on PNU-treated RPE-J cells. Cells were treated with PNU for 0.5, 1, 3, 8, and 12 hours before RNA was collected (RIN ≥ 7.0). Controls were DMSO-treated, MLA-treated, and nicotine-treated. RNA was processed by GeneWiz; fold-change data was analyzed in Excel and pathway analysis through Reactome software. Resulting signaling molecules were mapped for fold-change expression over time. To determine if PNU can act in a regenerative capacity, we examined the effect of PNU in an induced mouse model of glaucoma. Control retinas with glaucoma show an average loss of 25% (±5) of Thy1.2-positive RGCs 28-days post induction surgery, which were correlated with an average increase of IOP to 14.38 mmHg (±1.7). Glaucomatous retinas treated with PNU for 7 days showed regeneration and RGC cell counts returned to control levels. This research demonstrates a novel mechanism for mammalian retinal neurogenesis mediated through RPE cells and indicates the influence of several important signaling molecules. Further, this work shows that PNU treatment caused regeneration of RGCs after damage has taken place. This is significant as it points to a potential therapeutic approach to the millions affected by neurodegenerative retinal diseases.

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MiSFN abstract

Title: Blocking RAN translation enhances FMRP and reduces toxicity in unmethylated full mutation Fragile X stem cells.

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Expansion of a CGG repeat in the 5' UTR of the FMR1 gene underlies a heterogeneous set of human clinical disorders, including Fragile X Syndrome and Fragile X-Associated Tremor/Ataxia Syndrome. While full mutations (>200 repeat expansions) result in FXS typically lead to methylation and silencing of FMR1 expression, patients often display mosaicism of FMR1 methylation and CGG repeat length in individual cells. Large transcribed CGG repeats are potentially toxic as RNA or by triggering Repeat associated non-AUG initiated translation (CGG RAN). Moreover, large repeats can impede translation of FMRP even when transcription is sufficient. Therefore, effective therapies targeting large transcribed repeats will simultaneously block CGG RAN and enhance production of FMRP. To this end, our group developed a series of antisense oligonucleotides that selectively target RAN initiation sites (RAN ASOs) on the FMR1 transcript. Using patient-derived induced pluripotent stem cells (iPSCs) from an unmethylated full mutation (UFM) carrier, we generated human neurons that exhibit normal FMR1 mRNA transcription but very low FMRP expression. Application of RAN ASOs on these neurons effectively reduced accumulation of CGG RAN products and enhanced neuronal FMRP expression, suggesting that CGG RAN acts normally to inhibit FMRP synthesis. These biochemical correction were associated with enhanced UFM neuronal survival. Blocking endogenous CGG RAN also altered activity dependent FMRP synthesis in response to mGluR5 stimulation, a critical regulatory component of long-term depression. Together, these data suggest a native function for CGG RAN in regulating FMRP synthesis and demonstrate that targeting CGG RAN has the potential to correct multiple disease relevant features in Fragile X-associated disorders.

Sensory and Motor Systems

Abstract: Inhibitory control over the layer six corticothalamic feedback system

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The thalamus and neocortex are two reciprocally connected brain areas essential for normal sensation, movement and cognition. Altered communication between these areas has been associated with sensory processing deficits in certain diseases such as epilepsy and schizophrenia. Our lab utilizes the mouse somatosensory system, which is an ideal model to study the relationship between the thalamus and neocortex. A unique feature about this system is that the somatosensory cortex sends ten times as many axons back to the somatosensory nucleus of the thalamus (ventral posterior medial nucleus, or VPm) as it receives from VPm, suggesting the cortex may have a strong influence on its own sensory input. These descending projections originate from corticothalamic (CT) cells located in the deepest layer of cortex, layer six (L6). This CT cell system enables the cortex with the ability to modulate its own input by providing feedback to shape incoming thalamic information. It has recently become clear that the net influence of CT feedback on thalamic excitability (enhancement or suppression of thalamic throughput) is dynamic and depends critically on the rate at which CT cells fire (Crandall et al., 2015). Thus, to facilitate our understanding of CT systems, we must know how CT activity is controlled. This study sought to determine how feedforward inhibition mediated by local inhibitory cells in layer six is recruited by thalamus and its role in controlling the output of CT cells.

To target inhibitory cells, I used various transgenic mouse lines that each express fluorescent protein in one of the three distinct populations of GABAergic neurons in the neocortex- PV, SOM, and 5HT3aR. To gain optical control over the thalamic afferent to layer six, I performed viral injections encoding a variant of the light sensitive cation channel, Channelrhodopsin-2, into the VPm. I was then able to obtain acute brain slices for whole cell electrophysiology, allowing me to record activity of known populations of cells in layer six in response to optically-evoked thalamic stimulation. Recording from a fluorescently tagged inhibitory cell and a known excitatory cell simultaneously allowed me to compare the relative strength of thalamic input to each population of cells. Our results show that the relative strength of inputs to PV fast spiking inhibitory cells are much stronger than any other interneuron subtype. This suggests that these cells are likely the primary mediators of disynaptic feedforward inhibition onto L6 CT cells, providing a mechanism for how CT activity can be controlled.

Effect of Long Term Exercise on GDNF Expression and Innervation in Rat Skeletal Muscle

Alberto F. Cintrón-Colón and John M. Spitsbergen

Sarcopenia is a major cause of frailty and loss of independence in aging individuals. Results of recent studies suggest that changes in neural tissues may contribute to development of sarcopenia. Exercise provides neuroprotection by promoting neurogenesis, decreasing apoptosis, and modulating inflammation, however the mechanisms by which exercise generates these effects are not well understood. Neurotrophic factors are powerful regulators of neuronal maintenance and synaptic strength. Glial cell line-derived neurotrophic factor (GDNF) is a neurotrophic factor that has been shown to be a potent survival factor for somatic motor neurons that innervate skeletal muscle, but as age continues, GDNF levels in muscle tend to decrease. This study seeks to expand our understanding of the regulatory processes controlling GDNF expression, which will aid in identifying how these processes become disturbed with aging, injury or in neurodegenerative diseases. Our hypothesis is that long-term exercise will increase GDNF expression, and support neuromuscular junction (NMJ) structures. For these studies Sprague-Dawley rats (6-months of age) were exercised, in the form of voluntary running, for 6 months. Controls consisted of an age-matched sedentary group maintained in cages without access to running wheels. After 6 months of exercise hind-limb muscles were collected and processed for measurement of GDNF protein content via enzyme-linked immunosorbent assay. The length and area of motor end plates were quantified via staining with α-bungarotoxin. Immunohistochemical analysis was performed to detect co-localization of GDNF and choline acetyltransferase (ChAT), using antibodies against GDNF and ChAT. There was a significant ($p \leq 0.05$) increase in length of stained end plates in muscles from exercised rats (36.6 ± 3.5μm) when compared to that in the age-matched sedentary group (27.6 ± 1.15μm). There was a significant ($p \leq 0.05$) increase in area of stained end plates in muscles from exercised rats (616.3 ± 84.9) when compared to that in the age-matched sedentary group (430.3 ± 30.8μm^2). We also observed a trend towards an increase GDNF protein content in muscle from exercised rats compared to tissues from sedentary control rats; however, the effect was not significant. Although, co-localization between GDNF and ChAT appeared more apparent in tissues from exercised rats. These findings suggest that increased physical activity enhances structural neuroplasticity in NMJ elements and may possibly lead to enhanced neuromuscular function. Understanding the activity dependent regulation of neurotrophic factor expression and neural plasticity in the neuromuscular system may help in identifying novel targets for pharmacological development.

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Title: BACKWARD WALKING MEASURES ARE INDICATIVE OF FALLS IN MULTIPLE SCLEROSIS

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Purpose/Hypothesis: Accidental falls are common among individuals with multiple sclerosis (MS). Better screening tools to identify future fallers are needed to prevent injurious falls and to ensure proper referrals for rehabilitation. Although differences in backward walking have been identified among healthy controls and individuals with MS, the relationship of backward walking to falls has not been explored.

Subjects: Fifteen female participants (age 52.0± 9.0 years; symptom duration 16.5±10.8 years) with Relapsing Remitting MS participated in this study. 93% of the participants were taking disease-modifying therapies and 27% utilized walking devices during testing.

Materials/Methods: In a single session, we examined forward and backward walking performance over a GaitRite electronic walkway. Spatial and temporal gait parameters as well as coefficients of variation were calculated. Participants reported a 1-month fall history. Participants then kept a fall diary for the subsequent 6 months. Relationships among forward and backward walking and both retrospective and prospective falls were evaluated with Spearman correlations.

Results: While both forward and backward walking measures were strongly related to reports of retrospective falls, only backward walking was related to retrospective near-falls and prospective falls and near-falls. Backward walking velocity better differentiated fallers from non-fallers compared to forward walking velocity; a backward walking velocity of ≤0.86 m/s accurately distinguished fallers from non-fallers based on a retrospective 1-month fall history. A BW velocity of ≤0.74m/s accurately differentiated fallers from non-fallers on prospective fall reporting.

Conclusions: Backward walking speed better identifies MS fallers than forward walking speed. Backward walking may be a potentially useful tool to identify MS fallers.

Shedding Light on Behavior: The Neuronal Circuitry of Light-Guided Behavioral Responses in the Medicinal Leech

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The pervasiveness of visual systems across the animal kingdom highlights the adaptive value in the ability to detect and respond to electromagnetic radiation in the environment. Constrained by the common physics of light, there is enormous diversity in the structure of eyes in extant animals; yet, the fundamental components of visual systems (i.e. the neuronal circuits that analyze visual images) are remarkably conserved—emphasizing the importance of understanding the systems responsible for coordinating light-guided behavior. A central tenet of neuroethology is to determine the strategies employed by neuronal circuitry underlying decision-making processes. It is known that light-guided behavior results from the capacity of visual systems to encode behaviorally relevant image features, such as spatial frequency and luminal and spectral contrast; however, the processes by which these image features are encoded and decoded to execute coordinated behavioral responses is poorly understood. Here, we approach these questions using the medicinal leech, a highly tractable model animal that exhibits light-guided behaviors. Some of these behaviors include specific responses to ultraviolet (UV) light: Leeches will extend away from UV directed at the tail, while UV at the head elicits whole-body shortening—an example of opposite motor patterns depending on stimulus origin. Leeches also exhibit a mechanism for using color vision as a spatial cue, as they discriminate between green and UV light using dorsal/ventral spectral contrast to minimize ventral UV exposure and maintain body orientation. The leech collects visual information using and array of cephalic eyecups and a distributed, segmentally iterated array of dermal sensilla positioned to survey a 3-dimenstional visual field. Our work has shown that adaptation and spectral response properties of the primary receptors in this distributed visual system are spatially mapped. How does this peripheral mapping underlying image feature extraction cascade into the CNS to influence higher order executive functions? Our preliminary work shows evidence of integrated responses in higher order interneurons. We extend this observation and hypothesize that interneurons involved in these light-guided behaviors are influenced by the contextual state of the animal as determined by visual inputs. To facilitate this aim we are identifying a constellation of interneurons that receive visual input and evaluating how each responds to behaviorally relevant visual stimuli. Synaptic interactions between sensory axons and even higher order command neurons may provide the substrates for synaptic computation for image feature extraction by a simple nervous system lacking image-forming eyes.

FUNCTIONAL IMPLICATIONS OF MULTIPLE BURST PHENOTYPES IN THALAMIC RETICULAR NEURONS

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The Thalamic Reticular Nucleus (TRN) is a thin region of inhibitory neurons located between the dorsal thalamic nuclei and neocortex that plays a prominent role in modulating sensory processing. TRN neurons characteristically produce a high frequency (~200 Hz), transient burst discharge of action potentials at hyperpolarized membrane potentials due to activation of low-threshold T-type calcium channels. TRN neurons are not homogeneous, as there is a subset of neurons that do not produce burst discharge. In this study, we found a subpopulation of slowly-bursting neurons, characterized by maximal burst frequencies below 100Hz and a smaller amplitude calcium-dependent depolarization compared to regular bursting neurons. Here we investigate the physiologic significance of these multiple burst phenotypes by quantifying the downstream inhibition produced by the TRN neurons in ventrobasal nucleus (VB) neurons. We obtained whole cell recordings from VB neurons and electrically stimulated TRN axons at frequencies ranging from 10 Hz (non-bursting) to 300Hz (regular bursting). We found that increasing stimulus frequency produced a larger inhibitory response. Secondly, at all frequencies the inhibitory response was mediated by $GABA_A$ receptor activation; however, at higher frequencies, $GABA_B$ receptors were also activated. These data suggest that non bursting neurons would produce a short phasic inhibitory drive onto thalamocortical neurons, whereas regular bursting neurons would produce a larger and longer lasting inhibitory response mediated by both $GABA_A$ and $GABA_B$ activation. With these finding, we hope to better understand the consequence of TRN-dependent inhibition in the modulation of sensory processing with potential insight with regards to sensory processing disorders, as those seen in Autism.

DIFFERENTIAL RESPONSE OF ZEBRAFISH OLFACTORY SENSORY NEURON SUBTYPES AFTER INTRANASAL INFUSION WITH DETERGENT, Maser, T.L. and Byrd-Jacobs, C., Western Michigan University

Zebrafish have the natural ability to quickly regenerate olfactory sensory neurons (OSNs) after damage, making them an ideal model organism for studying neuronal plasticity in vertebrates. The zebrafish olfactory epithelium contains ciliated, microvillous, and crypt neurons that are distinct in structure and behavior. Ciliated OSNs detect bile salts that are important for social behaviors, microvillous OSNs detect amino acids that mediate food sensing abilities, and crypt OSNs are important for detecting sexual cues. Our hypothesis is that neurons mediating reproductive and social behavior are more sensitive to damage while neurons required for food detection are more resistant. The purpose of this study is to show whether microvillous OSNs are more resistant to damage, or if they appear more resistant because of quicker regeneration or reduced surface area compared to ciliated OSNs. To address this hypothesis, adult zebrafish were intranasally infused with 0.7% Triton X-100 once or on two consecutive days and were allowed to recover for 1 day post treatment. OSNs were identified using either anti-Hu (all OSNs), anti-Trpc2 (microvillous OSNs), anti-Gαs/olf (ciliated OSNs), and anit-TrkA (crypt OSNs). Comparisons in the amount of labeling were made between the treated side and the internal control side as well as with untreated control tissue using optical density analysis and cell counts. Results showed that anti-Gαs/olf labeling was significantly reduced following both single and double exposure to the detergent. Anti-TrkA labeling did appear to decrease with one TX-100 exposure, and was significantly reduced after two TX-100 exposures. Anti-Trpc2 labeling of microvillous neurons was not affected by a single dose of detergent, but there was a significant reduction in labeling after two consecutive detergent treatments. This suggests that microvillous OSNs are indeed susceptible to damage by detergent, but they may be protected from a single insult by the thick mat of cilia from the ciliated OSNs. We predict that the first infusion with detergent kills the ciliated neurons and removes the protective covering, exposing the microvillous neurons and causing them to be susceptible to the second detergent infusion. This study provides further support that OSNs display a differential response to injury, with microvillous sensory neurons showing resistance to chemical damage. This work has relevance to general neuroprotective mechanisms that ensure proper functioning of sensory input after damage.

Reprogramming of Feeding Behavior by Diet

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While we understand how changes in the environment such as temperature and light direct animal behavior by acting acutely on neural circuits, we know less about how the environment can lead to persistent changes in brain and behavior. Tackling this question has been challenging because it requires having a circuit-based understanding of the behavior and a mechanistic way to study how neural connections are changed by the environment. The reshaping of circuits that regulate food intake by a hyper-caloric diet in *Drosophila melanogaster* provides an attractive model for studying this question because the circuits are mapped, the behavior is easily quantifiable, and the environmental variables are simple to measure. We found that animals fed a Western style high-calorie diet show profound deregulation of feeding states: they incorrectly process the nutritional value of food, eat more, and become obese. We will present data showing how these behaviors are mediated by the metabolic-transcriptional reprogramming of distinct feeding circuits by diet and how their effect is persistent even after animals are returned to the control diet.

ELECTROPHYSIOLOGY AND ANATOMY OF PYRAMIDAL NEURONS IN SOMATOSENSORY CORTEX OF TWO MOUSE MODELS OF AUTISM

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Autism spectrum disorder (ASD) is characterized by a range of social and neurological deficits that can be caused by both environmental and genetic factors. Fragile X Syndrome (FXS) is the most common monogenic cause of ASD, resulting from a silencing mutation in the *FMR1* gene encoding the RNA-binding protein FMRP. Absence of FMRP at neuronal synapses leads to alterations in synaptic transmission and dendritic spine development, which are attributed to the human phenotypes including: hypersensitivity, learning deficits, and repetitive behaviors. Similar deficits have been observed in humans exposed to the drug valproic acid (VPA) during gestation. Specifically, mouse models of FXS and VPA-exposure display a hypersensitivity to touch stimuli, though the physiological basis of this has not yet been explored. Considering the role of primary somatosensory cortex (S1) in ASD behaviors, studies have proposed a physiological link between an altered dendritic spine phenotype, neuron excitability, and ASD behavior. In order to investigate this link, we generated a dataset including two time points for wildtype (WT), *Fmr1* knockout (KO), and VPA-exposed mice: during development at postnatal day 30 (P30), and in adulthood at P90+. Using a combined two-photon laser imaging and patch-clamp electrophysiology approach, we show a decrease in dendritic spine density and length for the *Fmr1*-KO and VPA conditions at P30 in layer 5 pyramidal neurons in S1. We also observe a trend towards altered intrinsic properties that requires further work to show how physiology and morphology are linked in the ASD condition.

COMBINING BIOLUMINESCENCE DRIVEN OPTOGENETIC STIMULATION WITH SWIM TRAINING FOR TREATMENT FOLLOWING SPINAL CORD INJURY IN RATS

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Spinal Cord Injury (SCI) is an injury that can result in paralysis below the site of injury. Out of all of the common methods used in medicine, most of the spinal cord injury treatment procedures have negative trade-offs that could permanently damage neurons. We are going to couple a non-invasive method to target neurons after injury along with swim therapy. In the case of this study we will be able to target interneurons of the spinal cord by combing an adeno associated virus (AAV) serotype 2/9 with a synapsin promoter to restrict gene expression and subsequent stimulation to interneurons of the spinal cord. The AAV also expresses a light-emitting luciferase fused to a channelrhodopsin, which is light sensitive. Neural cells will be stimulated by the addition of Coelenterazine (CTZ), which is the luciferase substrate. CTZ injections will ultimately lead to activation of synaptic networks of neurons that express the excitatory luminopsins. The Human Synapsin Promoter (hSYN1) can drive expression of the luminopsin, LMO3, which is stimulated by CTZ in adult rats. This will result in functional connectivity across the site of injury, aiding in the recovery of locomotion below the site of injury. After the interneural spinal cord stimulation, rigorous swim testing and therapy will be conducted in order to analyze the effects of the swim testing. Constant gait analyses will be done in order to evaluate the progression of the gait of the rats after injury and throughout the treatment.

The Presence of Photoreceptors in The Posterior Sucker of *Hirudo verbana* Inferred Through Functional Visual Response.

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The majority of animals receive and use light input for a variety of adapted behaviors including orientation in 3-dimensional space, predation, and escape tactics. Due to the physical nature of light, all visual systems fundamental neural basis have stark similarities. One of the core principles of neurophysiology is to determine how the nervous system controls the decision-making process leading to observed behaviors. In a previous study, it was observed that leeches have different responses to ultraviolet light stimulation on the anterior and posterior portion of their bodies suggesting that leech's decision hierarchies are influenced by light stimuli at both their head and tail. The presence of photoreceptors on the tail sucker could contribute to the difference in the observed behaviors. Medicinal leeches receive light input through modified sensilla across their entire body surface. Visual input is received from both its five pairs of bilateral eyecups and 14 photoreceptive sensilla embedded in the body wall of each of its 21 midbody segments, but what about its tail sucker? During embryogenesis four of the leeches, 32 segments fuse together to form its head and eyes, similarly, seven segments fuse together to form the posterior sucker. If no sensilla were lost during the fusion of the posterior seven body segments, the posterior sucker would possess one-quarter of the receptors in the entire body wall. There have been few studies on the hindbrain and tail sucker of the Medicinal Leech, however, there are known pathways in which the hindbrain and anterior brain communicate, like the interneuron S-cell which plays a role in rhythmic and mesenteric movements like crawling and swimming. In 10 trials, this study isolated the caudal region of the leech through partial ablation of the connective nerve between ganglion eleven and twelve and division of the body wall between body segment twelve and thirteen, then tested visual responses with and without the hindbrain isolated. Extracellular recordings were analyzed for S-cell response then standardized and compared. All 10 trials showed S-cell response to light stimuli while the hindbrain was isolated suggesting the tail sucker possesses sensilla.

Motor Learning as a Sensitive Behavioral Marker of Early Alzheimer's Disease

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Introduction: Alzheimer's disease (AD) is the sixth leading cause of death in the United States and is apparent in 60-80% of all cases of dementia. While early stages of AD are diagnosed by assessing impairments in cognitive functioning, neuropsychological batteries have only modest reliability, suggesting that they do not capture subtle changes in cognitive function that occur during the earliest stages of the disease. When learning a new motor skill, declarative memory processes allow us to make rapid improvements in performance initially. More gradual improvements are made during later stages of learning because of non-declarative memory processes. AD is associated with more significant impairments in declarative than non-declarative memory resources for learning and retaining information over short and long delays. Here, we investigate whether the early stages of motor learning are a sensitive behavioral marker of subtle cognitive impairments in AD, and whether short-term (i.e., within session) and long-term (after a 24 hour delay) retention of a newly acquired motor skill is also sensitive to the development of the disease.

Methods: The aim of this project is to recruit participants diagnosed with the MCI and the early stages of AD as well as control groups of cognitively healthy older adults and healthy young adults (n=20 per group). Motor learning can be investigated by having participants reach to visual targets while grasping a handle attached to a robotic device (KINARM, B-kin Technologies). Participants attempt to make a straight movement towards visual targets, while the robot applies a velocity-dependent force perpendicular to the direction of the target. While this load initially perturbs hand movement, participants gradually adapt by producing forces that counteract the load. On Day 1, participants adapt to the force-field and then perform a final block without the perturbation to 'washout' what was learned in the adaptation phase. On Day 2, participants return 24 hours later to perform the same motor learning task. All older participants also perform a standard neuropsychological assessment of cognitive function so that we can assess whether the motor learning task adds to the sensitivity of the neuropsychological battery to subtle cognitive changes in MCI and early AD.

Results: Here we present preliminary motor learning data from 20 healthy younger adults and compare their Day 1 data to previously acquired healthy older adult and AD participant data. The results suggest that the early stages of motor learning are impaired in AD, but not in healthy aging, as is short-term, within-session retention of the acquired skill. We also present Day 2 data from the younger adults only to demonstrate long term retention of the acquired motor skill. As we continue to recruit healthy older adults and participants with MCI and AD, we will assess the sensitivity of long-term retention measures of motor learning to early cognitive impairments. The overarching goal of this work is to determine whether short- and long-term memory measures in motor learning can distinguish between groups and supplement existing neuropsychological measures for diagnosing MCI and AD.

Techniques

Bioluminescence driven control of photosensory proteins

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Bioluminescence is light emitted by a luciferase oxidizing its substrate. We previously demonstrated that such "biological" light can activate optogenetic elements, such as channelrhodopsins and pumps, effecting membrane potential changes and resulting in activation or silencing of neurons in vitro and in vivo [1,2]. We explored whether bioluminescent light production can be utilized beyond activating ion-moving photoreceptors to the larger array of photosensory proteins employed as optical switches in cellular processes such as protein translocation and transcription [3].

In initial proof-of-concept experiments we co-transfected HEK293 cells with a blue light emitting luciferase and a blue light sensing photoreceptor. Light emitters were sbGLuc, a copepod luciferase variant, NanoLuc, a luciferase derived from shrimp, as well as two novel engineered synthetic luciferases. Photoreceptors were CRY/CIB, a light-gated dimerization system [4], and eLOV, based on light dependent protein unhinging [5]. Bioluminescence driven activation of these photoreceptors was measured as increased transcription of luminescent and fluorescent reporter proteins in direct comparison to LED driven activation.

Quantification of bioluminescence driven photoreceptor activation revealed that both light-gated switches, cryptochrome protein dimerization and light-oxygen-voltage J-alpha helix unfolding can be efficiently activated by biological light sources. Furthermore, the higher light emission of our synthetic luciferases resulted in better activation of transcription.

There are many ways to improve further on these basic results. Collectively, bioluminescence driven activation of the larger families of photoreceptors will expand their use for in vivo applications that benefit from non-invasive light sources and engagement of spatially distributed cells.

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The authors declare no conflicts of interest.

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QUANTIFYING STIMULUS-BASED NEURONAL ACTIVITY IN RAT BRAIN USING HIGH-RESOLUTION PHOTOACOUSTIC IMAGING

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Current functional imaging techniques, such as functional magnetic resonance imaging, rely upon activity-induced blood flood changes to neurons. This indirect measurement of neuronal activity inherently limits image resolution and specificity. However, advances in transgenic technology and photoacoustic (PA) methodology have offered new solutions to these limitations. Selective organic dyes and nanoparticles with high optical absorption in the near-infrared window, outside the range of which endogenous chromophores strongly absorb, can be valuable for generating a targeted signal with a high contrast-to-noise ratio. For example, virally-infected tumor cells can be made to express enzymes, such as LacZ, that are capable of cleaving colorless substrates into colored products in the presence of X-Gal. Therefore, since Fos is used as a marker of activated neurons, we propose using PA imaging to map activated neurons using a Fos-LacZ transgene reporter system in rats. Fusion of Fos with the lacZ gene gives active (Fos+) cells the ability to cleave pro-chromogenic substrates into PA-active dyes. In this study, we visualized neuronal activity PA signal in Fos-LacZ transgenic rats following two different stimulation methods.

We subjected Fos-LacZ rats to one of three conditions: Footshock, Cocaine bolus (one dose, 20 mg/kg), or home cage naïve. Ninety minutes after the stimulus presentation, the rats were injected in the medial prefrontal cortex (mPFC) with X-Gal. Brains were excised, then PA imaged *ex vivo*. We used an elevational scanning 18.5 MHz, 128 element L-22 linear array ultrasound transducer to record PA signal produced by pulsed laser illumination at both 690 nm and 850 nm nearly simultaneously through rapid scanning. Laser light was directed from a PA-optimized OPOTEK Phocus MOBILE laser by two fiber optic cable arrays placed on both sides of the probe to focus light approximately 1 cm beneath it. PA intensity within the mPFC of acquired images was quantified using ImageJ software.

We presently report quantified *in vivo* PA images of rat brains expressing X-Gal product prepared from footshocked, cocaine-treated, or naïve animals.

We discuss the feasibility of this reporter method for neuronal activity based on our acquired images, focusing on observed differences between stimulus-treated and naïve animals. With this technique, we propose a method of longitudinally monitoring activated (Fos+) neurons *in vivo* with high resolution and specificity.

ACTIVITY DEPENDENT NEURONAL MODULATION

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We have developed a bioluminescent protein based calcium indicator, Lumicampsin (LMC) by splitting a mutated Gaussia luciferase and introducing the Ca2+ sensing moiety Calmodulin-M13 (CaM-M13) in between the two split halves. Our results in vitro show that LMC is capable of producing a delta RLU/RLU_o of about200% which is higher than most luciferase based Ca2+ indicators. We generated 8 versions of LMC that have varying sensitivity to Ca2+ by using different CaM-M13s from already established sources (GCaMP6s, GCaMP6m, GCaMP6f, etc). To increase LMC's functionality, we have fitted it with various organelle localizing sequences to interrogate subcellular Ca2+ dynamics of the ER, mitochondria and the Golgi apparatus. LMC's superior light emission allowed activity dependent neuronal modulation by co-expressing LMC with various optogenetic elements in primary neuronal cultures. In our initial experiments, on multi-electrode arrays, we co-expressed LMC either with Mac (inhibitory proton pump) or ChR2-C138S (excitatory step function cation channel) and were able to modulate the opsins via LMC's Ca2+ dependent light emission. Currently we are working towards optimizing this system to achieve reliable and efficient coupling of Ca2+-induced light production and optogenetic effector activation.

TRANSSYNAPTIC NEURONAL COMMUNICATION VIA BIOLUMINESCENT OPTOGENETICS

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Neuronal communication mediates complex computations which underlies a range of physiological and behavioral properties. Complex routes of communication creates issues when trying to manipulate specific transsynaptic partners experimentally. Bioluminescence-driven Optogenetics (BL-OG) is the method of harnessing a genetically encoded light source, a luciferase, and a light-sensing component, an opsin, to interrogate functional neuronal transmission. The aim of this project is to exploit BL-OG to experimentally manipulate neuronal communication between transsynaptic partners. To achieve this aim, two different methods will be utilized: one being activity-independent and another being activity-dependent.

Activity-independent driven communication will be achieved by utilizing a method similar to GRASP (GFP Reconstitution Across Synaptic Partners). *Gaussia* luciferase (GLuc) can be split into inactive N- and C-terminal portions and tethered to a pre-synaptic neuron or post-synaptic-bound opsin respectively. With addition of coelenterazine (the substrate for GLuc) reconstitution of the luciferase across the synaptic cleft will occur and activation of the post-synaptic opsin and corresponding neuron can be achieved. By harnessing BRET (Bioluminescent Resonance Energy Transfer) and GRASP in tandem, a second form of activity-independent communication can be achieved. Attaching a split fluorescent protein to either a pre-synaptic bound or post-synaptic bound luciferase and an opsin will allow for the reconstitution of the protein – similar to the split luciferase. This BRET-based approach should cause an amplification in the luciferase-emitted light and activation of the post-synaptic opsin and neuron.

Activity-dependent communication can be achieved by targeting an intracellular luciferase to dense core and synaptic vesicles in the presynaptic neuron. When the neuron becomes depolarized, the luciferase can be released from the presynaptic neuron and activate a postsynaptic opsin and neuron. To achieve this, the N-terminal neurosecretory sequences of NARP (neuronal activity regulated pentraxin) and synaptobrevin will be utilized. The N-terminal NARP sequence will target luciferases to dense core vesicles while the synaptobrevin sequence will target to synaptic vesicles – allowing for two separate pathways to be explored. The development of these tools will provide knowledge of experimentally driven neuronal circuitry that can be used to advance therapeutic strategies within neuroscience for both physiological and behavioral needs.

PAMAM dendrimers: Characterization, labeling properties *in vitro* and applications *in vivo* for delivery of large biomolecules into the brain

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Dendrimers are 3-dimensional nanoparticles with branches having applications in the field of biomedical sciences. Dendrimers are available in different sizes, based on their generation (G) or number of branching points. G1 dendrimers are 1nm in diameter and G2 dendrimers are 2nm in diameter and so on. Previous evidences show that the G4 dendrimers having 100% amine surface (G4-NH_2) are highly toxic to cells *in vitro* and *in vivo* due to their highly positive charged amine groups. Therefore, to reduce the toxicity of the dendrimers, we modified the pure 100% amine surface of the dendrimers to a surface having neutral functional groups with 10% of the surface covered with -NH_2 and 90% of the surface covered with hydroxyl groups (-OH; known as G4-90/10). Our *in vitro* data shows that these surface modified dendrimers are taken up by the cells *in vitro* and *in vivo*. The toxicity assay show that these surface-modified dendrimers are 8-fold less toxic compared to the pure 100% -NH_2 surface dendrimers. Three weeks following unilateral intracranial injections into the striatum of these different sized dendrimers (G1-90/10 and G4-90/10), we observed that these dendrimers can migrate in the brain via corpus callosum at different rates depending on their size. Our research findings showed that the (1) dendrimers are taken up by the neuronal culture *in vitro*; (2) dendrimers alone can cross the BBB when injected via carotid artery and tail-vein, and are taken up by neurons and glial cells *in vivo*; and (3) the G1 and G4 dendrimers migrate in the brain following unilateral injections into the striatum up to 3 weeks, with a size dependent difference in migration between the G1 and the G4 dendrimers. Since the dendrimers are taken up easily by different cell types, we used this property to label and track stem cells in the brain following their transplantations in healthy mice. The current strategy for labeling stem cells are by using Hoechst (bisbenzamide). However, there are many reported issues and disadvantages associated with labeling cells with Hoechst. To circumvent these issues, we labeled the bone marrow derived mesenchymal stem cells (BM-MSCs) with Cyanine 5.5 (Cy5.5) tagged dendrimers. Following their uptake by the cells, we showed that the BM-MSCs were still able to proliferate and differentiate, proving that the dendrimers do not compromise the stemness of MSCs. Moreover, we also transplanted the Cy5.5 fluorescently tagged dendrimer MSCs into C57BL/6J mice and were able to track them in the brain using *in vivo* imaging system. Therefore, our results proved that dendrimers could be an alternate method to label and track the best suitable MSCs for treating Huntington's' disease. The dendrimers also have the ability to carry large cargo and biomolecules. Therefore, conjugating the drugs/biomolecules with the dendrimers could be a future aspect of delivering cargo systemically across the brain by crossing the BBB. The G4-90/10 dendrimers are capable of forming complex with plasmid DNA of various sizes. We have shown that these dendrimers can form stable complexes with any large-sized plasmids (having a reporter gene) and can deliver them to cells *in vitro* and *in vivo*. We also found that these complexes are non-toxic to the cells. Finally, we investigated the ideal route of administration of the dendrimers to the brain, possibly via systemic injections. These modified dendrimers were injected via tail vein (in multiple doses) into C57BL/6J mice. Our results showed that these dendrimers reach the brain by crossing the blood-brain barrier (BBB) and insert themselves into the brain cells, such as neuron and glial cells. Analyses of the presence of dendrimers in peripheral organs following showed that these dendrimers were present in the lungs, liver, spleen and kidneys in addition to the brain. Localizing into the kidneys showed that these dendrimers get eliminated out of the biological system thereby do not cause any side-effects as seen in any other type of nanoparticles that gets retained in the body.

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IDENTIFYING SOLUBILITY AND ABSORPTION PROPERTIES OF X-GAL PRODUCT FOR USE IN PHOTOACOUTIC IMAGING *IN VIVO*

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This project focuses on the optimization of a novel photoacoustic imaging technique to detect and quantify neuroactivation. Current neuroactivation imaging techniques rely upon activity-induced blood flow, which is an indirect measure. Our study uses a cFos/LacZ gene-reporter system to generate photoacoustically-active dyes from X-Gal in activated neurons, which can be directly identified and quantified, allowing for greatly increased specificity and spatial resolution.

X-Gal product was dissolved in 1:1, 1:9, and 9:1 concentrations of dimethyl sulfoxide (DMSO) and phosphate-buffered saline (PBS) to determine solubility. X-Gal was then added to seven solutions of 90% DMSO, 10% PBS, brought to a range of pHs from 5.5 to 8, and spectrophotographically analyzed to determine the absorbance curves of X-Gal across different pHs.

Our results show that X-gal is most soluble in a vehicle of 90% DMSO, with precipitates forming in the other solutions. The absorbance of X-Gal peaked at 7.0 pH with 0.1705 arbitrary absorbance units (AU), was similarly absorbent at 7.5 pH with 0.1695 AU, and was minimal at 5.5 pH with 0.1205 AU.

Our results indicate that so long as X-Gal is dissolved in a vehicle composed mostly of DMSO, it is viable in a biological model given its absorbance peak at a pH of 7.0-7.5 AU. This enables us to proceed with *in vivo* research using X-Gal product as our photoacoustically-active dye.

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